The efficacy of home disinfection techniques for pacifiers

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A research report submitted to the Faculty of Health Sciences, School of Oral Health Science, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Science of Dentistry, 2015.

DECLARATION

I, Deepa Bhoola, declare that this dissertation is my own work. It is being submitted for the degree of Master of Science of Dentistry at the University of the Witwatersrand, Johannesburg. It has not been submitted previously for any degree or examination at this or any other university.

An ethics wavier was granted by the Human Research Ethics committee, University of the Witwatersrand, Johannesburg. The certificate number is W-CJ-130626-1.

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DEDICATION

An investment in knowledge pays the best interest

(Benjamin Franlin)

To my dear parents, Mahendra & Manjula Bhoola who gave me the best investment, an opportunity to study.

ABSTRACT

Introduction

Non-nutritive pacifiers are used worldwide for infants to pacify them and provide comfort. In addition, pacifiers have analgesic effects on infants who are in pain, assist preterm babies in the development of sucking reflex and reduce the incidence of sudden infant death syndrome. They are usually sucked by the infant for a period of time during the day when it can become contaminated with saliva, oral microflora, pathogens and environmental bacteria. These bacteria form a biofilm on the pacifiers which is constantly fed by salivary compounds. Latex pacifiers are known to harbour increased numbers of contaminants compared to the silicon pacifiers because of the rough surface, flexible texture and susceptibility to micro-cracks. The immune systems of infants are not fully developed during these early years of life, and therefore infants who use pacifiers are known to have increased episodes of diarrhoea, common cold and flu. General cleaning and disinfection of pacifiers is important in the prevention of infections. The aim of this study was to evaluate the efficiency of various commercially available sterilizing systems and disinfection procedure in the decontamination of artificially contaminated latex and silicon pacifiers.

Methods

Each of the 54 latex and silicon pacifiers was purchased. Cultures of *Staphylococcus aureus, Escherichia coli* and *Candida albicans* were obtained from the Oral Microbiology laboratory and cultured using blood agar for *S. aureus* and *E. coli* and Sabouraud agar for *C. albicans*. A set of 6 pacifiers were used per test round. Pacifiers were artificially contaminated with test organisms. One pacifier was submerged into 150 ml sterile distilled water, mixed well using a magnetic

stirrer and a serial dilution technique was performed to obtain a quantity of organisms. These counts were used as a control count for the number of challenged organisms. The second pacifier was rinsed with tap water for 2 minutes, placed in 150 ml of sterile distilled water, centrifuged and cultured for the quantitative analysis. The third, fourth, fifth and sixth pacifier was each either boiled for 5 minutes, placed in hypochlorite containing Milton solution for 30 min, microwaved for 8 minutes or placed in 2 % chlorhexidine solution for 2 minutes respectively. Decontaminated pacifiers were placed in 150 ml of sterile distilled water, centrifuged and cultured for the quantitative analysis. Colony counts were performed, percentage kill and log reduction was calculated using the control counts. These experiments were repeated three times for all three test organisms and the two types of pacifiers. Percentage kill of disinfectants were compared using Kruskal Wallis nonparametric rank tests between the types of pacifiers, types of test organisms and the disinfection procedure.

Results

The results showed that rinsing with tap water removed 99.34 to 99.80% (log reduction of 2.35 to 2.8), 98.66 to 99.87% (log reduction of 2.28 to 2.91) and 99.63 to 99.96% (log reduction of 2.75) of *S. aureus, E. coli* and *C. albicans* respectively. For all the test organisms, boiling and microwaving obtained complete 100% kill. Milton showed a complete elimination of *S. aureus* (100%) but failed to kill *E. coli* (99.99%) and *C. albicans* (99.94%) completely. Finally, chlorhexidine completely eliminated *S. aureus* and *C. albicans* but was ineffective for *E. coli* (99.99%). When the percentage kill of latex and silicon were compared no significant difference was noted ($p>0.05$). Similarly, no significant difference in the percentage kill of *S. aureus, E. coli* and *C. albicans* was seen ($p>0.05$) in either latex or silicon pacifiers. However, when the methods of disinfection were compared to one another, all the methods performed better than

water rinse ($p=<0.01$). Yet no significant difference was noted between boiling, Milton, Microwave and chlorhexidine.

Conclusions

All the test methods including rinsing with water can reduce the number of contaminants. However, boiling and the use of microwave can completely eliminate common pathogens. These methods are cost effective, readily available and easy to use. Education to the care givers in the day care centres and to mothers with regards to the contamination and decontamination of pacifiers is important. It may reduce the transmission of pathogens and hence prevent and reduce the onset of infection among the pacifier using infants.

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NOMENCLATURE AND ABBREVIATIONS

AAP	American Academy of Pediatrics
AIDS	Acquired Immune Deficiency Syndrome
AOM	Acute otitis media
BPA	Bisphenol A
C. albicans	*Candida albicans*
CHX	Chlorhexidine
Cfu	Colony forming units
°C	Degrees celcius
E. coli	*Escherichia coli*
ml	Milliliters
%	Percentage
PAS	Periodic acid – Schiff
PBS	Phosphate Buffered Saline
PC	*Pseudomembranous candidiasis*
SAB	Sabourand agar
S. aureus	*Staphylococcus aureus*
SSSS	Staphylococcal scaled skin syndrome
S. mutans	*Streptococcus mutans*
SIDS	Sudden infant death syndrome
WHO	World Health Organization

PREFACE

Pacifiers are used by 75-85% of infants in Western countries which has advantages and disadvantages. One of the disadvantage of the use of pacifier is it becomes contaminated with pathogens and therefore regular disinfection of pacifiers is important. Many methods of disinfection are described and some are commercially available. However, there efficacy against common pathogens have not been comprehensively studied.

In this study latex and silicon pacifiers were artificially contaminated with common pathogens *Staphylococcus aureus, Candida albicans* and *Escherichia coli* and were disinfected using home disinfection techniques that are available and commonly practiced. This study showed some interesting results suggesting some effective, simple and cheap disinfection techniques.

The efficacy of home disinfection techniques for pacifiers

1 Introduction

Pacifiers/dummies/soothers are items designed for infants aged from around 2 weeks to around 5 years of age. They are used to pacify infants/toddlers when they are in discomfort; feeling insecure or crying, when they are sick, have colic or are teething. Sometimes they are given just before bedtime to sooth them. The pacifier is usually sucked by the infant for a period of time, and at many times during the day. It simulates the mother's breast nipple and therefore comforts the child. This sucking motion involves creating a seal with the lips while sucking, and swallowing at intervals. Once the pacifier has been introduced into the oral cavity, it comes in contact with saliva and with the oral microflora of the oral cavity. The oral microflora is composed of many different microorganisms including pathogenic species. The chemical composition of the saliva serves as nutrients for these microorganisms. These microorganisms including pathogenic species present in the saliva adhere to the pacifier and therefore the pacifier becomes colonized.

When the pacifier is not in use, it is usually kept in a pacifier container, or chained and attached to the infants' clothes hanging free and in reach of the infant to reuse at leisure. This can allow bacteria to grow on the pacifier between uses which could become potentially pathogenic to the child. Over time this growth of bacteria may result in a formation of a biofilm which are microbial aggregates included in an extracellular matrix (Costerton *et al.*, 1999). Furthermore, the pacifier can be contaminated with bacteria from the environment as they can stick to the pacifier as children often drop the pacifiers on the floor. Parents or caregivers may suck on the pacifier in attempt to clean it, and place it back into a child's mouth. Sucking on a fallen pacifier

may remove loose dirt and sand, but further contaminates the pacifier with additional oral bacteria from the adult. In daycare centers cross contamination between sick children can also occur through the use of pacifiers. Therefore, it is important to remove the bacteria and prevent the biofilm from maturing, since mature biofilm is more resistant to disinfection procedures. This study investigated the efficacy of commercially available sterilizing systems and disinfection procedures.

2 Literature Review

2.1 Pacifiers

Pacifiers are used by 75-85% of babies in Western countries (Niemela *et al.,* 2000). They constitute of a nipple, a shield/guard and a ring. The ring serves as a handle and is attached to the shield. This ring/handle made of polycarbonate, lies extra orally and is the area designed to be held when placing or removing the pacifier from the infant's month. . The shield, also made of polycarbonate, like the ring, lies extra-orally. It is generally 1.5cm in radius in all directions, as a safety measure preventing the infant from swallowing or choking on the pacifier. Therefore, the shield is sometimes referred to as a guard. Some pacifiers may have two perforations on either side of the shield. These perforations serve as ventilation holes preventing saliva from collecting behind the shield that can cause skin irritation and a rash around the infant's mouth. The nipple is the sucking part of the pacifier and is the part that is attached to the shield. It is made of silicon or latex and the shape resembles that of a lactating mother's nipple. These artificial nipples come in two distinct shapes, the round shape pacifier known as the conventional pacifier and the one with a flattened end on one side known as the orthodontic pacifier. Orthodontic pacifiers are recommended by dentists due to the ergonomic shape. The flattened shape permits the tongue to

be accommodated, allowing a more natural sucking posture (Figure 2.1). Some researchers have proved that the malocclusions seen in toddlers using these pacifiers are slightly less than, in toddlers who used a conventional pacifier (Adair *et al.,* 1992).

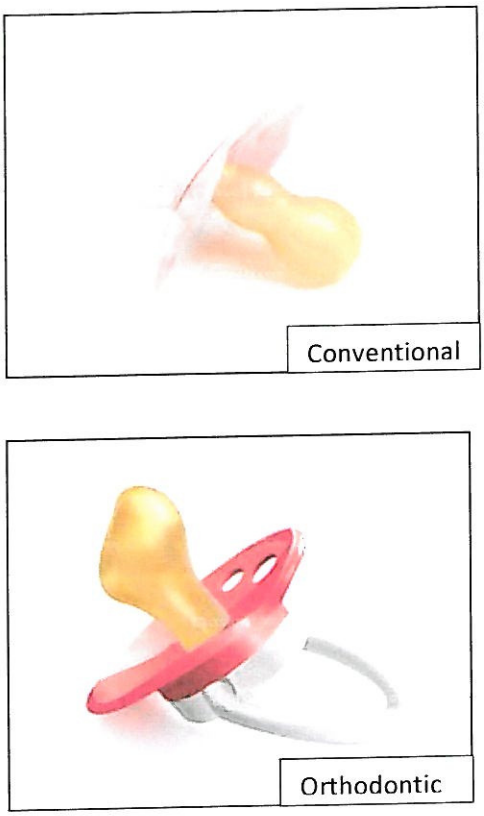


Figure 2.1: Conventional and orthodontic pacifier

2.1.1 Types of pacifiers

Two types of pacifiers are available, namely: latex and silicon (Figure 2.2). Silicon pacifiers, being the sturdier type of pacifiers, are easy to clean (Redmond *et al.,* 2009) and do not retain odours. Although latex pacifiers are softer and more flexible than silicon pacifiers and therefore

often preferred by infants, this softness results in the pacifier being worn out easily resulting in formation of micro cracks (Mainstone, 2004). These rough surfaces and micro cracks harbor bacteria, making the latex pacifiers difficult to clean. A study has shown that latex pacifiers have rugose and irregular surfaces which become colonized with microorganisms including *Candida albicans* and *C. parapsilosis* which cause oral thrush (da Silveira *et al.,* 2009). Comina *et al.,* 2006, collected used latex and silicon pacifiers and performed direct microscopic observations of the biofilm. The results revealed contamination of the latex nipple clearly showing a mature biofilm, in which bacterial cells (rods) were embedded in slime. The silicon pacifiers had a much lower level of slime contaminated with Staphylococcus. In this particular study the new latex pacifiers presented a clean but irregular surface with many grooves. In contrast, the new silicon pacifiers surfaces were also clean, but much smoother then the latex pacifiers.

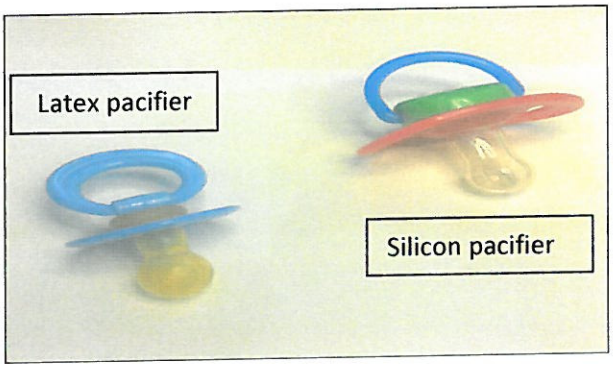


Figure 2.2: Latex and silicon pacifier

2.2 Advantages of pacifiers

As dentists, a common question asked by mothers is "Is it ok to give my child a dummy?" It is therefore important for a dentist and other health care providers to be aware of the advantages and disadvantages of pacifiers when advising parents. It is highly questionable as to whether a pacifier is a convenient and an efficient alternative to children sucking their thumb.

2.2.1 Analgesic effects

Non-nutritive sucking is a natural reflex for a fetus and newborn (Sexton and Natale, 2009). This is often noticed on ultra-sound scans as the fetus may be seen sucking on its hands or fingers. A pacifier may serve to fulfill this innate desire. According to Niemela *et al.,* 1995, the infant's need for sucking is greatest during the first 6 months of life. A subgroup of the American Academy of Pediatrics (AAP) has listed some minor medical procedures in which the use of pacifiers have analgesic effects on newborns and infants less than 6 months of age (Zempsky and Cravero, 2004; Kaufman *et al.,* 2002). These procedures include catheterization, circumcision, heel sticks, immunizations, insertion of an intravenous line, lumbar puncture, screening for retinopathy of prematurity and venipuncture. Furthermore, a shorter hospital stay for preterm infants using a pacifier has also been listed as an advantage (Sexton and Natale, 2009). A pacifier also has an analgesic effect on a teething toddler. However, the exact mode of action of sucking a pacifier, to act as an analgesic, is not fully understood.

2.2.2 Oromotor development

The sucking reflex assists preterm babies who are at risk of oromotor dysfunction to develop a better sucking reflex and therefore improve the oral feeding ability (Poore *et al.,* 2008). It does

not require a lot of energy to suck and assist in good coordination and muscular strength. Therefore, it is important that preterm infants start oral feeding as soon as possible to survive and get healthy quickly. It is believed that the oral feeds would increase the daily weight gain and therefore assist in the infant being discharged from hospital earlier. However, an evidence - based systematic review by Arvedson *et al.,* (2010) on the effects of motor interventions on feeding and swallowing in preterm infants, found none of the oral motor interventions provided consistent positive results on weight gain. The non-nutritional sucking did however show strong positive improvement in feeding and swallowing physiology, and reduced transition time to oral feeds. Furthermore, the continuous sucking increase the tension of the muscles of the upper airway and facilitates in keeping the tongue in a more forward position thereby protecting the airways (Jenik and Vain, 2009).

2.2.3 Reduction of sudden infant death syndrome

In literature, the reduction of sudden infant death syndrome (SIDS) in infants who use a pacifier while sleeping is well documented (Hauck *et al.,* 2005; Mitchell *et al.,* 2006; Sexton and Natale, 2009). However, the exact mechanism of this protective action is not fully understood. The diagnosis of SIDS is primarily a diagnosis of exclusion. This means that SIDS is given as diagnosis only after all other possible causes of death during sleep in an apparent healthy infant, have been ruled out. . Sudden infant death syndrome is one of leading causes of death in infants beyond the neonatal period in the United States (Arias *et al.,* 2003). The AAP guidelines suggests that offering pacifiers to an infant at the onset of sleep, soon after breastfeeding, has been established to reduce the risk of SIDS (AAP, 2005). The guidelines recommend establishing breastfeeding first for at least a month before introducing a pacifier, as later onset of

pacifier use appears to have fewer negative effects on breastfeeding (Howard *et al.,* 2003.) Reduction in SIDS due to the use of a pacifier is possibly due to less likelihood of rolling into the prone position, increased arousal, maintained airway patency, decreased gastro-oesophageal reflux and resultant sleep apnea, or increased respiratory drive with carbon dioxide retention (Hauck et al., 2005).

2.3 Disadvantages of pacifiers

2.3.1 Dental malocclusions

There are many disadvantages of pacifier usage. One of the commonly known disadvantages of long term use of pacifiers (for more than 2 years of age) is the development of dental malocclusions. The most notable changes are an increase in the prevalence of an anterior open bite (Zimmer *et al.,* 2011), posterior cross bite, narrow intercuspid width of the maxillary arch, and a high narrow palate (Poyak, 2006). However, orthodontic pacifiers are readily available with manufacturers claiming the use of such pacifiers reduce malocclusions compared to the use of conventional pacifiers. Orthodontic pacifiers have been scientifically designed to support the shape of babies' developing palates and jaws. The flattened shape not only simulates the shape of a mother's nipple when flattened in the mouth, but also encourages the most natural sucking action to help proper oral development. Studies indicate that children who use orthodontic pacifiers have a slightly smaller chance of developing an open bite than those who use conventional round pacifiers (Adair *et al.,* 1992). This difference is hard to demonstrate though, because neither type tends to cause noticeable problems unless sucked on for more than 2 years (Sexton and Natale, 2009).

2.3.2 Exposure to Bisphenol A

Bisphenol A (BPA) is an industrial chemical that has been used to make certain plastics and resins and can be found in pacifiers. It is added to enhance the malleability and therefore without this chemical the guard and the ring of pacifier would be hard and brittle. Some researchers have shown that BPA can seep out and pose a possible health risk such as cancer and is also associated with premature puberty (Aliprandini *et al.,* 2011), since the compound imitates estrogens. The infant's mouth is in direct contact with the shield creating a warm and humid environment which results in BPA migration. Studies have shown that migration of BPA increases with an increase in temperature and over time of pacifier use (Nam *et al.*, 2010). However, BPA free pacifiers are available in the market.

2.3.3 Colonization and transmission of pathogens and risk of infections

Pacifiers are known to carry microorganisms. Once the pacifier has been introduced into the oral cavity, it comes in contact with saliva and with the oral microflora. The oral microflora is composed of many different microorganisms including pathogenic species. These microorganisms adhere to the pacifier forming a slimy coating of bacteria called a biofilm. This becomes worse by cross contamination when care givers do not wash their hands when handling pacifiers. A bacterial biofilm is a structured community of bacterial cells enclosed in a self-produced polymeric matrix adherent to an inert or living surface (Costerton *et al.*, 1999). Biofilms constitute a protected mode of growth that allows bacterial growth and survival in a hostile environment. Biofilm infection can rarely be resolved by a host defense mechanism. Antibiotic therapy typically reverses the symptoms caused by the bacteria released from the biofilm, but fails to kill the bacteria (Marrie *et al.,* 1982). Moreover, the polymeric substances

that make up the matrix of the biofilm are known to retard the diffusion of antibiotics. It is possibly the reason that pacifier users are more prone to the common cold, flu and diarrhea as they can re-infect themselves with the pathogenic micro-organisms found in this biofilm coated pacifier. It has been noted that pacifier users have a significantly higher rate of *Candida* carriage in the oral cavity (Darwazeh *et al.,* 1995). In another study the presence of oral yeast and its relationship with sucking and feeding habits was investigated. This study showed no relationship in the prevalence of yeast with either breast feeding or bottle feeding, but the use of a pacifier was positively associated with the presence of yeast infections (Mattos-Graner *et al.,* 2001).

Research results regarding the use of pacifiers and the associated risk factor in otitis media is controversial. Acute otitis media (AOM) usually occurs when one has a viral upper respiratory tract infection with blockage of the Eustachian tube and escape of fluid in the middle ear, which becomes infected with bacteria. The most common bacteria found in this case are *Streptococcus pneumoniae, Haemophilus influenzae* and *Moraxella catarrhalis* (Lieberthal *et al.,* 2013). Studies have shown an increase in the risk of ear infection in infants using a pacifier. Indeed, some studies indicated an up to three times higher risk of ear infection in infants using pacifiers (Hanafin and Griffiths, 2002). The frequency of use of pacifiers was also directly proportional to the chances of developing otitis media (Hanafin and Griffiths, 2002). Parents are therefore often informed about the possible negative effects of the use of a pacifier once their child has been diagnosed with AOM, in order to avoid recurrent episodes (Rovers *et al.,* 2008). Interestingly, although many microorganisms were found on pacifiers collected from children with otitis media: the pathogens responsible for the otitis media were not present (Brook and Gorber, 1997).

2.3.4 Early development of dental caries

A relationship between the use of pacifiers and early development of dental caries has been suggested. However, in an evidence–based literature study, the question of whether the use of a pacifier plays a role in causing early dental caries, showed a weak correlation because of inconsistencies in the study methods. The inconsistencies among studies included: the variation of the definition of early dental caries and the variation of criteria to identify lesions. The frequency and duration of use of a pacifier was also not reported on clearly (Peressini, 2003). The use of a sweetner on the pacifier was not indicated and the age of the children varied among studies.

However, some studies did indeed list the use of pacifiers as a potential risk factor for the development of dental caries. In a prospective study, 18 month old children using pacifiers had a greater risk of developing dental caries by the age of 3 years compared to the children with the thumb sucking habit (Yonezu and Yakushiji, 2008). Ollila *et al.,* (1998), also reported that prolonged pacifier sucking was a significant risk factor for the development of early dental caries in children. Often these pacifiers are dipped into sweet substances which may be partly responsible for the development of dental caries (Aliprandini *et al.,* 2011). Whatever the reasons for a parent or a caregiver to introduce an infant to a pacifier; the prime importance is to not harm the infant. Well documented disadvantages of using a pacifier are acute otitis media, diarrhea and oral thrush. These disadvantages can be reduced if effective disinfecting methods are carried out to reduce the number of contaminants including pathogens. Therefore, it is important to understand the contaminating microorganisms.

2.4 Contamination of pacifiers with oral microorganisms

Once the pacifier enters the mouth it becomes contaminated with oral bacteria. Most of the time these oral bacteria live in harmony with the host, however, if the balance is disrupted they can cause infections. In addition, some of the bacteria such as respiratory tract pathogens are acquired by the oral cavity. These pathogens remain in the oral cavity as transient flora but they can cause infections as well as be transmitted to others. Therefore, anything placed in the mouth including pacifiers can become contaminated with oral flora as well as pathogens.

Bacteria will continue to colonize the pacifier until disinfected. While using a pacifier, a child is prone to dropping it on the floor. This results in contamination with environmental bacteria. Parents or caregivers may suck on the pacifier in an attempt to clean it, and place it back into a child's mouth. Sucking on a fallen pacifier may remove loose dirt and sand, but further contaminates the pacifier with additional oral bacteria from the adult. In daycare centers cross contamination between sick children can also occur through the use of pacifiers. A dirty pacifier grows a slimy biofilm which can become progressively more resistant to antibiotics and detergents. Bacterial sensitivity to antimicrobial agents has been shown to be much lower when they are constituents of a biofilm than when they are in aqueous suspension (Costerton *et al., 1*999).

2.4.1 Types of pathogenic contaminants

For the purpose of this study, the causative organism of sepsis, *Staphylococcus aureus, the* causative organism of oral thrush *Candida albicans* and the causative organism of diarrhea *Escherichia coli* will be discussed.

2.4.1.1 *Staphylococcus aureus*

Staphylococcus aureus, (*S. aureus*) is a Gram – positive, coccus – shaped bacteria. They are members of the Staphylococcaceae family. A typical characteristic of *S. arueus* on an agar plate is the yellow to golden appearance. Some strains are often surrounded by a clear zone as it is hemolytic on blood agar. They can grow at a temperature range of 15 to 45 degrees centigrade. *S. aureus* occurs in clusters (Mims *et al*, 2004) resembling grapes, because the cells divide successively in three perpendicular planes with the sister cells remaining attached to one another following each successive division (Todar, 2012).

S. aureus are facultative anaerobes which grow by aerobic respiration or by fermentation that yields principally lactic acid and it is also catalase- positive and mannitol- positive. A coagulase test determines pathogenic from non- pathogenic Staphylocci, of which *S. aureus* would test positive.

It is estimated that 20% of the human population carry *S. aureus* (Kluytmans *et al.,* 1997) which can be found as part of the anterior nares of the nasal passages (Kluytmans *et al.,* 1997; Cole *et al.,* 2001) and the respiratory tract. It may also occur as commensal, and under these circumstances does not always indicate an infection and therefore does not require treatment. However, it is a common cause of skin infections such as boils, pimples, impetigo, cellulites, folliculitis, carbuncles and abscesses (Todar, 2013). These bacteria may also cause food poisoning (Brooks *et al.,* 2001), and respiratory tract infections such as sinusitis. More life-threatening diseases caused by *S. aureus* are pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome and septicaemia (Mims *et al.*, 2004).

S. aureus is transmitted through air droplets and aerosol. The bacteria can be released into the air through saliva droplets when an infected person coughs or sneezes. These droplets of saliva contaminated with bacteria can infect other people. Another mode of transmission is through direct contact with objects including towel sheets and clothing that are infected with the bacteria. *S. aureus* is also still one of the five most common causes of nosocomial infections (infections contracted during hospital stay). Newborn infants are often colonized with *S aureus* originating from health care workers (Mernelius *et al.*, 2013). It can therefore be said that any caregiver can transfer the bacteria to the infant. This could be directly or through handling of the infants' personal items which includes a pacifier. Since *S. aureus* is favored by warm moist conditions, the use of a pacifier maintains such an environment which therefore acts as a harvesting device. This is exacerbated if the skin around the mouth is broken by disease such as eczema. In infants *S. aureus* infection can also cause a severe disease known as Staphylococcal scalded skin syndrome (Bukowski *et al.*, 2010).

Drug resistance in these bacteria is a known problem and therefore treatment is guided by sensitivity testing. Severe infections require prolonged intravenous therapy with β – lactamase – resistant antibiotics such as penicillin with clavulanic acid (augmentin). If resistance is not present then penicillin G is the treatment of choice (Brooks *et al.*, 2001). Methicillin resistant *S. aureus* is yet another known problem as well.

2.4.1.2 *Candida albicans*

Candida albicans (*C. albicans*) is a yeast, unicellular fungi that causes candidiasis or candidosis. *C. albicans* exists in two forms - a trait known as dimorphism. The yeast form of the organism is believed to be relatively innocuous, but the hyphal form is usually associated with invasion of

the host tissue. Candidiasis is the most common opportunistic oral fungal infection in humans. This fungus is found in the normal flora of about 50% of healthy individuals (Bork *et al.,* 1993). These individuals are carriers of the organisms in their mouths and may or may not present clinical evidence of infection. Clinicians have recognized that oral candidiasis may develop in people who are immune compromised.

The predisposing factors for the development of infection are the immune status of the host, the oral mucosal environment and the type of *C. albicans* strains. Therefore, Candidal infections are commonly seen in immunocompromised patients such as patients with HIV, cancer, organ transplants (Bagg *et al.*, 1999; Bork *et al.,* 1993), and diabetes mellitus (Bagg *et al.*, 1999). Individuals on broad spectrum antibiotics and with poor oral hygiene may also present with this infection. The clinical manifestations presents as white dots, patches or plaques also called thrush (Bagg *et al.,* 1999) found in the vaginal area or oral cavity. About 15% of newborns present with oral thrush which can be seen a week after delivery. Transmission occurs during delivery (Newman and Nisengard, 1988) as the pH of both the vagina and the newborn mouth supports the growth of *Candida* (Bork *et al.,* 1993). Thrush can also commonly be seen in infants who use bottle nipples and pacifiers (Bork *et al.,* 1993). Mothers often attempt to clean a bottle nipple or pacifier that has fallen on the floor by sucking it, which can transmit *Candida* from mother to child. Latex pacifiers are more significantly colonized with *Candida* and Staphylococcus than silicon pacifiers (Sio *et al.,* 1987; Comina *et al.,* 2006). This is believed to be due to latex pacifiers being softer and therefore prone to micro-cracks. It is these micro – cracks that serve as a niche for organisms to colonize.

Candidiasis has a variety of clinical manifestations, making diagnosis difficult at times. Below are the common oral manifestations: Pseudomembranous candidiasis, (PC) infection, presents with the common thrush like appearance. It is characterized by the presence of adherent white plaques that resemble cottage cheese or curdled milk on the oral mucosa. The white plaques are composed of tangled masses of hyphes, yeasts, desquamated epithelial cells, and debris. Rubbing with dry gauze can remove the plaques. This form of candidial infection can sometimes be noted when an individual has been exposed to broad spectrum antibiotics, as antibiotic treatment results in eliminating competing bacteria, or by impairment of the patient's immune system. The immune dysfunctions seen in leukemic patients or those infected with HIV are often associated with pseudomembranous candidiasis. Infants may also be affected because of their underdeveloped immune systems.

Erythematous Candidiasis, may or may not show white flecks or a white component as it is not a prominent feature of this particular organism, however this infection is more common than PC. Indeed, several clinical presentations may be seen. Acute atrophic candidiasis or antibiotic sore mouth typically follows a course of broad- spectrum antibiotics. Patients may complain of a burning sensation in the mouth with a diffuse loss of the filiform papillae of the dorsal tongue, resulting in redness.

Central papillary atrophy (median rhomboid glossitis), clinically appears in a well demarcated erythematous zone that affects the midline, posterior dorsal tongue and can often be asymptomatic. The erythema is due to the loss of the filiform papille in the area.

Angular cheilitis, can be seen in individuals with a loss of facial vertical height. This results in a skin fold that extends from the corners of the mouth. As saliva seeps out of the mouth, it builds

up in these folds. If these individuals are candidia carriers it may cause a reddish crusting appearance around these folds as the saliva accumulates in this area. In a similar fashion, infants who use pacifiers may present with a reddish rash-like appearance around their mouths where the pacifier shield sits. This occurs because some saliva seeps out the mouth and builds up behind the shield, which is in direct contact with the skin. Furthermore, denture stomatitis, is seen in individual who wear dentures, and has poor denture hygiene. The denture bearing areas appear red and inflamed with white patches. The denture indeed creates an environment conducive for candidial growth.

The diagnosis of candidiasis is usually established by the clinical signs in conjunction with an exfoliative cytologic examination. A specimen of culture is obtained by rubbing a sterile cotton swab over the lesion and then streaking the swab on the surface of a Sabouraud agar plate. *C. albicans* will grow as creamy, smooth surfaced colonies after 48 hours of incubation at room temperature. Candidal organisms can be seen microscopically in either an exfoliative cytological preparation or in tissue sections obtained from a biopsy specimen. On staining with the periodic acid - Schiff (PAS) method, the candidal hyphae and yeasts can be readily identified. This method stains carbohydrates, which is abundant in the fungal cell walls. This stain causes the organisms to appear bright-magenta in colour. To make the diagnosis of candidiasis, one must be able to see hyphae or pseudohyphae which are essentially elongated yeast cells.

Oral *C. albicans* can be treated with antifungal agents such as fluconazole and nystatin. If the use of broad spectrum antibiotics is the cause of infection, an interflora can be prescribed. Good oral hygiene is also important in aiding treatment. Therefore, any objects placed in the mouth, such as dentures, pacifiers and utensils should be thoroughly cleaned and disinfected.

2.4.1.3 *Escherichia coli*

In 1885, Theodor Escherich was the first to described *Escherichia coli* (*E. coli)*, as Bacterium coli commune, which he isolated from the feces of newborns (Todar 2012; Nodqvist 2013). It was later renamed *Escherichia coli*. *E. coli* is the head of this large bacterial family, the Enterobacteriaceae. This enteric species is a gram-negative, facultative anaerobic, rod–shaped bacterium (Brooks *et al.*, 2001; Gillespie and Bamford, 2007). A capsule or microcapsule is often present and a few strains produce profuse polysaccharide slime (Sussman, 1997). *E. coli* is known to be a harmless commensal, as part of the normal gut flora, since its natural habitat is the intestines of warm blooded animals and humans (Brooks *et al.*, 2001). These bacteria can be beneficial within the intestine (Hudault *et al.,* 2001; Reid *et al.,* 2001). It can also be found in the environment in water and soil. Some strains such as enterotoxigenic and enteropathogenic *E. coli* cause diarrhoea. In addition, they also cause urinary tract infections, neonatal meningitis and septicemia (Gehlbach *et al.,* 1973; Evans *et al.,* 2007).

The gastrointestinal tract of most warm–blooded animals and infants are colonized by *E. coli* within hours or a few days after birth. This is because the bacteria can adhere to the mucosa lining of the gut. The pathogenic strains can be contracted by the faecal–oral transmission route and the bacterium can also be ingested in food or water or obtained directly from other individuals handling the infant. Since *E. coli* is present in the environment in water and soil, toddlers can easily contract this pathogen. Studies have shown that pacifiers do become contaminated with fecal microorganisms and children who use pacifiers' contract diarrhoea more frequently as compared to the non-users (Tomasi *et al.,* 1994). Once established, an *E. coli* strain may persist for months or years as a commensal organism. Several types of *E. coli* existing as

part of the normal flora of the human gut have many beneficial functions, such as the production of vitamin K12. They also prevent pathogenic bacteria from establishing themselves in the intestine. A rapid shift may occur after an enteric (pathogenic *E. coli*) infection, or a shift in the state of health, or due to antimicrobial therapy that disturbs the normal flora (Gillespie & Bamford, 2007). However, nonpathogenic *E. coli* have a therapeutic use as a probiotic agent in medicine, mainly for the treatment of various gastroenterological diseases (Grozdanov *et al.*, 2004) including inflammatory bowel disease (Kamada *et al.*, 2005).

In a laboratory environment, *E. coli* can be grown in media with glucose as the sole organic constituent. It can also grow on peptone or meat extract media or in MacConkey agar. They can grow by both aerobic or anaerobic respiration (Brooks *et al.*, 2001). Under anaerobic conditions the bacterium will grow by means of fermentation, producing characteristic "mixed acids and gases" as end products (Brooks *et al.*, 2001). Optimal growth of *E. coli* occurs at 37°C within 24 hours of incubation.

A healthy adult will generally recover fully within 5 to 7 days after being infected with pathogenic *E. coli*. The individual may experience sudden abdominal pain/cramps, typically as the first symptom. A watery diarrhoea generally follows a few hours after the sudden abdominal pain. A day later there may be bright red bloody stools, caused by sores in the intestines. Nausea, vomiting and fever may also be present. Fatigue commonly follows diarrhoea as there is a loss of fluids and electrolytes (dehydration), causing the patient to feel sick and tired. Young children, elderly individuals and patients with weakened immune systems can develop potentially fatal complications and therefore may need medical attention. No current treatments can cure *E. coli*;

it has to run its course. Most doctors advise patients to get plenty of rest and fluids to prevent dehydration.

2.5 Decontamination of pacifiers

There are many different types of disinfection or sterilization methods available in the market that can decontaminate pacifiers. Consumers select a type depending on disinfection or sterilization time, efficiency, costing and methodology. The simple method of submerging the pacifier in boiling water for 5 minutes and possibly adding some salt or bicarbonate soda to the water is used by many mothers. Boiling kills vegetative bacteria and some spores depending on the exposure time. However, this method is simple, cost effective and short.

2.5.1 Disinfectants

2.5.1.1 Sodium hypochlorite

Submerging pacifiers in chemicals such as diluted chlorine based solutions for a period of time has been recommended and these solutions are commercially available. Sodium hypochlorite has been used widely in clinical care as a disinfecting agent and is known to be highly effective in cleaning surfaces contaminated with all types of micro-organisms (Rutala and Weber, 1997; 1999). The mechanism of antibacterial activity of chlorine containing compounds such as sodium hypochlorite is not fully understood. The postulated mechanism is thought to be due to inhibition of key enzymatic reactions within the cell as well as protein denaturation (Rutala and Weber, 1997). Efficacy of sodium hypochlorite decreases in the presence of light, heat, organic

and inorganic containments and the shelf life is reduced with decreasing concentrations (Johnson and Remeikis, 1993).

Chlorine based solutions such as Milton has been marketed for disinfecting baby teats, bottles, pacifiers and utensils. Milton which is readily available at local stores, is made of an aqueous solution of 1.0% sodium hypochlorite and 16.5% sodium chloride. The manufacturer's instructions are to dilute Milton in a noncorrosive plastic container with a lid. Items are disinfected by soaking in this solution for 30 minutes. However, pre-cleaning of pacifiers is very important and therefore recommended. Thrush is a common problem in babies, and the manufactures of Milton claims that Milton kills *C. albicans.* After removing from the Milton solution, pacifiers can be drained and used immediately without rinsing. The World Health Organisation (WHO) considers the acceptable level of chlorine for drinking water to be at 5 mg/l (0.5 % of the solution). Milton sterilizing solution is made up of 0.012% of chlorine which is far less than the level accepted by WHO. This explains why it is not necessary to rinse, and by rinsing there would be a risk of contaminating the baby's accessories again.

2.5.1.2 Chlorhexidine

Chlorhexidine is a broad spectrum disinfectant with well-established efficacy. Although it is not packaged or marketed as a pacifier disinfecting agent, studies on pacifiers and disinfectants have included chlorhexidine either as a positive control or as recommended disinfection for pacifiers (Chamele *et al.,* 2012). Chlorhexidine, a cationic bis-biguanide is an antimicrobial agent which has been used as a broad spectrum antiseptic since 1953. It is used extensively in the fields of medicine and dentistry in mouth rinses, antiseptic soaps and surface disinfectants. Furthermore, chlorhexidine has been used in the treatment of oral candidiasis since the 1970's.

In studies on polyguanides it was found that certain bisbiguanides have a very broad antimicrobial spectrum of activity. By structural variation these scientists arrived at the agent with the greatest bacteriostatic and bactericidal feature: 1,6 – bis – 4 – chloro – phenyldi – guanidonhexane, a synthetic cationic detergent usually referred to as chlorhexidine (Gjermo *et al.,* 1970; Brecx *et al.,* 1992). Rinsing the oral cavity with 0.12 % chlorhexidine reduces the number of bacteria in the saliva by up to 95 % in the course of few days. In a study by Grossman *et al.,* (1989) it was determined that use of chlorhexidine can reduce gingivitis, gingival bleeding and even dental plaque.

Chlorhexidine is a water soluble compound and is dissociable, allowing its positive charges to bind to teeth (Rolla *et al.,* 1970), oral soft tissues (Bonesvoll *et al.,* 1974), to the negatively charged bacterial cell wall (Mohammadi *et al.,* 2009) and to extracellular complexes (Loe *et al.,* 1970). The binding to bacteria interferes with membrane transport mechanism allowing leakage of low molecular weight substances, and at high concentrations it penetrates the cell wall precipitating the cellular cytoplasm causing cell death (Greenstein *et al.,* 1986; Gomes *et al.,* 2005). General and systemic side effects are rare. The most common side effect of chlorhexidine is the formation of an extrinsic, yellow–brown stain on the teeth and tongue. Disturbance of taste sensation (mainly sweet and salt) and a burning sensation of the tongue may occur on using the mouth wash and the bitter taste after the use chlorhexidine. It is this bitter after taste that is left on the pacifier which may result in an infant not accepting the pacifier.

2.5.2 Microwave sterilization

Microwave sterilizers which operate on a heating principle are also available. Silva *et al.,* (2013) studied the efficacy of the microwave in the infection of dentures and found that it can eliminate

C. albicans and *Streptococcus mutans* (*S. mutans*). Indeed a study by Chamele *et al.*, (2012) showed that 7 minutes of irradiation can kill *S. mutans* present on pacifiers, and this was comparable to chlorhexidine disinfection.

When comparing the mode of action of a microwave steamer and an electrical steamer they seem similar. Both require about 200ml of water to create steam. Heat, creating the steam is responsible for the sterilization in both techniques. The microwave method requires a sealable container (specifically designed as a microwave steamer) and a microwave, water and electricity. The electric steamer method requires the electric steamer device, water and electricity. Not much is known about the efficacy of these electrical steamer sterilizers.

2.6 Aim: This study evaluated various disinfection techniques for the effective disinfection of pacifiers.

2.6.1 Objectives:

- To determine the efficacy of various disinfection techniques in the disinfection of pacifiers.
- To compare the efficacy of various disinfection techniques in the disinfection of latex and silicon pacifiers.

3 Methods

3.1 Pacifiers

A hundred and eight pacifiers were purchased from a local supermarket. Fifty four were silicon and 54 were latex pacifiers of the snookums brand. The pacifiers were BPA free and treated as per the manufacturer's instructions. This involved boiling each pacifier for 5 minutes. A glass beaker with tap water was placed on a hot plate and the water was brought to boiling point (100°C). At boiling point pacifiers were submerged in the boiling water for 5 minutes. A sterile artery forceps was used to hold the pacifiers down. The latex and silicon pacifiers were boiled separately. After boiling, the pacifiers were aseptically removed and stored separately in a sterile glass jar ready for the experiments.

3.2 Cultures and inocula

Cultures of *S. aureus* ATCC 29213, *C. albicans* ATCC 90028 and *E. coli* ATCC 35218 were obtained from the Oral Microbiology laboratory. These are laboratory stock cultures that are purchased and not isolated from patients; therefore, full ethical clearance was not necessary. However, a waiver was obtained from the Human Research Ethics Committee of the University of the Witwatersrand (Ethics number: W-CJ-130626-1).

S. aureus and *E. coli* were grown on blood agar plates at 37°C for 24 hours whereas *C. albicans* was grown on Sabouraud Dextrose agar plates and incubated at 37°C for 48 hours. For each experiment fresh subcultures were prepared. To prepare inoculum, cultures were emulsified in 150 ml sterile distilled water and the turbidity was adjusted to 3 McFarland standards to obtain 10^7 cells per ml of suspension. For each experiment fresh inoculum was prepared.

3.3 Disinfectants

The disinfecting methods used included rinsing pacifiers under tap water for 2 minutes, submerging pacifiers in boiling water for 5 minutes, submerging pacifiers in Milton (chlorinated compound) for 30 minutes, placing pacifiers under microwave radiation for 8 minutes and submerging pacifiers into chlorhexidine gluconate (2.0%) for 2 minutes.

Milton (Figure 3.1) solution was purchased from a local supermarket and for each experiment fresh solutions were prepared according to the package insert, which included diluting 12.5 ml of Milton with 1 litre of water.



Figure 3.1: Milton

For the microwave technique, a special sterilizing microwavable unit (Figure 3.2) was purchased and used according to the manufacturer's instruction. This involved placing 200 ml water in the base, placing a pacifier on a rack provided with the sterilizing unit, closing the unit and placing the whole unit into the microwave for 8 minutes at 900 WATTS. By placing the pacifier on the rack, the pacifier did not come in contact with the water, which boils with radiation. However, heat generated steam, sterilizes the pacifiers.

Chlorhexidine gluconate (2% solution) was purchased from the local pharmacy (Corsodyl). It was used as positive control.

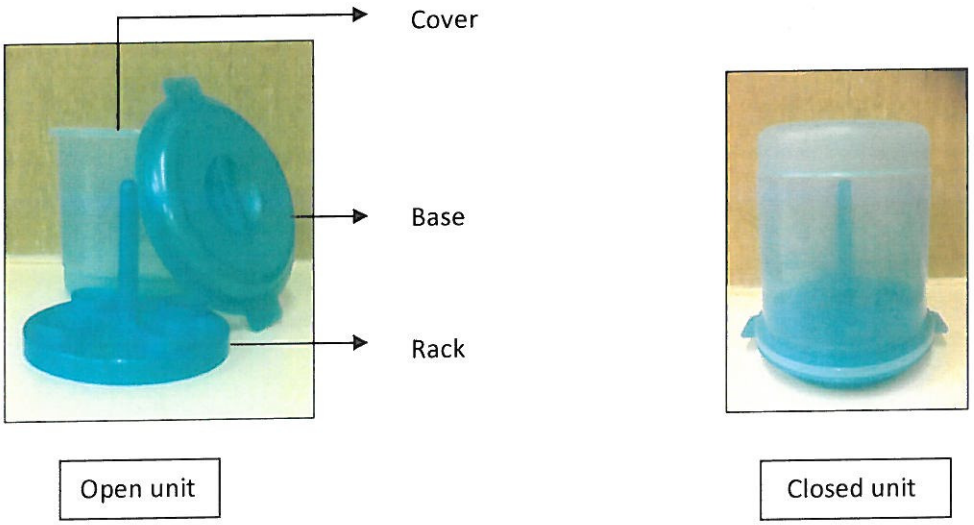
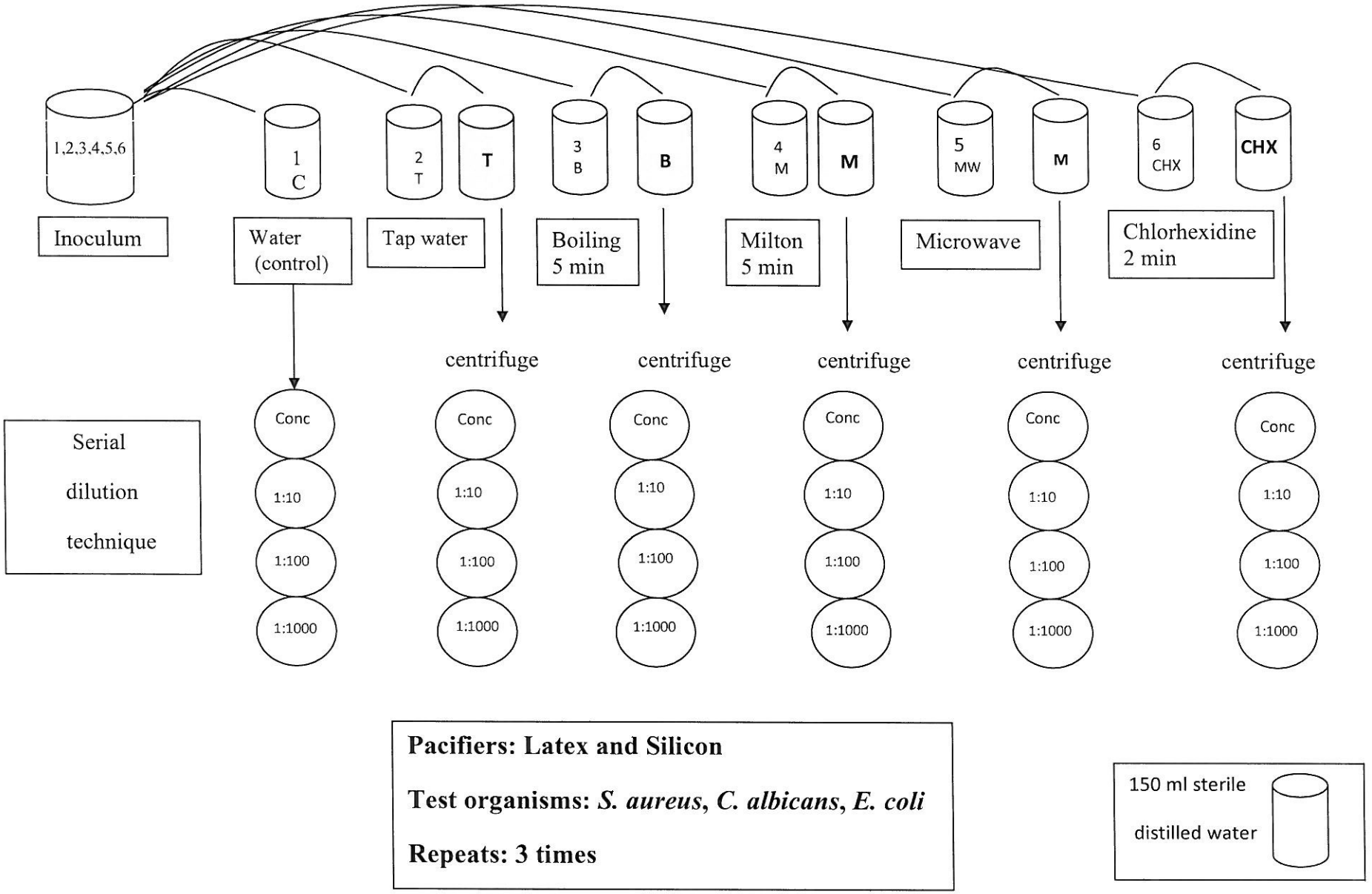


Figure 3.2: Microwave sterilization unit

Figure 3.3 Test procedures for the artificial contamination and decontamination of pacifiers



3.4 Test procedure

The test procedures were followed as described by Rweyendela *et al.,* (2009) but with modifications. The flow of the procedure is described in Figure 3.3. Six latex pacifiers were artificially contaminated by placing them individually in the inoculums for 2 minutes each, holding them down with a glass rod and removing them from the inoculum aseptically. The first pacifier was placed in 150 ml of sterile distilled water to obtain a control count (number of challenged organisms). This beaker was labelled as 'control' (Beaker C). The second contaminated pacifier was placed into 150 ml of normal tap water for 2 minutes. This pacifier was held by a sterile artery forceps and agitated by moving it in an up and downward movement to stimulate a rinsing effect. After 2 minutes the pacifier was aseptically removed and placed into 150 ml of sterile distilled water to obtain the remaining number of bacteria that were not removed by rinsing with tap water. This beaker was labelled 'tap water' (Beaker T). The third contaminated pacifier was submerged into 150 ml boiling water for 5 minutes. The pacifier was only placed in the water once the water reached boiling point (100°C) and was held down by a sterile artery forceps. After 5 minutes the pacifier was removed and placed into a beaker of 150 ml sterile distilled water marked 'boiling' (Beaker B). The forth contaminated pacifier was placed into 150 ml of a prepared chlorinated compound solution (Milton) for 30 minutes and held down by a sterile glass rod (Figure 3.4). The metal artery forceps was not used in this case because of the corrosive property of the chlorinated compound. The pacifier was removed and placed into a labelled beaker (Beaker M) containing 150 ml of sterile distilled water. The fifth contaminated pacifier was removed from the inoculum after 2 minutes and was placed into a microwavable sterilization unit. This unit is specifically designed to sterilize feeding bottles, teats and pacifiers. Two hundred millilitre tap water was placed into the unit as instructed by the

manufacturer. The pacifier was placed on the designed rack provided. The container was closed and placed in the microwave for 8 minutes at 900 watts. The container was removed from the microwave and was allowed to stand for 2 minutes as per instructions. This was to avoid any stream burns. After 2 minutes the container was opened and the pacifier was aseptically removed and placed into a beaker containing 150 ml sterile distilled water. This beaker was labelled 'microwave' (Beaker MW). Finally, the sixth pacifier was removed from the inoculum after 2 minutes and submerged into 150 ml of a chlorhexidine gluconate (CHX) solution for 2 minutes. A sterile glass rod was used to keep the pacifier submerged. This pacifier was then removed and placed into a beaker containing 150 ml of sterile distilled water. The beaker was labelled 'CHX' (Beaker CHX).

This disinfection test procedure was repeated three times for each organism (*E. coli, S. aureus* and *C. albicans*) using the latex and silicon pacifiers.



Figure 3.4: Pacifier held down by a glass rod in a beaker.

3.4.1 Bacterial and colony counts

After sterilization, a sterile magnetic stirrer was placed into the beakers as described in section 3.4 containing the pacifiers (beakers C, T, B, M, MW and CHX). The beakers were placed on a magnetic stirrer plate (Figure 3.5) and stirred for approximately 2 minutes. This stirring motion facilitated removing the microorganisms from the pacifiers and dispersing them into the water. Water from all the beakers was centrifuged at 5000 rpm for 15 minutes using conical tubes. The supernatant was removed and the sediment was suspended in 1 ml phosphate buffered saline (PBS). A serial dilution technique was performed to obtain counts of microorganisms. Briefly, 0.1 ml of solution was diluted in 0.9 ml PBS to obtain 1:10 dilution. Similarly 1:10 dilution was further diluted to 1:100 and 1:1000 dilutions. One hundred microliters of each dilution was transferred onto appropriate agar plates, spread and incubated appropriately. *E. coli* and *S. aureus* was cultured using blood agar plates which were incubated aerobically at 37°C for 24 hours. Whereas, *C. albicans* was cultured using Sabouraud dextrose agar plates which were incubated aerobically at 37°C for 48 hours. Colonies were counted and the colony forming units (cfu) were obtained by multiplying the colony counts with the dilution factors and taking the average count of the dilution plates. Organism counts were expressed as cfu per pacifier. The control count represented the level of artificial contamination. Bacterial counts in the tests represented the live bacteria due to the inadequate sterilization procedure. The percentage of bacteria/fungi killed was calculated using the formula: control count - test count/control count X100. Log reduction was also calculated.

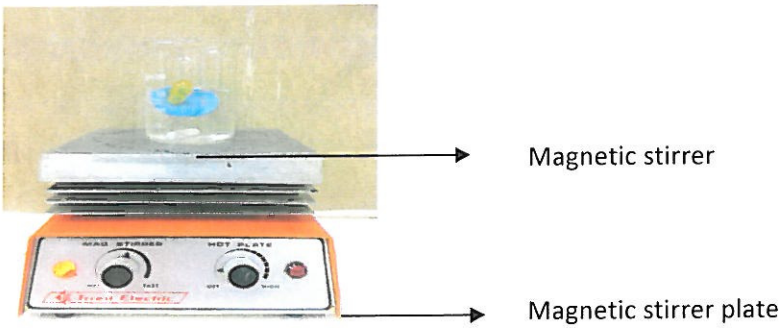


Figure 3.5: Magnetic stirrer plate

3.5 Statistical analysis

Descriptive analysis was performed to express the results. Percentage-kill of disinfectants were compared using Kruskal Wallis nonparametric rank tests between the types of pacifiers, types of test organisms and the disinfection procedure.

4 Results

The results are expressed as % kill as well as the log reduction in the microbial counts. Percentage kill are more comparable whereas log reduction is a true reflection of the actual reduction in the microbial counts. For example: a 2 log reduction of 1 000 000 challenged organisms shows that 10 000 organisms are still surviving which will calculate to a 99% kill. However, a 2 log reduction of 100 challenged organisms will calculate to a 100% kill. Therefore, for the % kill results, the number of challenged organisms should be given, otherwise the results are meaningless. In contrast, the log reduction results provide complete information, even if the number of challenged organisms is not provided.

4.1 Disinfection of latex pacifiers artificially contaminated with *Staphylococcus aureus*

Latex pacifiers contaminated with *S. aureus* were disinfected using 5 different procedures and the results are shown in Table 4.1 and Table 4.1a. The results show that placing pacifiers in boiling water for 5 minutes, or in Milton for 30 minutes, or in a sterilization unit in the microwave for 8 minutes, or submerging them in chlorhexidine for 2 minutes, were all effective methods of eradicating *S. aureus* from latex pacifiers (% kill = 100; log reduction 6.56). However, when the pacifiers were rinsed in tap water for 2 minutes, a complete removal was not obtained (mean % kill = 99.34; log reduction 2.35).

Table 4.1: **Disinfection of latex pacifiers artificially contaminated with *Staphylococcus aureus* using various disinfection techniques (% kill).**

Disinfection	Repeats	No. of challenged org. (cfu/ pacifier) a	No. of surviving org. (cfu/pacifier) b	No. org. killed (cfu/ pacifier) a-b	% kill $\frac{a-b}{a}$ X100
Tap water	1	2745000	6800	2738200	**99.75**
	2	2940000	7009	2932991	**99.76**
	3	5700000	85470	5614530	**98.50**
Mean±SD					**99.34 ± 0.73**
Boiling	1	2745000	0	2745000	100
	2	2940000	0	2940000	100
	3	5700000	0	5700000	100
Mean±SD					**100 ± 0**
Milton	1	2745000	0	2745000	100
	2	2940000	0	2940000	100
	3	5700000	0	5700000	100
Mean±SD					**100 ± 0**
Microwave	1	2745000	0	2745000	100
	2	2940000	0	2940000	100
	3	5700000	0	5700000	100
Mean±SD					**100 ± 0**
Chlorhexidine	1	2745000	0	2745000	100
	2	2940000	0	2940000	100
	3	5700000	0	5700000	100
Mean±SD					**100 ± 0**

Inoculum: Mean±SD = 3795000

Table 4.1a: Disinfection of latex pacifiers artificially contaminated with *Staphylococcus aureus* using various disinfection techniques (log reduction)

Disinfection	Repeats	No. of challenged org. (log cfu/ pacifier) a	No. of surviving org. (log cfu/pacifier) b	Log reduction a-b
Tap water	1	6.44	3.83	**2.61**
	2	6.47	3.85	**2.62**
	3	6.76	4.93	**1.83**
Mean±SD				**2.35**
Boiling	1	6.44	0	6.44
	2	6.47	0	6.47
	3	6.76	0	6.76
Mean±SD				**6.56**
Milton	1	6.44	0	6.44
	2	6.47	0	6.47
	3	6.76	0	6.76
Mean±SD				**6.56**
Microwave	1	6.44	0	6.44
	2	6.47	0	6.47
	3	6.76	0	6.76
Mean±SD				**6.56**
Chlorhexidine	1	6.44	0	6.44
	2	6.47	0	6.47
	3	6.76	0	6.76
Mean±SD				**6.56**

Inoculum: Mean±SD = 6.56

4.2 Disinfection of latex pacifiers artificially contaminated with *Escherichia coli*

Latex pacifiers contaminated with *E. coli* were disinfected using 5 different procedures and the results are shown in Table 4.2 and Table 4.2a. The results show that placing pacifiers in boiling water for 5 minutes or placing them in a sterilization unit in the microwave for 8 minutes were effective in eradicating *E. coli* from latex pacifiers (% kill = 100; log reduction 7.54). However, when the pacifiers were rinsed in tap water for 2 minutes, placed in Milton for 30 minutes or

submerged in chlorhexidine for 2 minutes, a complete removal was not obtained (mean % kill = 99.87, 99.99 and 99.99 respectively).

Table 4.2: Disinfection of latex pacifiers artificially contaminated with *Escherichia coli* using various disinfection techniques (% kill).

Disinfection	Repeats	No. of challenged org. (cfu/ pacifier) a	No. of surviving org. (cfu/pacifier) b	No. org. killed (cfu/ pacifier) a-b	% killed $\frac{a-b}{a} X100$
Tap water	1	30150000	46400	30103600	**99.85**
	2	36150000	28560	36121440	**99.92**
	3	37150000	55650	37094350	**99.85**
Mean±SD					**99.87 ± 0.04**
Boiling	1	30150000	0	30150000	100
	2	36150000	0	36150000	100
	3	37150000	0	37150000	100
Mean±SD					**100 ± 0**
Milton	1	30150000	0	30150000	**100**
	2	36150000	64	36149936	**99.99**
	3	37150000	0	37150000	**100**
Mean±SD					**99.99 ± 0.01**
Microwave	1	30150000	0	30150000	100
	2	36150000	0	36150000	100
	3	37150000	0	37150000	100
Mean±SD					**100 ± 0**
Chlorhexidine	1	30150000	0	30150000	100
	2	36150000	0	36150000	100
	3	37150000	1500	37148500	99.99
Mean±SD					**99.99 ± 0.01**

Inoculum: Mean±SD =34483333

Table 4.2a: Disinfection of latex pacifiers artificially contaminated with *Escherichia coli* using various disinfection techniques (log reduction)

Disinfection	Repeats	No. of challenged org. (log cfu/ pacifier) a	No. of surviving org. (log cfu/pacifier) B	Log reduction a-b
Tap water	1	7.48	4.67	**2.81**
	2	7.56	4.46	**3.10**
	3	7.57	4.76	**2.81**
Mean±SD				**2.91**
Boiling	1	7.48	0	7.48
	2	7.56	0	7.56
	3	7.57	0	7.57
Mean±SD				**7.54**
Milton	1	7.48	0	**7.48**
	2	7.56	1.81	**5.75**
	3	7.57	0	**7.57**
Mean±SD				**6.93**
Microwave	1	7.48	0	7.48
	2	7.56	0	7.56
	3	7.57	0	7.57
Mean±SD				**7.54**
Chlorhexidine	1	7.48	0	7.48
	2	7.56	0	7.56
	3	7.57	3.18	**4.39**
Mean±SD				**6.48**

Inoculum: Mean±SD = 7.54

4.3 Disinfection of latex pacifiers artificially contaminated with *Candida albicans*

Table 4.3 shows that tap water and Milton were not effective in disinfecting latex pacifiers contaminated with *C. albicans* (% kill = 99.63 and 99.96 respectively, with the log reduction 2.75 and 3.57 respectively). However, heat based disinfections such as boiling and microwave radiation as well as chlorhexidine were effective in eliminating *C. albicans* completely.

Table 4.3: Disinfection of latex pacifiers artificially contaminated with *Candida albicans* using various disinfection techniques (% kill).

Disinfection	Repeats	No. of challenged org. (cfu/ pacifier) a	No. of surviving org. (cfu/pacifier) b	No. org. killed (cfu/ pacifier) a-b	% killed $\frac{a-b}{a} X100$
Tap water	1	414000	580	413419	99.86
	2	155400	1434	153965	99.08
	3	266400	116	266284	99.96
Mean±SD					**99.63 ± 0.48**
Boiling	1	414000	0	414000	100
	2	155400	0	155400	100
	3	266400	0	266400	100
Mean±SD					**100 ± 0**
Milton	1	414000	72	413928	99.98
	2	155400	102	155298	99.93
	3	266400	46	266354	99.98
Mean±SD					**99.96 ± 0.03**
Microwave	1	414000	0	414000	100
	2	155400	0	155400	100
	3	266400	0	266400	100
Mean±SD					**100 ± 0**
Chlorhexidine	1	414000	0	414000	100
	2	155400	0	155400	100
	3	266400	0	266400	100
Mean±SD					**100 ± 0**

Inoculum: Mean±SD = 278600

Table 4.3a: Disinfection of latex pacifiers artificially contaminated with *Candida albicans* using various disinfection techniques (log reduction)

Disinfection	Repeats	No. of challenged org. (log cfu/ pacifier) a	No. of surviving org. (log cfu/pacifier) b	Log reduction a-b
Tap water	1	5.62	2.76	**2.85**
	2	5.19	3.16	**2.03**
	3	5.43	2.06	**3.36**
Mean±SD				**2.75**
Boiling	1	5.62	0	5.62
	2	5.19	0	5.19
	3	5.43	0	5.43
Mean±SD				**5.41**
Milton	1	5.62	1.86	**3.76**
	2	5.19	2.01	**3.18**
	3	5.43	1.66	**3.77**
Mean±SD				**3.57**
Microwave	1	5.62	0	5.62
	2	5.19	0	5.19
	3	5.43	0	5.43
Mean±SD				**5.41**
Chlorhexidine	1	5.62	0	5.62
	2	5.19	0	5.19
	3	5.43	0	5.43
Mean±SD				**5.41**

Inoculum: Mean±SD = 5.41

4.4 Disinfection of silicon pacifiers artificially contaminated with *Staphylococcus aureus*

The results are described in Table 4.4 and Table 4.4a. Boiling, Milton, microwave and chlorhexidine were effective in eliminating *S. aureus* from artificially contaminated silicon pacifiers. However, tap water rinsing was ineffective giving % kill results of 99.80 and a log reduction of only 2.8.

Table 4.4: Disinfection of silicon pacifiers artificially contaminated with *Staphylococcus aureus* using various disinfection techniques

Disinfection	Repeats	No. of challenged org. (cfu/ pacifier) a	No. of surviving org. (cfu/pacifier) b	No. org. killed (cfu/ pacifier) a-b	% killed $\frac{a-b}{a}$ X100
Tap water	1	32550000	34960	32515040	**99.98**
	2	80100000	57280	80042720	**99.93**
	3	21150000	107100	21042900	**99.49**
Mean±SD					**99.80 ± 0.27**
Boiling	1	32550000	0	32550000	100
	2	80100000	0	80100000	100
	3	21150000	0	21150000	100
Mean±SD					**100 ± 0**
Milton	1	32550000	0	32550000	100
	2	80100000	0	80100000	100
	3	21150000	0	21150000	100
Mean±SD					**100 ± 0**
Microwave	1	32550000	0	32550000	100
	2	80100000	0	80100000	100
	3	21150000	0	21150000	100
Mean±SD					**100 ± 0**
Chlorhexidine	1	32550000	0	32550000	100
	2	80100000	0	80100000	100
	3	21150000	0	21150000	100
Mean±SD					**100 ± 0**

Inoculum: Mean±SD = 44600000

Table 4.4a: Disinfection of silicon pacifiers artificially contaminated with *Staphylococcus aureus* using various disinfection techniques (log reduction)

Disinfection	Repeats	No. of challenged org. (log cfu/ pacifier) a	No. of surviving org. (log cfu/pacifier) b	Log reduction a-b
Tap water	1	7.51	4.54	**2.97**
	2	7.90	4.76	**3.14**
	3	7.33	5.03	**2.3**
Mean±SD				**2.8**
Boiling	1	7.51	0	7.51
	2	7.90	0	7.90
	3	7.33	0	7.33
Mean±SD				**7.58**
Milton	1	7.51	0	7.51
	2	7.90	0	7.90
	3	7.33	0	7.33
Mean±SD				**7.58**
Microwave	1	7.51	0	7.51
	2	7.90	0	7.90
	3	7.33	0	7.33
Mean±SD				**7.58**
Chlorhexidine	1	7.51	0	7.51
	2	7.90	0	7.90
	3	7.33	0	7.33
Mean±SD				**7.58**

Inoculum: Mean±SD = 7.58

4.5 Disinfection of silicon pacifiers artificially contaminated with *Escherichia coli*

Table 4.5 and Table 4.5a show that *E. coli* contaminated silicon pacifiers were difficult to decontaminate because the % kill for tap water, Milton and chlorhexidine were 98.66, 99.99 and 99.99 (log reduction of 2.28, 7.31 and 6.57) respectively. However, boiling and microwave radiation was once again effective in achieving a complete kill (100%).

Table 4.5: Disinfection of silicon pacifiers artificially contaminated with *Escherichia coli* using various disinfection techniques

Disinfection	Repeats	No. of challenged org. (cfu/ pacifier) a	No. of surviving org. (cfu/pacifier) b	No. org. killed by (cfu/ pacifier) a-b	% killed $\frac{a-b}{a}$ X100
Tap water	1	109200000	150480	109049520	**99.86**
	2	48450000	146850	48303150	**99.70**
	3	70650000	2521600	68128400	**96.43**
Mean±SD					**98.66 ± 1.94**
Boiling	1	109200000	0	109200000	100
	2	48450000	0	48450000	100
	3	70650000	0	70650000	100
Mean±SD					**100 ± 0**
Milton	1	109200000	0	109200000	**100**
	2	48450000	0	48450000	**100**
	3	70650000	44	70649956	**99.99**
Mean±SD					**99.99 ± 0.01**
Microwave	1	109200000	0	109200000	100
	2	48450000	0	48450000	100
	3	70650000	0	70650000	100
Mean±SD					**100 ± 0**
Chlorhexidine	1	109200000	0	109200000	**100**
	2	48450000	0	48450000	**100**
	3	70650000	7500	70642500	**99.99**
Mean±SD					**99.99 ± 0.01**

Inoculum: Mean±SD = 76100000

Table 4.5a: Disinfection of silicon pacifiers artificially contaminated with *Escherichia coli* using various disinfection techniques (log reduction)

Disinfection	Repeats	No. of challenged org. (log cfu/ pacifier) a	No. of surviving org. (log cfu/pacifier) b	Log reduction a-b
Tap water	1	8.04	5.18	**2.86**
	2	7.69	5.17	**2.52**
	3	7.85	6.40	**1.45**
Mean±SD				**2.28**
Boiling	1	8.04	0	8.04
	2	7.69	0	7.69
	3	7.85	0	7.85
Mean±SD				**7.86**
Milton	1	8.04	0	**8.04**
	2	7.69	0	**7.69**
	3	7.85	1.64	**6.21**
Mean±SD				**7.31**
Microwave	1	8.04	0	8.04
	2	7.69	0	7.69
	3	7.85	0	7.85
Mean±SD				**7.86**
Chlorhexidine	1	8.04	0	**8.04**
	2	7.69	0	**7.69**
	3	7.85	3.88	**3.97**
Mean±SD				**6.57**

Inoculum: Mean±SD =7.86

4.6 Disinfection of silicon pacifiers artificially contaminated with *Candida albicans*

The results in Table 4.6 and Table 4.6a shows that boiling, microwave radiation and chlorhexidine were successful in eliminating *C. albicans* from silicon pacifiers. Tap water and Milton were ineffective in eliminating *C. albicans* from silicon pacifiers (% kill = 99.96 and 99.94 and a log reduction of 2.75 and 4.43 respectively).

Table 4.6: Disinfection of silicon pacifiers artificially contaminated with *Candida albicans* using various disinfection techniques

Disinfection	Repeats	No. of challenged org. (cfu/ pacifier) a	No. of surviving org. (cfu/pacifier) b	No. org. killed (cfu/ pacifier) a-b	% killed $\frac{a-b}{a} X100$
Tap water	1	485500	2618	482882	**99.46**
	2	1597500	4949	1592551	**99.69**
	3	1402500	483	1402017	**99.97**
Mean±SD					**99.96 ± 0.03**
Boiling	1	485500	0	485500	100
	2	1597500	0	1597500	100
	3	1402500	0	1402500	100
Mean±SD					**100 ± 0**
Milton	1	485500	897	484603	**99.82**
	2	1597500	0	1597500	**100**
	3	1402500	63	1402437	**99.99**
Mean±SD					**99.94 ± 0.1**
Microwave	1	485500	0	485500	100
	2	1597500	0	1597500	100
	3	1402500	0	1402500	100
Mean±SD					**100 ± 0**
Chlorhexidine	1	485500	0	485500	100
	2	1597500	0	1597500	100
	3	1402500	0	1402500	100
Mean±SD					**100 ± 0**

Inoculum: Mean±SD = 1161833

Table 4.6a: Disinfection of silicon pacifiers artificially contaminated with *Candida albicans* using various disinfection techniques (log reduction)

Disinfection	Repeats	No. of challenged org. (log cfu/ pacifier) a	No. of surviving org. (log cfu/pacifier) b	Log reduction a-b
Tap water	1	5.69	3.42	**2.27**
	2	6.20	3.69	**2.51**
	3	6.15	2.68	**3.46**
Mean±SD				**2.75**
Boiling	1	5.69	0	5.69
	2	6.20	0	6.20
	3	6.15	0	6.15
Mean±SD				**6.01**
Milton	1	5.69	2.95	**2.74**
	2	6.20	0	**6.20**
	3	6.15	1.80	**4.35**
Mean±SD				**4.43**
Microwave	1	5.69	0	5.69
	2	6.20	0	6.20
	3	6.15	0	6.15
Mean±SD				**6.01**
Chlorhexidine	1	5.69	0	5.69
	2	6.20	0	6.20
	3	6.15	0	6.15
Mean±SD				**6.01**

Inoculum: Mean±SD = 6.01

4.7 Summary results of disinfection of artificially contaminated pacifiers

Table 4.7 and Table 4.7a shows the summary of results obtained in this study. Tap water consistently proved to be ineffective in disinfecting pacifiers. When the results of all the test organisms are combined, purely from the observation, boiling and microwave radiation proved to be the 2 most effective methods of sterilization. Milton was effective only 33.33% (1/3 of the organisms) of the time, whereas chlorhexidine showed better efficacy (66.66% of the time – 2/3 of the organisms) than Milton.

Table 4.7: Summary results of disinfection of artificially contaminated pacifiers (% kill).

Test organism	Type of pacifier	No. of challenged organisms (Mean cfu/pacifier)	Mean ± SD % killed (n = 3)				
			Tap water	Boiling water	Milton	Microwave	Chlorhexidine (CHX)
S. aureus	Latex	3795000	99.34 ± 0.73	100 ± 0	100 ± 0	100 ± 0	100 ± 0
	Silicon	44600000	99.80 ± 0.27	100 ± 0	100 ± 0	100 ± 0	100 ± 0
E. coli	Latex	34483333	99.87 ± 0.04	100 ± 0	99.99 ± 0.01	100 ± 0	99.99 ± 0.01
	Silicon	76100000	98.66 ± 1.94	100 ± 0	99.99 ± 0.01	100 ± 0	99.99 ± 0.01
C. albicans	Latex	278600	99.63 ± 0.48	100 ± 0	99.96 ± 0.03	100 ± 0	100 ± 0
	Silicon	1161833	99.96 ± 0.03	100 ± 0	99.94 ± 0.1	100 ± 0	100 ± 0

Table 4.7a: Summary results of disinfection of artificially contaminated pacifiers (log reduction).

Test organism	Type of pacifier	No. of challenged organisms (Mean log cfu/pacifier)	Mean ± log reduction (n = 3)				
			Tap water	Boiling water	Milton	Microwave	Chlorhexidine (CHX)
S. aureus	Latex	6.56	2.35	6.56	6.56	6.56	6.56
	Silicon	7.58	2.8	7.58	7.58	7.58	7.58
E. coli	Latex	7.54	2.91	7.54	6.93	7.54	6.48
	Silicon	7.86	2.28	7.86	7.31	7.86	6.57
C. ablicans	Latex	5.41	2.75	5.41	3.57	5.41	5.41
	Silicon	6.01	2.75	6.01	4.43	6.01	6.01

4.8 Comparison of disinfectants and statistical analysis

Percentage-kill of disinfectants were compared using Kruskal Wallis non-parametric rank tests between the types of pacifiers, types of test organisms and the disinfection procedure.

When the disinfection of latex and silicon pacifiers were compared, the results showed no significant difference in the % kill with a p value of 0.85 (Figure 4.1). This suggests that the type of pacifier has no effect on the disinfection method.

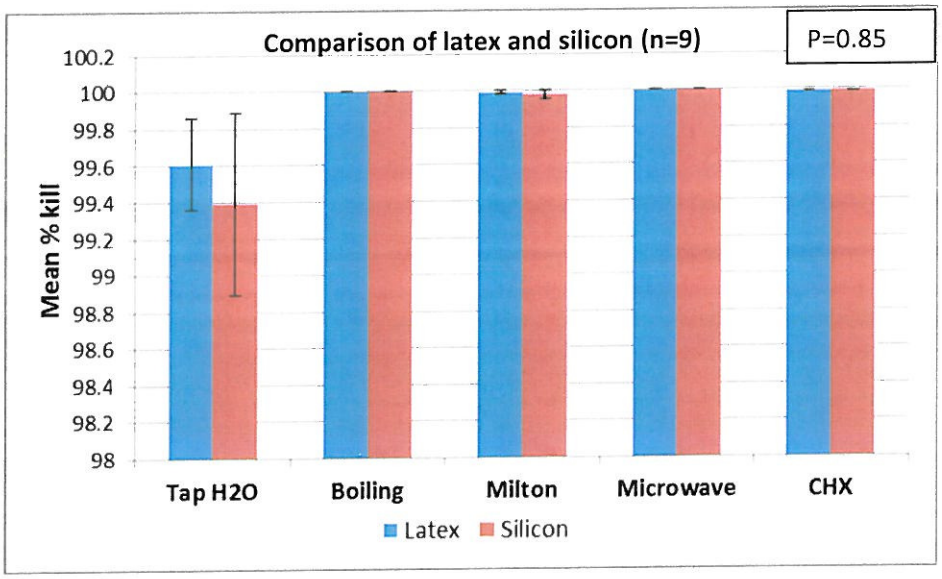


Figure 4.1 Comparison of different methods in the disinfection of latex and silicon pacifiers

When the disinfection methods in decontamination of pacifiers contaminated with *S. aureus, E. coli* and *C. albicans* were compared (Figure 4.2), the results showed that there was no significant difference (p=0.46). This suggests that there was no difference in the results of the test organisms.

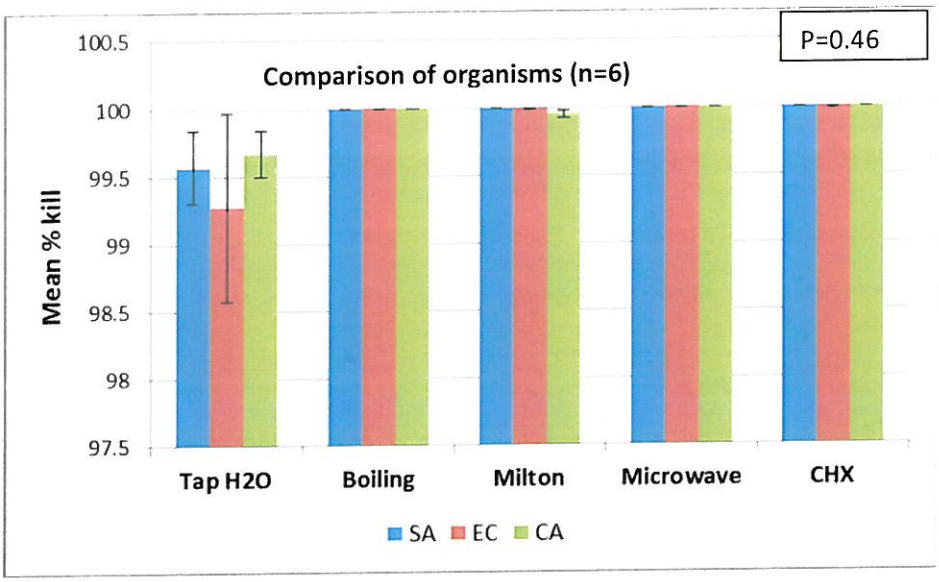


Figure 4.2 **Comparison of different methods in the disinfection of pacifiers contaminated with *S. aureus* (SA), *E. coli* (EC) and *C. albicans* (CA)**

Results in Figure 4.1 and 4.2 suggest that the types of pacifiers and the test organisms behaved similarly and there was no significant difference in the results of disinfection. Therefore, all the results were pooled together to compare the methods of disinfection.

When all the results for each of the disinfection methods were pooled together (**latex** – SA x3, EC x3, CA x3 and **silicon** – SA x3, EC x3, CA x3) and compared to one another (Figure 4.3), tap water significantly differed from all the other sterilization methods used in this study ($p=0.01$ and $p<0.01$ respectively). However, there was no significant difference in the performance of the disinfection methods such as boiling, Milton, microwave and chlorhexidine.

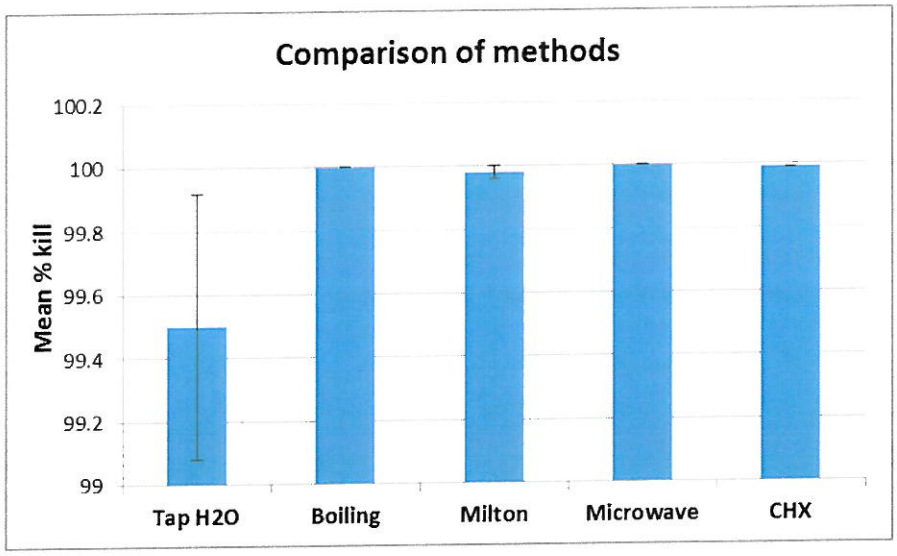


Figure 4.3 Comparison of different methods in the disinfection of pacifiers.

5 Discussion

Pacifiers are non-nutritive objects designed for infants around 2 weeks of age to 5 years. They are used to pacify infants when they are crying due to discomfort or feeling insecure. In addition, they are used to increase oromotor coordination and muscular strength in feeding abilities, and to wean them off breast feeding (Sexton and Natale, 2009; Poore *et al.,* 2008). When it is placed in the oral cavity, the pacifier is in direct contact with the oral saliva and its oral micro flora. These microorganisms adhere and colonise the surfaces of pacifier (Brook and Gober, 1997; Mattos–Graner *et al.*, 2001; Comina *et al.*, 2006). While studies related to pacifier contamination can be found in the literature not much is published on the available cleaning methods and their effectiveness. Pacifiers are made of silicon or latex with shape resembling that of a lactating mother's nipple. Due to their difference in texture, surface structure and durability, the

colonization with bacteria may differ. Therefore, this study investigated the efficacy of home disinfection procedures including commercially available chemicals in the disinfection of latex and silicon pacifiers.

5.1 Disinfection of latex and silicon pacifiers

When the % kill results were compared between the two types of pacifiers, no statistical difference was found (Figure 4.1, Table 4.7). For both the latex and the silicon pacifiers, boiling and microwave radiation were adequate. Similarly, disinfection with water rinse, Milton and chlorhexidine was inadequate. Although the latex pacifiers are known to get worn out easily and harbour biofilms of microorganisms compared to the silicon pacifiers (da Silveira *et al*, 2009; Comina *et al, 2006*), the test disinfection procedures provided similar results.

The second important aspect to consider is the formation of a bacterial biofilm on the pacifiers. A biofilm formation is much more easily established on a rough surface and therefore more commonly found on old used latex pacifiers. Mature biofilms containing bacteria and a slime layer have been found on old used latex pacifiers, whereas silicon pacifiers had a much lower level of slime contaminated with *S. aureus* and *C. albicans* (Comina *et al.*, 2006). Biofilms are difficult to remove and a mature biofilm is more resistant to disinfection procedures. This suggests that the test disinfection procedures may not be as efficient in the disinfection of old used pacifiers. Therefore, pacifiers should be disinfected regularly from the time they are new to prevent the establishment of a biofilm. This was seen in the study by Sio *et al.,* (1987), where the presence of *C. albicans* was investigated on used pacifiers. They found that the use of silicon pacifiers was associated with a lower rate of *C. albicans* when compared to latex pacifiers. Furthermore, over time, used pacifiers develop a biofilm. This biofilm formation acts as a

protective layer for the bacteria in a structural complex and therefore become progressively more resistant to cleaning methods. It is known that the presence of a biofilm impairs antimicrobial agents penetrating the full depth of the biofilm, thereby retarding diffusion.

However, test pacifiers in the current study were new and they did not have micro-cracks and irregular surfaces that could shelter bacteria from the disinfection process, which explains the similar results for the latex and silicon pacifiers. The results also showed that rinsing with tap water certainly did not achieve complete removal of bacteria but solution (Milton and CHX), and heat based disinfection would be effective. Furthermore, the results showed that heat based methods such as boiling and microwaving are the 2 most effective methods of disinfection compared to the solutions containing antimicrobial compounds (Milton and chlorhexidine) which will be discussed further in the disinfection section (See section 5.4).

5.2 Disinfection of pacifiers contaminated with different types of bacteria

When the % kill results were compared between the contaminants, no significant difference was found, which suggests that the methods were either adequate or inadequate for all three test organisms (Figure 4.2, Table 4.7). However, close observation of the results showed that *S. aureus* was the easiest to kill because most methods were adequate in killing this specific bacterium. *E. coli* was the most difficult organism to eradicate, as it showed resilience to Milton and chlorhexidine. Boiling and microwave (steam based) proved to be the best disinfection procedure for all the test contaminants. Clearly, tap water rinsing eliminated the least bacteria from the pacifiers irrespective of the type of pacifier and type of bacterium containment.

Resilience of *E. coli* to the disinfection procedures is of concern because this particular bacterium is often associated with diarrhoea (86% of infants) which is common in pacifier users (Mathur *et al, 1*990). Festini *et al*, (2012) also showed that 62% of infants who used pacifiers had at least one episode of diarrhoea in their first year of life. If the disinfection procedures are not adequate, these faecal contaminants can be transmitted from one infant to the other, particularly in the day care centers. Although the widely used disinfectant chlorhexidine, which was included as a positive control, reduced 6 to 7 logs of *E. coli* (Table 4.7a), it failed to completely eradicate the organism. This is also of concern with regards to hand washing antiseptic soaps which generally contains this chemical compound. *C. albicans* is known to colonize pacifiers, (Comina *et al*, 2006; da Silviera *et al,* 2009) which explains the high Candida carriage and potential reservoir for the risk of infection in the infants who use pacifiers (Darwazeh *et al,* 1995). Although Milton reduced 99.94 to 99.96% of *C. albicans*, the log reduction was only 3.57 to 4.43 (Table 4.7a), meaning that if the pacifier becomes contaminated with a high number of *C. albicans*, there will be a considerable number of organisms remaining on the pacifier, after the use of Milton.

5.3 Comparison of disinfection procedures

When the efficacy of the disinfection methods were compared (Figure 4.3, Table 4.7), the results showed that boiling, Milton, microwave radiation and chlorhexidine were significantly better than tap water rinse ($p<0.01$). For the tap water rinsing, the pacifier was agitated in an upwards and downwards motion for 2 minutes to mimic a rinsing action. Not much has been done to investigate the effectiveness of rinsing with tap water to disinfect pacifiers or to clean surfaces. However, rinsing is a cheap and easy cleaning method that is possibly carried out by many

individuals from a lower socioeconomic background. Rinsing does reduce the number of bacteria (lowest reduction – log 2.28 of *E. coli* from silicon pacifier). However, water has no antimicrobial action and rinsing with tap water is purely an operator dependent mechanical action. Furthermore, rinsing with water will remove the planktonic form of bacteria and may fail to remove the bacterial biofilm. Similar results were reported by Chamele *et al.,* (2012) with toothbrush and pacifier disinfection, although the test organisms were the cariogenic bacteria, *S. mutans*. They reported that rinsing with water was less effective than chlorhexidine and microwave radiation.

Placing a pacifier in boiling water for 5 minutes is also an easy and cheap alternative and the results showed that it was effective regardless of the type of pacifier and the contaminants. It is a simple, easy, practical, and economical method and therefore used in many developing countries to sterilize metal instruments. For example, in Khartoum, Sudan, 2% of dentists and in Lagos, Nigeria, 19.3% of dentists use boiling as a method of sterilization (Elkarim *et al.*, 2004, Sofola and Savage, 2003). A recent survey in India showed that up to 70.2% of dentists use boiling as a sterilization method (Singh *et al.*, 2012). Simple boiling for 30 minutes is known to kill all vegetative forms of bacteria but it will only kill some spores. However, the aim of disinfection of pacifiers is to kill all the pathogens and it is not necessary to have a sterile pacifier. In food industries submerging oranges in boiling water for 30 seconds before squeezing juice for bottling have been suggested to reduce the number of faecal contaminants such as *E. coli* (Bagci and Temiz, 2011). In our study, not only *E. coli* but other pathogens such as *S. aureus* and *C.*

albicans were completely eliminated after 5 minutes of boiling. Therefore, boiling can be used as an effective method of disinfection.

The third sterilization method tested in this study was with Milton. Milton's composition is 1.0 % sodium hypochlorite and 16.5 % sodium chloride. The results revealed that Milton completely eliminated *S. aureus* but failed to kill *E. coli* and *C. albicans*. Hecker *et al.,* (2012) suggested that the effect of sodium hypochlorite in endodontic treatment depends on the duration of contact. Corcoran *et al.,* (2014) demonstrated that although sodium hypochlorite can reduce *Salmonella enterica*, a food poison causing pathogen, it could not eradicate an early biofilm or a mature biofilm even after 90 minutes of contact time. Results of this present study (Figure 4.7 and Figure 4.7a) also showed that the sodium chloride/sodium hypochlorite containing product Milton (66.66% of test samples) was less effective when compared to boiling, microwave and chlorhexidine, with some surviving *E.coli* and *C. albicans* on both latex and silicon pacifiers. Further studies need to be carried out to investigate the effective concentration and the exposure time of Milton needed to eradicate these 2 organisms from pacifiers.

The use of a microwave sterilization unit to clean teats, pacifiers, milk bottles and teething rings has become a popular method in recent years. Microwave irradiation is claimed to be a simple, effective and inexpensive (if one already has a microwave), disinfection method. One hundred percent kill was obtained with this method for all three organisms on both latex and silicon pacifiers. It is important to note that in this study only one parameter (8 minutes at 900 watts)

was tested. This result cannot be generalized to all microwaves which may have different parameters. The manufacturers of these microwave sterilization units claim that it keeps items sterilized for up to 24 hours provided that the unit cover has not been opened. By placing the pacifier in the unit with water, the organisms were disinfected by both irradiation and steam. The use of a microwave to disinfect medical and dental items have been of interest in health field. For example Rosaspina *et al.,* (1994) demonstrated that 90 seconds of microwave exposure provided effective sterilization of surgical scalpel blades which were contaminated with *C. albicans*. Their study revealed that irradiation induced a morphological modification and eventually rupture of the cell after 9 minutes. Bhasin *et al.,* (2013) revealed that a 5 minute exposure to irradiation can eliminate *C. albicans* and *P. aeruginosa*, while elimination of *S. aureus* took longer (7 minutes). Many studies have found microwave irradiation to be effective in disinfecting used dentures, dental impression material and dental stone (Ribeiro *et al.*, 2009; Silva *et al.,* 2006; Neppelenbroek *et al.*, 2003; Yildirim-Bicer *et al.*, 2014).

Finally, in this study, pacifiers were submerged in chlorhexidine for 2 minutes. This disinfectant solution contained 2 mg/ml of chlorhexidine gluconate with 5.4% m/v ethanol as a preservative. Chlorhexidine eliminated *S. aureus* and *C. albicans* completely from both latex and silicon pacifiers. However, for *E. coli*, chlorhexidine gluconate was 99.99% effective on both latex and silicon pacifiers. Chlorhexidine is an antiseptic agent with a broad spectrum antimicrobial activity. Chlorhexidine destroys bacteria by breaking their membranes and inducing cytoplasmic precipitation (Gomes *et al.*, 2005, Cheung *et al.*, 2012). Due to its well established efficacy, this chemical was used as a positive control. The efficacy of chlorhexidine may be organism and

contact time dependent. *Streptococcus mutans* can be eliminated from pacifiers (Nelson-Filho *et al.*, 2011, Chamele *et al.*, 2012) whereas chlorhexidine may fail to completely eliminate other bacteria such as gastrointestinal pathogens (Sato *et al,* 2005). In addition, gram negative bacilli are known to have a low susceptibility to chlorhexidine (Brown *et al.*, 1989). Oral cavities of cancer patients are known to be colonised with gram negative bacteria, particularly enteric pathogens including *E. coli* even after the continuous use of chlorhexidine mouth rinse (Brown *et al.*, 1989, Raybould *et al.*, 1994). For this reason, *E. coli* might have survived on those pacifiers used in this study, even after the use of chlorhexidine.

5.4 Comparison between different disinfection procedures

All the disinfection methods used in this study, including tap water, reduced the number of test organisms regardless of the material construct of the pacifier. However, disinfection with a chemical or heat proved to be superior to water alone. The purpose of disinfection is the removal of pathogens and reduction of the number of bacteria. Pacifiers do not have to be sterile; however, some of the methods used in this study showed complete elimination of test organisms (microwave and boiling). None of the test organism was sporulating bacteria therefore this discussion only includes vegetative forms of bacteria. It is clear that heat based disinfection was better than chemical disinfection. Both boiling and the use of a microwave are relatively cheap, easy to use and most people have the required equipment in their homes.

Although chemicals (Milton and chlorhexidine) reduced the number of organisms, they did not show complete elimination particularly of pathogens such as *E. coli* (Milton and chlorhexidine) and *C. albicans* (Milton). Montagner *et al.,* (2009) evaluated the antifungal action of

chlorhexidine and hypochlorite on acrylic resin specimens, contaminated with *C. albicans*. They observed that sodium hypochlorite was a more efficacious disinfectant against *C. albicans* than 2.0 % chlorhexidine solution. Interestingly, in our study the results revealed that Milton (sodium Hypochlorite) did not eliminate *C. albicans* but 2.0 % chlorhexidine was indeed effective. Milton is widely used and the manufacturer claims that it eliminates *C. albicans* but the results of this study failed to show that. In addition, it is an additional cost to the consumer. Chlorhexidine is generally not used for the disinfection of pacifiers for many reasons. It is costly and inadequate for the enteric pathogens as also shown in the study. Furthermore, it has an unpleasant taste, sometime causes temporary numbness in the oral cavity, alter the taste and stain teeth.

6 Limitations, Future research and Conclusions

6.1 Limitations

- The sucking motion of the tongue creates friction on the pacifier creating micro-cracks which allows better bacterial attachment. The presence of saliva may also create a better bacterial attachment. In this study results were based on smooth unused pacifiers. The results reported here are on the vegetative form of planktonic bacteria rather than the biofilm.

- Mimicking and creating micro-cracks was considered. However, to standardize these cracks posed a challenge.

- The pacifiers were placed into the solution of contaminants for 2 minutes and no rubbing was involved which would be different from the used pacifiers.

6.2 Future research

- The effective exposure time and the concentration of Milton need further analysis.
- Use of chlorhexidine and the limitation particularly the ineffectiveness against gram negative bacteria can be studied.
- Results of microwave sterilization (steam based) can be compared to another method where the pacifier is placed in water in the microwave (boiling based). This may eliminate the cost of a microwavable steamer.
- Further studies need to be carried out to assess the efficacy of these methods used on pacifiers with biofilms of bacteria which are difficult to kill.

6.3 Conclusions

Effective cleaning and disinfection of pacifiers remain an important concern. While one is aware that pacifiers do carry and acts as a reservoir for bacterial growth, the usage of these devices are commonly seen. This study has shown that all the test interventions, including rinsing with tap water can reduce the number of contaminants. However, the use of disinfectant solutions such as hypochlorite-based commercially available products such as Milton and chlorhexidine as well as heat-based methods such as boiling and microwaving can significantly reduce the presence of the test microorganisms. Among these methods, boiling and the use of the microwave proved to be the best in killing the common pathogens *S. aureus, E. coli* and *C. albicans* completely. These methods are cost effective, readily available and easy to use. Education to the care givers in the day care centers and to mothers with regards to the contamination and decontamination of

pacifiers is important. It may reduce the transmission of pathogens and hence prevent and reduce the onset of infection among pacifier using infants.

7 Appendix

7.1 **Human Research Ethics Committee Wavier form** (Attached on next page)

University
of the Witwatersrand,
Johannesburg

Human Research Ethics Committee (Medical)
(formerly Committee for Research on Human Subjects (Medical)

Secretariat: Research Office, Room: SH10005 10th floor, Senate House • Telephone: +27 11 717-1234 • Fax: +27 11 339-5708
Private Bag 3, Wits 2050, South Africa

Ref: W-CJ-130626-1

26/06/2013

TO WHOM IT MAY CONCERN:

Waiver: This certifies that the following research does not require clearance from the Human Research Ethics Committee (Medical).

Investigator: **Dr M Patel, Deepa Boola (student no 0103169Y).**

Project title: **The efficacy of home disinfection techniques for pacifiers.**

Reason: This is an *in vitro* laboratory study using commercially acquired pacifiers and stock bacterial cultures. No humans are involved.

UNIVERSITY OF THE WITWATERSRAND
PROF PE CLEATON-JONES
HREC (MEDICAL)
2013 -06- 26
JOHANNESBURG

Professor Peter Cleaton-Jones
Chair: Human Research Ethics Committee (Medical)

copy: Anisa Keshav, Zanele Ndlovu, Wits Research Office

7. 2 Media and reagents

Blood Agar

39 g	Columbai agar (Oxoid ltd, UK)
1000 ml	Distilled water
5g	Sterile defibrinated blood

Columbia agar base was suspended in 1000 ml distilled water. It was dissolved completely by bringing to boil. It was sterilized by autoclaving at 15 lb and 121^0C for 10 minutes, allowed to cool to 50^0C and 5 % sterile defibrinated blood was added. The agar was poured into plates and allowed to set. Plates were labelled and refrigerated until use.

Sabouraud Agar

65 g Sabouraud Agar

1000 m*l* Distilled water

Sabouraud agar was suspended in 500 ml distilled water. The other 500 ml of distilled water was boiled. Both were mixed and boiled together. Agar was aliquoted, sterilized at 121^0C, 15 lb pressure for 20 minutes, cooled to 45^0C and plates were poured.

Phosphate Buffered Saline (PBS)

4.2 g	Sodium chloride
0.078 g	Sodium dihydrogen phosphate
0.64 g	Sodium hydrogen phosphate
500 m*l*	Distilled water

Solids were suspended and autoclaved at 15 1b, 121^{0}C for 15 minutes and dispensed.

7.3 Statistical analysis

(Attachments on pages below page 61 - 64)

7.4 Plagiarism declaration

(Attachments on pages below page 65)

7.5 Plagiarism report generated on Turnitin

(Attachments on pages below page 66 - 86)

```
      name:  <unnamed>
       log:  /Users/macbookpro/Documents/Stata/Pacifier.log
  log type:  text
 opened on:  17 Nov 2014, 14:13:05

. use "Pacifier.dta"

.
. *1 DESCRIPTIVES
.
. tab type

      Type |      Freq.     Percent        Cum.
-----------+-----------------------------------
   Silicon |         45       50.00       50.00
     Latex |         45       50.00      100.00
-----------+-----------------------------------
     Total |         90      100.00

. tab org

       Org. |      Freq.     Percent        Cum.
------------+-----------------------------------
  S. aureus |         30       33.33       33.33
    E. coli |         30       33.33       66.67
C. albicans |         30       33.33      100.00
------------+-----------------------------------
      Total |         90      100.00

. tab method

      Method |      Freq.     Percent        Cum.
-------------+-----------------------------------
   Tap water |         18       20.00       20.00
     Boiling |         18       20.00       40.00
      Milton |         18       20.00       60.00
   Microwave |         18       20.00       80.00
Chlorhexidine |         18       20.00      100.00
-------------+-----------------------------------
       Total |         90      100.00

.
. sum kill, detail

                            % kill
-------------------------------------------------------------
      Percentiles      Smallest
 1%        96.43          96.43
 5%        99.49           98.5
10%        99.79          99.08       Obs                  90
25%        99.99          99.46       Sum of Wgt.          90

50%          100                      Mean            99.89667
                        Largest       Std. Dev.       .4223423
75%          100             100
90%          100             100      Variance         .178373
95%          100             100      Skewness       -6.753457
99%          100             100      Kurtosis       53.12129

.
. *2.Does type of material have any influence on the % kill?
.
. table type, c(mean kill sd kill)
```

```
------------------------------------
     Type | mean(kill)    sd(kill)
----------+-------------------------
  Silicon |    99.8733    .5397643
    Latex |      99.92    .2614208
------------------------------------

.
. kwallis kill, by(type)

Kruskal-Wallis equality-of-populations rank test

  +---------------------------+
  |    type | Obs | Rank Sum  |
  |---------+-----+-----------|
  | Silicon |  45 |  2066.00  |
  |   Latex |  45 |  2029.00  |
  +---------------------------+

chi-squared =     0.022 with 1 d.f.
probability =     0.8813

chi-squared with ties =     0.034 with 1 d.f.
probability =     0.8538

.
. *NO SIGNIFICANT EFFECT OF TYPE ON %KILL
.
.
. *3. Does organsism have any influence on the % kill?
. table org, c(mean kill sd kill)

------------------------------------
      Org. | mean(kill)    sd(kill)
-----------+-------------------------
  S. aureus |    99.9137    .2883902
    E. coli |    99.8523    .6499903
C. albicans |     99.924    .1966195
------------------------------------

.
. kwallis kill, by(org)

Kruskal-Wallis equality-of-populations rank test

  +-------------------------------+
  |         org | Obs | Rank Sum  |
  |-------------+-----+-----------|
  |   S. aureus |  30 |  1477.50  |
  |     E. coli |  30 |  1339.50  |
  | C. albicans |  30 |  1278.00  |
  +-------------------------------+

chi-squared =     1.020 with 2 d.f.
probability =     0.6006

chi-squared with ties =     1.552 with 2 d.f.
probability =     0.4602

.
. *NO SIGNIFICANT EFFECT OF ORGANISMS ON %KILL
.
.
```

```
. *4 Does treatment have different effects on the % kill?
. table method, c(mean kill sd kill)

----------------------------------------
       Method | mean(kill)    sd(kill)
--------------+-------------------------
    Tap water |    99.5022    .8519013
      Boiling |       100           0
       Milton |    99.9822    .0438655
    Microwave |       100           0
Chlorhexidine |    99.9989    .0032345
----------------------------------------

.
. kwallis kill, by(method)

Kruskal-Wallis equality-of-populations rank test

  +---------------------------------+
  |        method | Obs | Rank Sum  |
  |---------------+-----+-----------|
  |     Tap water |  18 |    184.50 |
  |       Boiling |  18 |   1062.00 |
  |        Milton |  18 |    792.50 |
  |     Microwave |  18 |   1062.00 |
  | Chlorhexidine |  18 |    994.00 |
  +---------------------------------+

chi-squared =     44.934 with 4 d.f.
probability =      0.0001

chi-squared with ties =     68.411 with 4 d.f.
probability =      0.0001

.
. *SIGNIFICANT EFFECT OF TREATMENT METHOD ON %KILL, WE NEED TO FIND THE EFFECTIVE
> METHODS
.
.
. *5 Which treatment is significanly better than water (w)?
. oneway kill method, tab sch

           |          Summary of % kill
    Method |        Mean   Std. Dev.       Freq.
-----------+------------------------------------
 Tap water |   99.502222   .85190132          18
   Boiling |         100           0          18
    Milton |   99.982222   .04386547          18
 Microwave |         100           0          18
 Chlorhexi |   99.998889    .0032345          18
-----------+------------------------------------
     Total |   99.896667   .42234228          90

                       Analysis of Variance
    Source              SS         df      MS            F     Prob > F
------------------------------------------------------------------------
Between groups      3.50479846      4   .876199614       6.02     0.0003
 Within groups      12.3703985     85    .1455341
------------------------------------------------------------------------
    Total           15.875197      89      .178373

Bartlett's test for equal variances:  chi2(2) = 228.4909  Prob>chi2 = 0.000

note: Bartlett's test performed on cells with positive variance:
```

```
      2 multiple-observation cells not used

                        Comparison of % kill by Method
                                  (Scheffe)
Row Mean-|
Col Mean |   Tap wate    Boiling     Milton   Microwav
---------+--------------------------------------------
 Boiling |    .497778
         |      0.007
         |
  Milton |        .48   -.017778
         |      0.010      1.000
         |
Microwav |    .497778          0    .017778
         |      0.007      1.000      1.000
         |
Chlorhex |    .496666   -.001111    .016666   -.001111
         |      0.007      1.000      1.000      1.000

.
. *ALL TREATMENT BETTER THAN WATER STATISTICALLY.
. *SOME TREATMENTS ARE NOT SUPERIOR TO EACH OTHER SUCH AS MILTON AND BOILING WATER
.
.
. log close
      name:  <unnamed>
       log:  /Users/macbookpro/Documents/Stata/Pacifier.log
  log type:  text
 closed on:  17 Nov 2014, 14:13:05
-------------------------------------------------------------------------------
```

Faculty of Health Sciences, Postgraduate Office
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PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I DEEPA BHOOLA (Student number: 0103169Y) am a student registered for the degree of MSc Dent in the academic year 2015.

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.
- I have included as an appendix a report from "Turnitin" (or other approved plagiarism detection) software indicating the level of plagiarism in my research document.

Signature: _____ Date: 30/3/15

8 References:

• Adair SM, Milano M, Duskhu JC. Evalulation of the effects of orthodontic pacifiers on the primary dentition of 24 to 59 month old children: preliminary study. Pediatric Dentistry 1992; 14(1): 13-18.

• Aliprandini P, Ferreira FB, Bertol LS, Kindlein Junior W. Comparison of design, materials selection and characterization of pacifiers produced in Brazil. Australasian Medical Journal 2011; 4(2): 76-80.

• American Academy of Pediatrics. The changing concept of sudden infant death syndrome. Pediatrics 2005; 116(5): 1245–1255.

• Arias E, MacDorman MF, Strobino DM, Guyer B. Annual summary of vital statistics-2002. Pediatrics 2003; 112: 1215–1230.

• Arvedson J, Clark H, Lazarus C, Schooling T, Frymark T. Evidence – based systematic review; effects of oral motor interventions on feeding and swallowing in preterm infants. American Journal of Speech-Language Pathology 2010; 19(4): 321–340.

• Bagci U, Temiz A. Microbiological quality of fresh – squeezed orange juice and efficacy of fruit surface decontamination methods in microbiological quality. Journal of food Protection 2011; 74(8): 1238-1244.

• Bagg J, MacFarlane TW, Poxton IR, Miller CH, Smith AJ. Essentials of Microbiology for Dental Students. Oxford Medical Publications. 1999.

• Bhasin A, Vinod V, BhasinV, Mathew X, Sajjan S, Ahmed ST. Evaluation of effectiveness of microwave irradiation for disinfection of silicone elastomeric impression material. Journal of Indian Prosthodontic Society 2013; 13 (2); 89-94.

• Bonesvoll P. Oral pharmacology of chlorhexidine. Journal of Clinical Periodontology 1977; 4: 49-65.

• Bork K, Hoede N, Korting GW, Burgdorf WHC, Young SK. Diseases of the oral mucosa and the lips. W. B. Saunders: Philadelphia. 1993.

• Brecx M, Brownstone E, MacDonald L. Efficacy of Listerine, Merdial and chlorhexidine mouth rinses as supplements to regular tooth-cleaning measures. Journal of Clinical Periodontology 1992; 19: 202-207.

• Brooks GF, Butel JS, Morse SA. Jawetz, Melnick and Adelberg's Medical Microbiology 22nd Edition. McGraw- Hill Companies, 2001.

• Brook I, Gober AE. Bacterial colonization of pacifiers of infants with acute otitis media. The Journal of Laryngology and Otology 1997; 111: 614-615.

• Brown AT, Sims RE, Raybould TP, Lillich TT, Henslee PJ, Ferretti GA. Oral gram – negative bacilli in bone marrow transplant patients given chlorhexidine rinses. Journal of Dental Research 1989; 68(7): 1199–1204.

• Bukowski M, Wladyka B, Dubin G. Exfoliative Toxins of Staphylococcus aureus. Toxins 2010; 2(5): 1148-1165.

• Chamele J, Bhat C, Saraf T, Jadhav A, Beg A, Jagtap C, Ubeja R, Patil P. Efficacy of microwaves and chlorhexidine for disinfection of pacifiers and toothbrushes: an in vitro study. The Journal of Contemporary Dental Practice 2012; 13(5): 690-694.

• Cheung HY, Wong MM, Cheung SH, Liang LY, Lam YW, Chiu SK. Differential actions of chlorhexidine on the cell wall of Bacillus subtilis and Escherichia coli. Public library Science 2012; 7(5): e36659. doi: 10.1371/journal.pone.0036659.

• Cole AM, Tahk S, Oren A, Yoshioka D, Kim Y-H, Park A, Ganz T. Determinants of Staphylococcus aureus nasal carriage. Clinical Vaccine Immunology 2001; 8(6): 1064–1069.

• Comina E, Marion K, Renaud FN, Dore J, Bergeron E, Freney J. Pacifiers: a microbial reservoir. Nursing Health Science 2006; 8(4): 216–223.

• Corcoran M, Morris D, De Lappe N, O'connor J, Lalor P, Dockery P, Cormican M. Commonly used disinfections fail to eradicate Salmonella enteric biofilms from food contact surface materials. Applied and Environmental Microbiology 2014; 80(4): 1507-1514.

• Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilm: a common cause of persistent infection. Science 1999; 284: 1318 -1322.

• Darwazeh AM, al-Bashir A. Oral candidial flora in healthy infants. Journal of Oral Pathology and Medicine 1995; 24(8): 361-364.

• Da Silva CH, Paranhos Hde F. Efficacy of biofilm disclosing agent and of three brushes in the control of complete denture cleaning. Journal of Applied Oral Science 2006; 14(6): 454-459.

• Da Silveira LC, Charone S, Maia LC, Soares RM, Portela MB. Biofilm formation by Candida species on silicone surfaces and latex pacifier nipples: an in vitro study. The Journal of Clinical Pediatric Dentistry 2009; 33(3): 235-340.

• Elkarim IA, Abdulla ZA, Yahia NA, Al Qudah A, Ibrahim YE. Basic infection control procedures in dental practice in Khartoum – Sudan. International Dental Journal 2004; 54(6): 413-417.

• Evans Jr D, Evans DG, Escherichia coli. Medical Microbiology, 4th edition, The University of Texas, Medical Branch at Galveston. 2007.

• Festini F, Giusti F, Paoletti E, Biancalani L, Poggi GM. Is early use of pacifier risk factor for diarrhea? Minerva Pediatrics 2012; 64(3): 303-305.

• Gehlbach SH, MacCormack JN, Drake BM, Thompson WV. Spread of disease by fecal-oral route in day nurseries. Health Service Reports 1973; 88: 320-322.

• Gillespie S and Bamford K. Medical Microbology & Infection at a Glance, 3rd edition. Blackwell Publishing, 2007.

• Gjermo P, Baastad KL, Rolla G. The plaque inhibiting capacity of 11 antimicrobial components. Journal of Periodontal research 1970; 5: 102-109.

• Gomes BPFA, Vianna ME, Matsumoto Cu, Rossi Vde P, Zaia AA, Ferraz CC, Souza Filho FJ. Disinfection of gutta – percha cones with chlorhexidine and sodium hypochlorite. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics 2005; 100: 512–517.

• Greenstein G, Berman C, Jaffin R. Chlorhexidine a adjunct to periodontal therapy. Journal of Periodontology 1986; 57: 370-377.

• Grossman E, Meckel AH, Isaacs RL, Ferretti GA, Sturzenberger OP, Bollmer BW, Moore DJ, Lijana RC, Manhart MD. A clinical comparison of antibacterial mouthrinses: effects of chlorhexidine, phenolics and sanguinarine on dental plaque and gingivitis. Journal of Periodontology 1989; 60(8): 435-340.

• Grozdanov L, Raasch C, Schulze J, Sonnenborn U, Gottschalk G, Hacker J, Dobrindt U. Analysis of the genome structure of the nonpathogenic probiotic Escherichia coli strain Nissle 1917. Journal of Bacteriology 2004; 186: 5432-5441.

• Hanafin S, Griffiths P. Does pacifier use cause ear infection in young children? British Journal of Community Nursing 2002; 7(4):206, 208-11.

• Hauck FR, Omojokun OO, Siadaty MS. Do pacifiers reduce the risk of sudden infant death syndrome? Pediatrics 2005; 116(5): 716–723.

• Hecker S, Hiller KA, Galler KM, Erb S, Mader T, Schmalz G. Establishment of an optimized ex vivo system for artificial root canal infection evaluated by use of sodium hypochlorite and the photodynamic therapy. International Endodontic Journal 2013; 46 (5): 449–457.

• Howard CR, Howard FM, Lanphear B, Eberly S, deBlieck EA, Oakes D, Lawrence RA. Randomized clinical trial of pacifier use and bottle-feeding or cupfeeding and their effects on breastfeeding. Pediatrics 2003; 111(3): 511-518.

• Hudault S, Guignot J, Servin AL. Escherichia coli strains colonizing the gastrointestinal tract protect germ-free mice against Salmonella triphimurium infection. Gut 2001; 49(1):47-55.

• Jenik AG, Vain N. The pacifier debate. Early human development 2009; 85(10): 89-91.

• Johnson BR and Remeikis NA. Effective shelf – life of prepared sodium hypochlorite solution. Journal of Endodontic 1993; 19(11): 40–43.

• Kamada N, Inoue N, Hisamatsu T, Okamoto S, Matsuoka K, Sato T, Chinen H, Hong KS, Yamada T, Suzuki T, Watanabe N, Tsuchimoto K, Hibi T. Nonpathogenic Escherichia coli strain Nissle 1917 prevents murine acute and chronic colitis. Inflammatory Bowel Diseases 2005; 11:455-463.

• Kaufman GE, Cimo S, Miller LW, Blass EM. An evaluation of the effects of sucrose on neonatal pain with 2 commonly used circumcision methods. American Journal of Obstetrics and Gynaecology 2002; 28(3): 564–568.

• Kluytmans J, van Belkum A, Verbrugh H. Nasal carriage of Staphylococcus aureus: epidemiology, underlying mechanisms, and associated risks. Clinical Microbiology Reviews 1997; 10(3): 505–520.

• Lieberthal AS, Carroll AE, Chonmaitree T, Ganiats TG, Hoberman A, Jackson MA, Joffe MD, Miller DT, Rosenfeld RM, Sevilla XD, Schwartz RH, Thomas PA, Tunkel DE. The Diagnosis and Management of Acute Otitis Media. Pediatrics 2013; 131: 964.

• Loe H, Schiott CR. The effect of mouthrinses and topical application of chlorhexidine on the development of dental plaque and gingivitis in man. Journal of Periodontology 1970; 5: 79-83.

• Mainstone A. Domestic hazard analysis of infant feeding utensiles. British Journal of Midwifery 2004; 12(6): 368-372.

• Marrie TJ, Nelligan J, Costerton JW. A scanning and transmission electron microscopic study of an infected endocardial pacemaker lead. Circulation 1982; 66(6): 1339-1341.

• Mathur GP, Mathur S, Khanduja GS. Non – nutritive suckling and use of pacifiers. Indian Pediatrics 1990; 27(11): 1187-1189.

• Mattos-Graner RO, de Moraes AB, Rontani RM, Birman EG. Relation of oral yeast infection in Brazilian infants and use of a pacifier. ASDC Journal of Dentistry for Children 2001; 68(1): 33-36,10.

• Mernelius S, Löfgren S, Lindgren PE, Blomberg M, Olhager E, Gunnervik C, Lenrick R, Thrane MT, Isaksson B, Matussek A. The effect of improved compliance with hygiene guidelines on transmission of Staphylococcus aureus to newborn infants: The Swedish Hygiene Intervention and Transmission of S. aureus study. American Journal of Infection Control 2013;41(7): 585-590.

• Mims C, Dockrell HM, Goering RV, Roitt I, Wakelin D, Zuckerman M. Medical Microbiology Third Edition. Elsevier Limited, 2004.

• Mitchell EA, Blair PS, L'Hoir MP. Should pacifiers be recommended to prevent sudden infant death syndrome? Pediatrics 2006; 117: 1755-1758.

• Mohammadi Z, Abbott PV. The properties and applications of chlorhexidine in endodontics. International Endodontic Journal 2009; 42: 288-302.

• Montagner H, Montagner F, Braun KO, Peres PE, Gomes BP. In vitro antifungal action of different substances over microwave-cured acrylic resins. Journal of Applied oral Science 2009; 17: 432–435.

• Nam SH, Seo YM, Kim MG. Bisphenol A migration from polycarbonate baby bottle with repeated use. Chemosphere 2010; 79(9): 949-952.

• Nelson-Filho P, da Silva LA, Ds Silva RA, da Silva LL, Ferreira PD, Ito IY. Efficacy of microwaves and chlorhexidine on the disinfection of pacifiers and toothbrushes: an in vitro study. Pediatics Dentistry 2011; 33(1): 10-13.

• Neppelenbroek KH, Pavarina AC, Spolidorio DM, Vergani CE, Mima EG, Machado AL. Effectiveness of microwave sterilization on three hard chairside reline resins. International Journal of Prosthodontics 2003; 16(6): 616-620.

• Newman and Nisengard. Oral Microbiology and Immunology. W.B Saunders: Philadelphia.1988.

• Niemela M, Pihakari O, Pokka T, Uhari M, Uhari M. Pacifier as a risk factor for acute otitis media: A randomized, controlled trial of parental counseling. Pediatrics 2000; 106: 483-488.

• Niemela M, Uhari M, Mottonen MA. Pacifier increases the risk of recurrent acute otitis media in children in daycare centers. Pediatrics 1995; 96:884-888.

• Nordqvist C. What is E.coli? Medical News Today.2013

• Ollila P, Niemela M, Uhari M, Larmas M. Prolonged pacifier-sucking and use of nursing bottle at night: possible risk factors for dental caries in children. Acta Odontologica Scandinavica 1998; 56(4): 233-237.

• Peressini S. Pacifier use and early childhood caries: An evidence – based study of the literature. Journal of Canadian Dental Association 2003; 69(1): 16-9.

• Poore M, Zimmerman E, Barlow SM, Wang J, Gu F. Patterned orocutaneous therapy improves sucking and oral feeding in preterm infants. Acta Paediatrica 2008; 97(7): 920-927.

• Poyak J. Effects of pacifiers on early oral development. International Journal of Orthodontics (Milwaukee, WiS) 2006; 17(4): 13-16.

• Raybould TP, Carpenter AD, Ferretii GA, Brown AT, Lillich TT, Henslee J. Emergence of gram- negative bacilli in the mouths of bone marrow transplant recipients suing chlorhexidine mouthrinse. Oncology Nursing Forum 1994; 21(4): 691-696.

• Redmond EC, Griffith CJ, Riley S. Contamination of bottles used for feeding reconstituted powdered infant formula and implications for public health. Perspectives Public Health 2009; 129(2): 85-94.

• Reid G, Howard J, Gan BS. Can bacteria interference prevent infection? Trends Microbiology 2001; 9: 424-428.

• Ribeiro DG, Pavarina AC, Dovigo LN, Palomari Spolidorio DM, Giampaolo ET, Vergani CE. Denture disinfection by microwave irradiation: a randomized clinical study. Journal of Dentistry 2009 Sep; 37(9): 666 -672.

• Rolla G, Loe H, Schiott CR. The affienity of chlorhexidine for hydroxyapatite and salivary mucins. Journal of periodontal Res 1970; 5: 90-95.

• Rosaspina S, Salvatorelli G, Anzanel D, Bovolenta R. Effect of microwave radiation on Candida albicans. Microbiology 1994; 78(314): 55 -59.

• Rovers MM, Numans ME, Langenbach E, Grobbee DE, Verheij TJM, Schilder AGM. Is pacifier use a risk factor for acute otitis media? A dynamic cohort study. Family Practice 2008; 25: 233-236.

• Rutala WA, Weber DJ. Infection control: the role of disinfection and sterilization. Journal of Hospital Infections 1999; 43: 43-55.

• Rutala WA, Weber DJ. Uses of inorganic hypochlorite (bleach) in health – care facilities. Clinical Microbiology 1997; 10 (4): 597 – 610.

• Rweyendela IH, Patel M, Owen CP. Disinfection of irreversible hydrocolloid impression material with chlorinated compounds. South African Dental Journal 2009; 64(5): 208, 210-212.

• Sato S, Pedrazzi V, Guimaraes Lara EH, Panzeri H, Ferreira de Albuquerque R Jr, Ito IY. Antimicrobial spray for toothbrush disinfection: an in vivo evaluation. Quintessence International 2005; 36(10): 812-816.

• Sexton S, Natale R. Risks and Benefits of Pacifiers. American Family Physician 2009; 79(8): 681-685.

• Silva M, Consani R, Sardi J, Mesquita M, Macêdo A, Takahashi J. Microwave irradiation as an alternative method for disinfection of denture base acrylic resins. Minerva Stomatology 2013; 62(1-2): 23-29.

• Singh BP, Khan SA, Agrawal N, Siddharth R, Kumar L. Current biomedical waste management practice and cross – infection control procedures of dentist in India. International Dental journal 2012; 62 (3): 111 -116.

• Sio JO, Minwallla FK, George RH, Booth IW. Oral Candida: is dummy carriage the culprit? Archives of disease in childhood 1987; 62(4): 406– 408.

• Sofola OO,Savage KO. Assessment of the compliance of Nigerian dentists with infection control: a preliminary study. Infection Control and Hospital Epidemiology 2003; 24(10): 737–740.

• Sussman M. Escherichia coli: mechanisms of virulence. Department of Microbiology, Medical School, Newcastle upon Tyne; Cambribridge university press:1997.

• Todar K. Staphylococcus aureus and Staphylococcal Disease.Text Book of Bacteriology. Madison, Wisconsin 2012. http://textbookofbacteriology.net/staph.html

• Tomasi E, Victora CG, Post PR, Olinto MT, Béhague D.The use of pacifiers in children: fecal contamination and association with diarrhea. Revista de Saúde Pública 1994; 28(5): 373-379.

• Victora CG, Tomasi E, Olinto MT, Barros FC. Use of pacifiers and breastfeeding duration. Lancet 1993; 341(8842): 404-406.

• Yildirim – Bicer AZ, Peker I, Akca G, Celik I. In vitro antifungal evaluation of seven different disinfectants on acrylic resins. BioMed Research International 2014; 2014:519098. doi: 10.1155/2014/519098

• Yonezu T, Yakushiji M. Longitudinal study on influence of prolonged non-nutritive sucking habits on dental caries in Japanese children from 1.5 to 3 years of age. The Bulletin of Tokyo Dental College 2008; 49(2): 59-63.

• Zempsky WT, Cravero JP. Relief of pain and anxiety in pediatric patients in emergency medical systems. Pediatrics 2004; 114(5): 1348-1356.

• Zimmer S, Barthel CR, Ljubicic R, Bizhang M, Raab WH. Efficacy of a novel pacifier in the prevention of anterior open bite. American Academy of Pediatric Dentistry 2011; 33(1): 52-5.

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The efficacy of home disinfection techniques for pacifiers 1 Introduction Pacifiers/dummies/soothers are items designed for infants aged from around 2 weeks to around 5 years of age. They are used to pacify infants/toddlers when they are in discomfort; feeling insecure or crying, when they are sick, have colic or are teething. Sometimes they are given just before bedtime to sooth them. The pacifier is usually sucked by the infant for a period of time, and at many times during the day. It simulates the mother's breast nipple and therefore comforts them. This sucking motion involves creating a seal with the lips while sucking, and swallowing at intervals. Once the pacifier has been introduced into the oral cavity, it comes in contact with saliva and with the oral microflora of the oral cavity. The oral microflora is composed of many different microorganisms including pathogenic species. The chemical composition of the saliva serves as nutrients for these microorganisms. These microorganisms including pathogenic species present in the saliva adheres to the pacifier and therefore the pacifier becomes colonized. When the pacifier is not in use, it is usually kept in a pacifier container, or chained and attached to the infants' clothes hanging free and in reach of the infant to reuse at leisure. This can allow bacteria to grow on the pacifier between uses which could become potentially pathogenic to the child. Over time this growth of bacteria may result in a formation of a biofilm which are microbial aggregates included in an extracellular matrix (Costerton et al 1999). Furthermore the pacifier can be contaminated with bacteria from the environment as they can stick to the pacifier as children often drop the pacifiers on the floor. Parents or caregivers may suck on the pacifier in attempt to clean it, and place it back into a child's mouth. Sucking on a fallen pacifier may remove loose dirt and sand, but further contaminates the pacifier with additional oral bacteria. In daycare centers cross contamination between sick children can also occur through the use of pacifiers. Therefore it is important to remove the bacteria and prevent the biofilm from maturing, since mature biofilm is more resistant to disinfection procedures. This study investigated the efficacy of commercially available

sterilizing systems and disinfection procedures. 2 Literature Review 2.1 Pacifiers Pacifiers are used by 75-85% of babies in Western countries (Niemela et al 2000). They constitute of a nipple, a shield/guard and a ring. The ring serves as a handle and is attached to the shield. It lies extra orally and is the area designed to be held when placing or removing the pacifier from the infant's month. It is made of polycarbonate. The shield is also made of polycarbonate. The shield, like the ring, lies extra-orally. It is generally 1,5cm in radius in all directions. This is a safety measure which prevents the infant from swallowing or choking on the pacifier. It is therefore sometimes referred to as a guard. Some pacifiers may have two perforations on either side of the shield. These perforations serve as ventilation holes, protecting the skin around the infant's mouth. Without them, saliva can collect behind the shield, irritating the skin and causing a rash. The nipple is the sucking part of the pacifier and is the part that is attached to the shield. It is made of silicon or latex and the shape resembles that of a lactating mother's nipple. These nipples come in two distinct shapes, the round shape pacifier known as the conventional pacifier or the flattened end on one side pacifier known as the orthodontic pacifier. Orthodontic pacifiers are recommended by dentists due to the ergonomic shape. The flattened shape permits the tongue to be accommodated, allowing a more natural sucking posture (Figure 2.1). Some researchers have proved that the malocclusions seen in toddlers using these pacifiers are slightly less than, toddlers that used a conventional pacifier, (Adair et al, 1992). Both these shapes of the nipples are manufactured using either silicon or latex. Figure 2.1: Conventional and orthodontic pacifier 2.1.1 Types of pacifiers There are two types of pacifiers available, latex and Silicon (Figure 2.2). Silicone pacifiers are sturdier than latex pacifier. They also clean more easily (Redmond et al 2009) and do not retain odours. However latex pacifiers are more soft and flexible, which an infant may prefer. This softness results in the pacifier being worn out easily resulting in micro cracks (Mainstone, 2004). These rough surfaces and micro cracks harbor bacteria, making it difficult to clean. A study has shown that latex pacifiers have rugose and irregular surfaces which become colonized with microorganisms including Candida albicans and C. parapsilosis which cause oral thrush (da Silveira et al 2009). Comina et al 2006, collected used latex and silicone pacifiers and performed direct microscopic observations of the biofilm. The results revealed contamination of latex nipple clearly showing a mature biofilm, in which bacterial cells (rods) were embedded in slime. The silicone pacifiers had a much lower level of slime contaminated with Staphylococcus. In this study the new latex pacifiers presented a clean surface however the surface was irregular and showed many grooves. Whereas surfaces of the new silicone pacifiers were also clean, but were much smoother then the latex pacifiers. Figure 2.2: Latex and silicone pacifier 2.2 Advantages of pacifiers As dentists, a common question asked by mothers is "is it ok to give my child a dummy?" It is therefore important for a dentist and other health care providers to be aware of the advantages and disadvantages of pacifiers when advising parents. It is highly questionable as to whether a pacifier is a convenient and an efficient alternative to children sucking their thumb. 2.2.1 Analgesic effects Nonnutritive sucking is a natural reflex for a fetus and newborn (Sexton and Natale, 2009). This can be noted on ultra-sound scans as the fetus may be seen sucking on their hands or fingers. A pacifier may serve to fulfill this innate desire. According to Niemela et al 1995, the infant's need for sucking is greatest during the first 6 months of life. A subgroup of

3**the American Academy of Pediatrics (AAP)** has listed **the use of pacifiers**

to have analgesic effects on newborns and infants less than 6 months of age who are undergoing minor medical procedures (Zempsky and Cravero, 2004, Kaufman et al 2002) such as catheterization, immunizations, lumbar puncture and circumcision. It also has an analgesic effect on a teething toddler. Shorter hospital stay for preterm infants has also been listed as an advantage (Sexton and Natale, 2009). However the exact mode of action is not fully understood. 2.2.2 Oromotor development The sucking reflex assists preterm babies who are at risk of oromotor dysfunction to develop better sucking reflex and therefore improve an oral feeding ability (Poore et al 2008). It does not require a lot of energy to suck and assist in good coordination and muscular strength. Therefore it is important that preterm infants start oral feeding as soon as possible to survive and get healthy quickly. It is believed that the oral feeds would increase the daily weight gain and therefore assist in the infant being discharged from hospital earlier. However in evidence – based systematic review by Arvedson et al (2010) on the effects of motor

interventions on feeding and swallowing in preterm infants, found none of the oral motor interventions provided consistent positive results on weight gain. The non nutritional sucking did however show strong positive findings for improvement in some feeding and swallowing physiology and found that non nutritional sucking did reduce transition time to oral feeds. Also the continuous sucking would increase the tension of the muscles of the upper airway. A pacifier also facilitates in keeping the tongue in a more forward position and in doing so protects the airways (Jenik and Vain, 2009). 2.2.3 Reduction of sudden infant death syndrome In literature, the reduction of sudden infant death syndrome (SIDS) in infants who use a pacifier while sleeping is well documented (Hauck et al 2005; Mitchell et al 2006; Sexton and Natale, 2009). However the exact mechanism is not fully understood. The diagnosis of SIDS is primarily a diagnosis of exclusion. This means that it is given only after all other possible causes of the baby's death have been ruled out, in an apparently healthy infant, usually during sleep. Sudden infant death syndrome is one of leading cause of death in infants beyond the neonatal period in the United States (Arias et al 2003). AAP guidelines suggests that offering pacifiers to an infant at the onset of sleep, soon after breastfeeding has been established to reduce the risk of SIDS (AAP, 2005). The guidelines recommend at least establishing breastfeeding first for a month before introducing a pacifier, as later onset of pacifier use appears to have fewer negative effects on breastfeeding (Howard et al 2003.) Reduction in SIDS due to the use of a pacifier could be due to the decrease in the likelihood of rolling into the prone position, increased arousal, maintained airway patency, decreased gastroesophageal reflux and resultant sleep apnea, or increased respiratory drive with carbon dioxide retention (Hauck et al 2005). 2.3 Disadvantages of pacifiers 2.3.1 Dental malocclusions There are many disadvantages of pacifier usage have been suggested. One of the commonly known disadvantages of the long term use of pacifiers (for more than 2 years of age) is the development of dental malocclusions. The most notable changes are an increase in the prevalence of an anterior open bite (Zimmer et al 2011), posterior cross bite, narrow intercuspid width of the maxillary arch, and a high narrow palate (Poyak, 2006). However orthodontic pacifiers are readily available in the market, claiming the use of these pacifiers do reduce these malocclusions when compared to the use conventional pacifiers.

3**Orthodontic pacifiers have been scientifically designed to support the shape of babies' developing** palate **and jaws. The flattened shape not only simulates the shape of a mother's nipple when flattened in the mouth, but also encourages the most natural sucking action to help proper oral development. Studies indicate that children who use orthodontic pacifiers have a** slightly **smaller chance of developing an open bite than who use conventional round pacifiers,**

(Adair et al 1992).

3**This difference is hard to demonstrate, though, because neither type tends to cause** noticeable **problems unless sucked** on **for** more than 2 **years**

(Sexton and Natale 2009). 2.3.2 Exposure to Bisphenol A Bisphenol A (BPA) is an industrial chemical that has been used to make certain plastics and resins and can be found in pacifiers. It is added to enhance the malleability and therefore without this chemical the guard and the ring of pacifier would be hard and brittle. Some researchers have shown that BPA can seep out and pose a possible health risk such as cancer and is also associated with premature puberty (Aliprandini et al 2011), since the compound imitates estrogens. The infant's mouth is in direct contact with the shield creating a warm and humid environment which results in BPA migration. Studies have shown that migration of BPA increase with increase in temperature and, over time with pacifier use (Nam et al, 2010). There are BPA free pacifiers available in the market. 2.3.3 Colonization and transmission of pathogens and risk of infections Pacifiers

are known to carry microorganisms. Once the pacifier has been introduced into the oral cavity, it comes in contact with saliva and with the oral microflora of the oral cavity. The oral microflora is composed of many different microorganisms including pathogenic species. These microorganisms adhere to the pacifier forming a slimy coating of bacteria called a biofilm. This becomes worse by cross contamination when care givers do not wash their hands when handling pacifiers. Bacterial biofilm is a structured community of bacterial cells enclosed in a self - produced polymeric matrix and adherent to an inert or living surface (Costerton et al 1999). Biofilms constitute a protected mode of growth that allows bacterial growth and survival in a hostile environment. Biofilm infection can rarely be resolved by a host defense mechanism. Antibiotic therapy typically reverses the symptoms caused by the bacteria release from the biofilm, but fails to kill the bacteria (Marrie et al 1982). The polymeric substances that make up the matrix of the biofilm are known to retard the diffusion of antibiotics. It is possibly for this reason that pacifier users are more prone to the common cold, flu and diarrhea as they can re- infect themselves with the pathogenic micro-organisms found in this biofilm coated pacifier. It has been noted that pacifier users have a significantly higher rate of Candida carriage in the oral cavity (Darwazeh et al 1995). In another study the presence of oral yeast and its relationship with sucking and feeding habits was investigated. This study showed no relationship with the prevalence of yeast with breast feeding or bottle feeding but the use of a pacifier was positively associated with the presence of yeast infections (Mattos-Graner et al 2001). Research regarding the use of pacifiers and the associated risk factor in otitis media is controversial. Acute otitis media (AOM) usually occurs when one has a (viral) upper respiratory tract infection with blockage of the Eustachian tube and escape of fluid in the middle ear, which becomes infected with bacteria. The most common bacteria found in this case are Streptococcus pneumoniae, Haemophilus influenzae and Moraxella catarrhalis (Lieberthal et al 2013). Studies have shown an increase in risk of ear infection in infants using a pacifier. Some studies indicated this increase to be up to three times higher in infants using pacifiers (Hanafin and Griffiths, 2002). The frequency of use of the pacifier was also directly proportional to the chances of developing otitis media (Hanafin and Griffiths, 2002). Parents are therefore often informed about the possible negative effects of the use of a pacifier once their child has been diagnosed with AOM to avoid recurrent episodes (Rovers et al 2008). Interestingly, pacifiers collected from children with otitis media failed to show presence of pathogens responsible for otitis media. However, oral microorganisms were found on these pacifiers (Brook and Gorber, 1997). 2.3.4 Early development of dental caries Relationship between the use of pacifiers and early development of dental caries has been suggested. In an evidence–based study of literature, the question of whether the use of a pacifier plays a role in causing early dental caries showed weak correlation because of inconsistencies in the study methods. The inconsistencies included: the variation of the definition of early dental caries among studies and the variation of criteria to identifying lesions. The use of a pacifier frequency and duration was also not reported on clearly (Peressini, 2003). The use of a sweeter on the pacifier was not indicated and the age of the children varied among studies. However some studies listed the use of pacifier as a potential risk factor for the development of dental caries. In a prospective study, 18 month old children with pacifiers had a greater risk of developing dental caries by the age of 3 years compared to the children with thumb sucking habit, (Yonezu and Yakushiji, 2008). Ollila et al 1998, also reported that prolong pacifier sucking was a significant risk factor for the development of early dental caries in children. Often these pacifiers are dipped into sweet substances which may be partly responsible for the development of dental caries (Aliprandini et al 2011). Whatever the reasons, a parent or a caregiver may introduce a pacifier to an infant; the prime importance is to not harm the infant. Well documented disadvantages of using a pacifier, are acute media otitis, diarrhea and oral thrush. These disadvantages can be reduced if effective disinfecting methods are carried out to reduce the number of contaminants including pathogens. Therefore it is important to understand the contaminating microorganisms. 2.4 Contamination of pacifiers with oral microorganisms Once the pacifier enters the mouth it becomes contaminated with oral bacteria. Most of the time these oral bacteria live in harmony with the host however if the balance is disrupted they can cause infections. In addition, some of the bacteria such as respiratory tract pathogens are acquired by the oral cavity. They remain in the oral cavity as transient flora but they can cause infections as well as they can be transmitted to others. Therefore anything placed in the mouth including pacifiers can become contaminated with oral flora as well as pathogens. These bacteria will continue to colonize the pacifier until disinfected. While using a pacifier, a child is prone to dropping it on the floor. This results in contamination

with environmental bacteria. Parents or caregivers may suck on the pacifier in attempt to clean it, and place it back into a child's mouth. Sucking on a fallen pacifier may remove loose dirt and sand, but further contaminates the pacifier with additional oral bacteria. In daycare centers cross contamination between sick children can also occur through the use of pacifiers. A dirty pacifier grows a slimy biofilm which can become progressively more resistant to antibiotics and detergents. Bacterial sensitivity to antimicrobial agent has been shown to be much lower when they are constituents of a biofilm than when they are in aqueous suspension (Costerton et al 1999). 2.4.1 Types of pathogenic contaminants For the purpose of this study, the causative organism of sepsis, Staphylococcus aureus, the causative organism of oral thrush, Candida albicans and the causative organism of diarrhea Escherichia coli will be discussed. 2.4.1.1 Staphylococcus aureus Staphylococcus aureus, (S. aureus) is a Gram – positive, coccus – shaped bacteria. They are members of the Staphylococcaceae family. A typical characteristic of S. arueus on agar plate are that they are yellow to a golden in appearance. Some strains are often surrounded by a clear zone as it is hemolytic on blood agar. They can grow at a temperature range of 15 to 45 degrees. S. aureus occurs in clusters resembling grapes. They grow in clusters (Mims et al, 2004) because the cells divide successively in three perpendicular planes with the sister cells remaining attached to one another following each successive division (Todar, 2013). S. aureus is facultative anaerobes that grow by aerobic respiration or by fermentation that yields principally lactic acid and it is catalase- positive and mannitol-positive. A coagulase test determines pathogenic from non- pathogenic Staphylocci, which S. aureus would test positive. It is estimated that 20% of the human population carry S. aureus (Kluytmans et al, 1997) which can be found as part of the anterior nares of the nasal passages (Kluytmans et al, 1997; Cole et al 2001) and respiratory tract. It may occur as commensal, and under these circumstances does not always indicate infection and therefore does not require treatment. However

1it is a common cause of skin infections such as boils, pimples,

impetigo, cellulites, folliculitis, carbuncles, abscesses (Todar, 2013). It also may cause food poisoning (Brooks GF et al, 2001), and respiratory tract infections such as sinusitis. More life-threatening diseases are pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome and septicaemia (Mims et al, 2004). S. aureus is transmitted through air droplets and aerosol. The bacteria can be released in the air through saliva droplets when an infected person coughs or sneezes. These droplets of saliva contaminated with bacteria can infect other people. Another mode of transmission is through direct contact with objects including towel sheets and clothing that are infected with the bacteria. S. aureus is also still one of the five most common causes of nosocomial infections (infections contracted during hospital stay). Newborn infants are often colonized with Staphylococcus aureus originating from health care workers (Mernelius et al, 2013). It can therefore be said that any caregiver can transfer the bacteria to the infant. This could be directly or through handling of the infants' personal items which includes a pacifier. Since S. aureus is favored by warm moist conditions, the use of a pacifier maintains such an environment which therefore acts as a harvesting device. This is exacerbated if the skin around the mouth is broken by disease such as eczema. In infants S. aureus infection can also cause a severe disease known as Staphylococcal scalded skin syndrome (Bukowski et al, 2010). Drug resistance in these bacteria is a known problem therefore treatment is guided by sensitivity testing. Severe infections require prolonged intravenous therapy with β – lactamase – resistant antibiotic such as penicillin with clavulanic acid (augmentin). If resistance is not present then penicillin G is the treatment of choice (Brooks GF et al, 2001). Methicillin resistant S. aureus is a known problem as well. 2.4.1.2 Candida albicans Candida albicans (C. albicans) is a yeast, unicellular fungi that causes candidiasis or candidosis. C. albicans exists in two forms a trait known as dimorphism. The yeast form of the organism is believed to be relatively innocuous, but the hyphal form is usually associated with invasion of the host tissue. Candidiasis is the most common opportunistic oral fungal infection in the humans. This fungus is found in the normal flora of about 50% of healthy individuals (Bork et al, 1993). These individuals are carriers of the organisms in their mouths and may or may not present any clinical evidence of infection. Clinicians have recognized that oral candidiasis may develop in people who are immune compromised. The predisposing factors for the development of infection are the immune status of the host, the oral mucosal environment and the type of

C. albicans strains. Therefore Candidal infections are commonly seen in immunocompromised patients such as patients with HIV, cancer and organ transplant (Bagg et al, 1999; Bork et al 1993), and diabetes mellitus (Bagg et al, 1999). Individuals on broad spectrum antibiotics and with poor oral hygiene may also present with this infection. The clinical manifestations presents as white dots, patches or plaques also called thrush (Bagg et al, 1999). Thrush can also be found in the vaginal area or oral cavity. About 15% of newborns present with oral thrush which can be seen a week after delivery. Transmission occurs during delivery (Newman and Nisengard, 1988) as the pH of the vagina and the newborn mouth supports the growth of Candida (Bork et al, 1993). Thrush can also commonly be seen in infants that use bottle nipples and pacifiers (Bork et al, 1993). Often mothers attempt to clean a bottle nipple or pacifier that has fallen on the floor by sucking it which can transmit Candida from mother to child. Latex pacifiers have been found to be significantly colonized with Staphylococcus and Candida than pacifiers made of silicon (Sio et al, 1987; Comina et al, 2006). This is believed to be because latex pacifiers are softer and therefore can easily get micro - cracks. It's these micro – cracks that serve as a niche for organisms to colonize. C. albicans has a variety of clinical manifestations, making diagnosis difficult at times. Below are the common oral manifestations. Pseudomembranous candidiasis, (PC), presents with the common thrush like appearance. It is characterized by the presence of adherent white plaques that resemble cottage cheese or curdled milk on the oral mucosa. The white plaques composed of tangled masses of hyphes, yeasts, desquamated epithelial cells, and debris. Rubbing with dry gauze can remove the plaques. This form of candidial infection can sometimes be noted when an individual has been exposed to broad spectrum antibiotics, as it results in eliminating competing bacteria, or by impairment of the patient's immune system. The immune dysfunctions seen in leukemic patients or those infected with the human immunodeficiency virus are often associated with pseudomembranous candidiasis. Infants may also be affected because of their underdeveloped immune systems. Erythematous Candidasis, may or may not show white flecks or a white component as it is not a prominent feature. It is more common than PC. Several clinical presentations may be seen. Acute atrophic candidiasis or antibiotic sore mouth typically follows a course of broad- spectrum antibiotic. Patients may complain of a burning sensation in the mouth. A diffuse loss of the filiform papillae of the dorsal tongue, resulting in a redness appearance. Central papillary atrophy (median rhomboid glossitis), clinically appears a well demarcated erythematous zone that affects the midline, posterior dorsal tongue and often asymptomatic. The erythema is due to the loss of the filiform papille in the area. Angular cheilitis, can be seen in individuals with a loss of facial vertical height. This results in a skin fold that extends from the corners of the mouth. As saliva seeps out of the mouth it builds up in these folds. If these individuals are candidia carriers it may cause an reddish crushing appearance around these folds as the saliva accumulates in this area. In a similar fashion infants that use pacifiers may present with a reddish rash like appearance around their mouths where the pacifier shield sits. This occurs because some saliva seeps out the mouth and builds up behind the shield, which is in direct contact with the skin. Denture stomatitis, is seen in individual that wear dentures, who has poor denture hygiene. The denture bearing areas appear red and inflamed with white patches. The denture creates an environment conducive for candidial growth. The diagnosis of candidiasis is usually established by the clinical signs in conjunction with exfoliative cytologic examination. A specimen of culture is obtained by rubbing a sterile cotton swab over the lesion and then streaking the swab on the surface of a Sabouraud agar plate. C. albicans will grow as creamy, smooth surfaced colonies after 48 hours of incubation at room temperature. Candidal organism can be seen microscopically in either an exfoliative cytological preparation or in tissue sections obtained from biopsy specimen. On staining with the periodic acid - Schiff (PAS) method, the candidal hyphae and yeasts can be readily identified. This method stains carbohydrates, which is abundance in the fungal cell walls. This stain causes the organisms to appear bright-magenta in colour imparted by the stain. To make the diagnosis of candidiasis, one must be able to see hyphae or pseudohyphae which are essentially elongated yeast cells. Oral C. albicans can be treated with antifungal agents such as fluconazole and nystatin. If the use of broad spectrum antibiotic is the cause of infection, an interflora can be prescribed. Good oral hygiene is also important in aiding treatment. Therefore any objects placed in the mouth such as dentures, pacifiers and utensils should be thoroughly cleaned. 2.4.1.3 Escherichia coli In 1885, Theodor Escherich was the first to described Escherichia coli (E. coli), as Bacterium coli commune, which he isolated from the feces of newborns (Todar 2012; Nodqvist 2013). It was later renamed Escherichia coli. E. coli is the head of the large bacterial family,

Enterobacteriaceae. This enteric species is a gram- negative, facultative anaerobic, rod – shaped bacterium (Brooks GF et al, 2001; Gillespie & Bamford, 2007). A capsule or microcapsule is often present and a few strains produce profuse polysaccharide slime (Sussman 1997) E. coli is known to be a harmless commensal, part of the normal gut flora, as its natural habitat is the intestine of warm blooded animals and humans (Brooks GF et al, 2001). These bacteria can be beneficial within the intestine (Hudault et al, 2001; Reid et al 2001). It can also be found in the environment in water and soil. Some strains such as enterotoxigenic and enteropathogenic E. coli cause diarrhoea. In addition they also cause urinary tract infections, neonatal meningitis and septicemia (Gehlbach et al, 1973; Evans et al, 2007). The gastrointestinal tract of most warm – blooded animals and infants are colonized by E. coli within hours or a few days after birth. This is because the bacteria can adhere to the mucosa lining of the gut. Fecal – oral transmission is a route through which pathogenic strains can be contracted. The bacterium can also be ingested in food or water or obtained directly from other individuals handling the infant. Since E. coli is present in the environment in water and soil, toddlers can easily contract this pathogen. Studies have shown that pacifiers do become contaminated with fecal microorganisms and children who use pacifier contract diarrhoea more frequently as compared to the non-users (Tomasi et al, 1994). Once established, an E. coli strain may persist for months or years as a commensal. Some strains of E. coli that exist in the

2**human gut have beneficial functions,** for example **the production of vitamin** K12. **They also prevent** pathogenic **bacteria,**

from establishing themselves in the intestine. A rapid shift may occur after enteric (pathogenic E. coli) infection, or a shift in states of health, or due to antimicrobial therapy that disturbs the normal flora (Gillespie & Bamford, 2007). However, nonpathogenic E. coli have therapeutic use as a probiotic agent in medicine, mainly for the treatment of various gastroenterological diseases (Grozdanov et al, 2004) including inflammatory bowel disease (Kamada et al, 2005). In a laboratory environment, E. coli can be grown in media with glucose as the sole organic constituent. It can also grow on peptone or meat extract media or MacConkey agar. They can grow by aerobic or anaerobic respiration (Brooks GF et al, 2001). Under anaerobic conditions it will grow by means of fermentation, producing characteristic "mixed acids and gases as an end products (Brooks GF et al, 2001). Optimal growth of E. coli occurs at 37oC within 24 hours of incubation A healthy adult generally will recover fully within 5 to 7 days after been infected with pathogenic E. coli.

2**The individual may experience** sudden **abdominal pain/** cramps, **typically** as **the first symptom.**

A watery diarrhoea generally follows

2**a few hours after the sudden abdominal pain.**

2**A day later there may be bright red bloody stools, caused by sores in the intestines. Nausea vomiting**

and fever may also be present. Fatigue is common following diarrhoea as there is a

2**loss of fluids and electrolytes (dehydration), making the patient feel sick and tired.**

In

> 2young children, elderly individuals and patients with weakened immune systems can develop potentially fatal

complications and therefore may need medical attention.

> 2No current treatments can cure E. coli, it has to run its course. Most doctors advise patients to get plenty of rest and

fluids to prevent dehydration. 2.5 Decontamination of pacifiers There are many different types of disinfection or sterilization methods available in the market that can decontaminate pacifiers. Consumers select a type depending on disinfection or sterilization time, efficiency, costing and methodology. The simple method of submerging the pacifier in boiling water for 5 minutes and possibly adding some salt or bicarbonate soda to the water is used by many mothers. Boiling kills vegetative bacteria but not spores. However, this method is simple, cost effective and short. 2.5.1 Disinfectants 2.5.1.1 Sodium hypochlorite Submerging pacifiers in chemicals such as diluted chlorine based solutions for a period of time has been recommended and these solutions are commercially available. Sodium hypochlorite has been used widely in clinical care as a disinfecting agent and is known to be highly effective in cleaning surfaces contaminated with all types of micro- organisms (Rutala and Weber 1999; 1997). The mechanism of antibacterial activity of chlorine containing compounds such as sodium hypochlorite is not fully understood. The postulated mechanism by which its activity does occur is thought to be due to inhibition of key enzymatic reaction within the cell and protein denaturation (Rutala and Weber, 1997). Efficacy of sodium hypochlorite decreases the presence of light, heat, organic and inorganic containments and the shelf life is reduced with decreasing concentrations (Johnson and Remeikis 1993). Chlorine based solutions such as Milton has been marketed for disinfecting baby teats, bottles, pacifiers and utensils. Milton is readily available at local stores, which

> 1is made of an aqueous solution of 1.0% sodium hypochlorite and 16.5% sodium chloride. The

manufactures instructions are to dilute in a noncorrosive plastic container with a lid. Items are disinfected by soaking in this solution for 30 minutes. However, pre-cleaning of pacifiers is very important and therefore recommended. Thrush is a common problem in babies, and the manufactures of Milton claims that Milton kills C. albicans.

> 1After removing from the Milton solution, pacifiers can be drained and used immediately

without rinsing. The

> 1World Health Organisation (WHO) considers that the acceptable level of chlorine for drinking water is at 5 mg/l

> 1(0.5 % of the solution). Milton sterilizing solution is made up

of

> 10.012% of chlorine which is far less than the level accepted by WHO. This explains why it is not necessary to rinse, as there would be a risk of contaminating the baby' s accessories again by rinsing them.

2.5.1.2 Chlorhexidine Chlorhexidine is a broad spectrum disinfectant with well-established efficacy. It is not packaged or marketed as a pacifier disinfecting agent. However, studies on pacifiers and disinfectants have included chlorhexidine either as a positive control or recommended disinfection for pacifies (Chamele et al 201). Chlorhexidine, a cationic bis – biguanide is an antimicrobial agent which has been used as a broad spectrum antiseptic since 1953. It is used extensively in the fields of medicine and dentistry in mouth rinses, antiseptic soaps and surface disinfectants. Chlorhexidine has been used in the treatment of oral candidiasis since the 1970's. In studies on polygunides it was found that certain bisbiguanides had a very broad antimicrobial spectrum. By structural variation they arrived at the agent with the greatest bacteriostatic and bactericidal feature, 1,6 – bis – 4 – chloro – phenyldi – guanidonhexane, a synthetic cationic detergent usually referred to as chlorhexidine (Gjermo et al 1970, Brecx et al 1992). Rinsing the oral cavity with 0.12 % chlorhexidine reduces the number of bacteria in the saliva by up to 95 % in the course of few days. In a study by Grossman et al, (1989) showed that use of chlorhexidine can reduce gingivitis, gingival bleeding and dental plaque. Chlorhexidine is a water soluble compound and is dissociable, allowing it's positive charges to bind to teeth (Rolla et al, 1970), oral soft tissues (Bonesvoll et al, 1974) and to the negatively charged bacterial cell wall (Mohammadi et al, 2009) and extracellular complexes (Loe et al, 1970). The binding to bacteria interferes with membrane transports mechanism allowing leakage of low molecular weight substances and at high concentrations it penetrates the cell wall precipitating the cellular cytoplasm causing cell death (Greenstein et al, 1986; Gomes et al 2005). General and systemic side effects are rare. The most common side effect of chlorhexidine is the formation of an extrinsic, yellow – brown stain on teeth and tongue. Disturbance of taste sensation (mainly sweet and salt) and a burning sensation of the tongue may occur on using the mouth wash. Another side effect is the bitter taste after the use chlorhexidine. It is this bitter aftertaste that is left on the pacifier which may result in an infant not accept the pacifier. 2.5.2 Microwave sterilization Microwave sterilizers are also available which operates on a heating principle. Silva et al (2013) studied the efficacy of microwave in the infection of dentures and found that it can eliminate C. albicans and Streptococcus mutans (S. mutans)A study by Chamele et al, (2012) showed that 7 minutes of irradiation can kill S. mutans present on pacifiers and it was comparable to chlorhexidine disinfection. When comparing the mode of action of a microwave steamer and an electrical steamer they seem similar. Both require about 200 ml of water to create steam. Heat, creating the steam is responsible for the sterilization in both techniques. The microwave method requires a sealable container (specifically designed as a microwave steamer) and a microwave, water and electricity. The electric steamer method requires the electric steamer device, water and electricity. Not much is known about the efficacy of these sterilizers. 2.6 Aim: This study evaluated various disinfection techniques for the disinfection of pacifiers. 2.6.1 Objectives: ? To determine the efficacy of varies disinfection techniques in the disinfection of pacifiers. ? To compare the efficacy of varies disinfection techniques in the disinfection of latex and silicon pacifiers. 3 Methods 3.1 Pacifiers A hundred and eight pacifiers were purchased from a local supermarket. They were 54 silicone and 54 latex pacifiers of the snookums brand. The pacifiers were BPA free and treated as per the manufacturer's instruction. This involved boiling each pacifier for 5 minutes. A glass beaker with tap water was placed on a hot plate and the water was brought to boiling point (100oC). At boiling point pacifiers were submerged in the boiling water for 5 minutes. A sterile artery forceps was used to hold the pacifiers down. The latex and silicon pacifiers were boiled separately. After boiling, the pacifiers were aseptically removed and stored separately in a sterile glass jar ready for the experiments. 3.2 Cultures and inocula Cultures of S. aureus ATCC 29213, C. albicans ATCC 90028 and E. coli ATCC 35218 were obtained from the Oral Microbiology laboratory. These are laboratory stock cultures that are purchased and not isolated

from patients therefore full ethical clearance was not necessary. However, a waiver was obtained from the Human Research Ethics committee of the University of the Witwatersrand (Ethics number: W-CJ-130626-1). S. aureus and E. coli were grown on blood agar plates at 37oC for 24 hours whereas C. albicans was grown on Sabouraud Dextrose agar plates and incubated at 37oC for 48 hours. For each experiment fresh subcultures were prepared. To prepare inoculum, cultures were emulsified in 150 ml sterile distilled water and the turbidity was adjusted to 3 MacFarlane standards to obtain 107 cells per ml of suspension. For each experiment fresh inoculum was prepared. 3.3 Disinfectants The disinfecting methods used included rinsing pacifiers under tap water for 2 minutes, submerging pacifiers in boiling water for 5 minutes, submerging pacifiers in Milton (chlorinated compound) for 30 minutes, placing pacifiers under microwave radiation for 8 minutes and submerging pacifiers into chlorhexidine gluconate (2.0%) for 2 minutes. Milton (Figure 3.1) solution was purchased from a local supermarket and for each experiment fresh solutions were prepared according to the package insert as recommended. This included diluting 12.5 ml of Milton with litre of water. Figure 3.1: Milton For the microwave technique, a special sterilizing microwavable unit (Figure 3.2) was purchased and used according to the manufacturer's instruction. This involved placing 200 ml water in the base, then placing a pacifier on a rack provided with the sterilizing unit, closing the unit and placing into the microwave for 8 minutes at 900 WATTS. By placing the pacifier on the rack, the pacifier did not come in contact with the water, which boils with radiation. However, heat generated steam, sterilizes the pacifiers. Chlorhexidine gluconate was purchased from the local pharmacy (Corsodyl). Figure 3.2: Microwave sterilization unit Cover Base Rack Open unit Closed unit 24 Figure 3.3 Test procedures for the artificial contamination and decontamination of pacifiers 1,2,3,4,5,6 Inoculum Serial dilution technique 1 2 3 B 4 6 C T T B M M 5 MW M 1 W CHX CHX Water Tap water 5 Milton Microwave Chlorhexidine (control) 0 Boiling 2 min m 5 min 5 min l centrifuge centrifuge centrifuge centrifuge centrifuge Conc Conc Conc Conc Conc Conc 1:10 1:10 1:10 1:10 1:10 1:10 1:100 1:100 1:100 1:100 1:100 1:100 1:1000 1:1000 1:1000 1:1000 1:1000 1:1000 Pacifiers: Latex and Silicon Test organisms: S. aureus, C. albicans, E. coli Repeats: 3 times 150 ml sterile distilled water 3.4 Test procedure The test procedures were followed as described by Rweyendela et al (2009) with modifications. The flow of the procedure is described in Figure 3.3. Six latex pacifiers were artificially contaminated by placing them separately in the inoculums for 2 minutes each and holding them down with a glass rod and removed them from the inoculum aseptically. The first pacifier was placed in 150 ml of sterile distilled water to obtain control count (number of challenged organisms). This beaker was labelled as control (Beaker C). The second contaminated pacifier was placed into 150 ml of normal tap water for 2 minutes. This pacifier was held by a sterile artery forceps and agitated by moving it in an up and downward movement to stimulate a rinsing effect. After 2 minutes the pacifier was aseptically removed and placed into 150 ml of sterile distilled water to obtain the remaining number of bacteria that were not removed by rinsing with tap water. This beaker was labelled tap water (Beaker T). The third contaminated pacifier was submerged into 150 ml boiling water for 5 minutes. The pacifier was placed in only once the water reached boiling point (100oC) and was held down by a sterile artery forceps. After 5 minutes the pacifier was removed and placed into a beaker of 150 ml of sterile distilled water marked boiling (Beaker B). The forth contaminated pacifier was placed into 150 ml of a prepared chlorinated compound solution (Milton) for 30 minutes and held down by a sterile glass rod (Figure 3.4). The metal artery forceps was never used in this case because of the corrosive property of chlorinated compound. The pacifier was removed and placed into a labelled beaker (Beaker M) containing 150 ml of sterile distilled water. The fifth contaminated pacifier was removed from the inoculum after 2 minutes and was placed into a microwavable sterilization unit. This unit is specifically designed to sterilise feeding bottles, teats and pacifiers. 200 ml tap water was placed into the unit as instructed by the manufacture. The pacifier was placed on the designed rack. The container was closed and placed in the microwave for 8 minutes at 900 watts. The container was removed from the microwave and was allowed to stand for 2 minutes as instructed. This was to avoid any stream burns. After 2 minutes the container was opened and the pacifier was aseptically removed and placed into a beaker containing 150 ml sterile distilled water. This beaker was labelled microwave (Beaker MW). Finally, the sixth pacifier was removed from the inoculum after 2 minutes and submerged into 150 ml of a chlorhexidine gluconate (CHX) solution for 2 minutes. A sterile glass rod was used to keep the pacifier submerged. This pacifier was then removed and placed into a beaker containing 150 ml of sterile distilled water. The beaker was labelled CHX (Beaker CHX). This disinfection test procedure was repeated three

times for each organism (E. coli, S. aureus and C. albicans) using the latex and silicon pacifiers. Figure 3.4: Pacifier held down by a glass rod in a beaker. 3.4.1 Bacterial and colony counts After sterilization, a sterile magnetic stirrer was placed into the beakers as described in section 3.4 containing the pacifiers (beaker C, T, B, M, MW and CHX). The beakers were placed on a magnetic stirrer plate (Figure 3.5) and stirred for approximately 2 minutes. This stirring motion facilitated in removing the microorganisms from the pacifiers and dispersing them into the water. Water from all the beakers was centrifuged at 5000 rpm for 15 minutes using conical tubes. The supernatant was removed and the sediment was suspended in 1 ml phosphate buffered saline (PBS). Serial dilution technique was performed to obtain counts of microorganisms. Briefly, 0.1 ml of solution was diluted in 0.9 ml PBS to obtain 1:10 dilution. Similarly 1:10 dilution was further diluted to 1:100 and 1:1000 dilutions. One hundred microliters of each dilution was transferred onto appropriate agar plates, spread and incubated appropriately. E. coli and S. aureus was cultured using blood agar plates which were incubated aerobically at 37oC for 24 hours. Whereas, C. albicans was cultured using Sabouraud dextrose agar plates which were incubated aerobically at 37oC for 48 hours. Colonies were counted and the colony forming units (cfu) were obtained by multiplying the colony counts with the dilution factors and taking the average count of the dilution plates. Organism counts were expressed as cfu per pacifier. Control count represented the level of artificial contamination. Bacterial counts in the tests represented the live bacteria due to the inadequate sterilization procedure. The percentage of bacteria/fungi killed was calculated using the formula: control count - test count/control count X100. Log reduction was also calculated. Figure 3.5: Magnetic stirrer plate Magnetic stirrer 3.5 Statistical analysis Magnetic stirrer plate Descriptive analysis was performed to express the results. Percentage-kill of disinfectants were compared using Kruskal Wallis nonparametric rank tests between the types of pacifiers, types of test organisms and the disinfection procedure. 4 Results The results are expressed as % kill as well as the log reduction in the microbial counts. Percentage kill are more comparable whereas log reduction is a true reflection of the actual reduction in the microbial counts. For example 2 log reduction of 1000000 number of challenged organisms shows that 10000 organisms are still surviving which will calculate to 99% kill. However, 2 log reduction of 100 number of challenged organisms will calculate to 100% kill. Therefore for the % kill results, number of challenged organisms should be given otherwise the results are meaningless. Whereas, the log reduction results, provide complete information, even if the number of challenged organisms are not provided. 4.1 Disinfection of latex pacifiers artificially contaminated with Staphylococcus aureus Latex pacifiers contaminated with S. aureus were disinfected using 5 different procedures and the results are shown in Table 4.1and Table 4.1a. The results showed that placing pacifiers in boiling water for 5 minutes or in Milton for 30 minutes or in a sterilization unit in the microwave for 8 minutes or submerging them in chlorhexidine for 2 minutes all were effective in eradicating S. aureus from latex pacifiers (% kill = 100 or log reduction of ± 6.56). However, when the pacifiers were rinsed in tap water for 2 minutes, a complete removal was not obtained (mean % kill = 99.34 or log reduction of 2.35). Table 4.1: Disinfection of latex pacifiers artificially contaminated with Staphylococcus aureus using various disinfection techniques (% kill). Disinfection Repeats No. of challenged org. (cfu/ pacifier) A No. of surviving org. (cfu/pacifier) B No. org. killed (cfu/ pacifier) a-b % kill a-b a X100 Tap water 1 2745000 6800 2738200 99.75 2 2940000 7009 2932991 99.76 3 5700000 85470 5614530 98.50 Mean±SD 99.34 ± 0.73 Boiling 1 2745000 0 2745000 100 2 2940000 0 2940000 100 3 5700000 0 5700000 100 Mean±SD 100 ± 0 Milton 1 2745000 0 2745000 100 2 2940000 0 2940000 100 3 5700000 0 5700000 100 Mean±SD 100 ± 0 Microwave 1 2745000 0 2745000 100 2 2940000 0 2940000 100 3 5700000 0 5700000 100 Mean±SD 100 ± 0 Chlorhexidine 1 2 2745000 2940000 0 0 2745000 2940000 100 100 3 5700000 0 5700000 100 Mean±SD 100 ± 0 Inoculum: Mean ±SD were 3795000 Table 4.1a: Disinfection of latex pacifiers artificially contaminated with Staphylococcus aureus using various disinfection techniques (log reduction) Disinfection Repeats No. of challenged org. (log cfu/ pacifier) A No. of surviving org. (log cfu/pacifier) B Log reduction a-b Tap water 1 6.44 3.83 2.61 2 6.47 3.85 2.62 3 6.76 4.93 1.83 Mean±SD 2.35 Boiling 1 6.44 0 6.44 2 6.47 0 6.47 3 6.76 0 6.76 Mean ±SD 6.56 Milton 1 6.44 0 6.44 2 6.47 0 6.47 3 6.76 0 6.76 Mean±SD 6.56 Microwave 1 6.44 0 6.44 2 6.47 0 6.47 3 6.76 0 6.76 Mean±SD 6.56 Chlorhexidine 1 6.44 0 6.44 2 6.47 0 6.47 3 6.76 0 6.76 Mean±SD 6.56 Inoculum: Mean±SD were 6.56 4.2 Disinfection of latex pacifiers artificially contaminated with Escherichia coli Latex pacifiers contaminated with E. coli were disinfected using 5 different procedures and the results are shown in Table 4.2 and Table 4.2a. The results showed that placing pacifiers in boiling

water for 5 minutes or placing in a sterilization unit in the microwave for 8 minutes were effective in eradicating E. coli from latex pacifiers (% kill = 100 or log reduction of 7.54). However, when the pacifiers were rinsed in tap water for 2 minutes, placed in Milton for 30 minutes or submerged in chlorhexidine for 2 minutes a complete removal was not obtained (mean % kill = 99.87, 99.99 and 99.99 respectively). Table 4.2: Disinfection of latex pacifiers artificially contaminated with Escherichia coli using various disinfection techniques (% kill). Disinfection Repeats No. of challenged org. (cfu/ pacifier) A No. of surviving org. (cfu/pacifier) B No. org. killed (cfu/ pacifier) a-b % killed a-b a X100 Tap water 1 30150000 46400 30103600 99.85 2 36150000 28560 36121440 99.92 3 37150000 55650 37094350 99.85 Mean±SD 99.87 ± 0.04 Boiling 1 30150000 0 30150000 100 2 36150000 0 36150000 100 3 37150000 0 37150000 100 Mean±SD 100 ± 0 Milton 1 30150000 0 30150000 100 2 36150000 64 36149936 99.99 3 37150000 0 37150000 100 Mean±SD 99.99 ± 0.01 Microwave 1 30150000 0 30150000 100 2 36150000 0 36150000 100 3 37150000 0 37150000 100 Mean±SD 100 ± 0 Chlorhexidine 1 30150000 0 30150000 100 2 36150000 0 36150000 100 3 37150000 1500 37148500 99.99 Mean±SD 99.99 ± 0.01 Inoculum: Mean±SD were 34483333 Table 4.2a: Disinfection of latex pacifiers artificially contaminated with Escherichia coli using various disinfection techniques (log reduction) Disinfection Repeats No. of challenged org. (log cfu/ pacifier) a No. of surviving org. (log cfu/pacifier) b Log reduction a-b Tap water 1 7.48 4.67 2.81 2 7.56 4.46 3.10 3 7.57 4.76 2.81 Mean±SD 2.91 Boiling 1 7.48 0 7.48 2 7.56 0 7.56 3 7.57 0 7.57 Mean±SD 7.54 Milton 1 7.48 0 7.48 2 7.56 1.81 5.75 3 7.57 0 7.57 Mean±SD 6.93 Microwave 1 7.48 0 7.48 2 7.56 0 7.56 Mean±SD 3 7.57 0 7.57 7.54 Chlorhexidine 1 7.48 0 7.48 2 7.56 0 7.56 3 7.57 3.18 4.39 Mean±SD 6.48 Inoculum: Mean±SD were 7.54 4.3 Disinfection of latex pacifiers artificially contaminated with Candida albicans Table 4.3 shows that tap water and Milton were not effective in disinfecting latex pacifiers contaminated with C. albicans (% kill – 99.63 and 99.96 and 2.75 and 3.57 log reduction respectively). However, heat based disinfections such as boiling and microwave radiation as well as chlorhexidine were effective in eliminating C. albicans completely. Table 4.3: Disinfection of latex pacifiers artificially contaminated with Candida albicans using various disinfection techniques (% kill). Disinfection Repeats No. of challenged org. (cfu/ pacifier) A No. of surviving org. (cfu/pacifier) B No. org. killed (cfu/ pacifier) a-b % killed a-b a X100 Tap water 1 414000 580 413419 99.86 2 155400 1434 153965 99.08 3 266400 116 266284 99.96 Mean±SD 99.63 ± 0.48 Boiling 1 414000 0 414000 100 2 155400 0 155400 100 3 266400 0 266400 100 Mean±SD 100 ± 0 Milton 1 414000 72 413928 99.98 2 155400 102 155298 99.93 3 266400 46 266354 99.98 Mean±SD 99.96 ± 0.03 Microwave 1 414000 0 414000 100 2 155400 0 155400 100 3 266400 0 266400 100 Mean±SD 100 ± 0 Chlorhexidine 1 414000 0 414000 100 2 155400 0 155400 100 3 266400 0 266400 100 Mean±SD 100 ± 0 Inoculum: Mean±SD were 278600 Table 4.3a: Disinfection of latex pacifiers artificially contaminated with Candida albicans using various disinfection techniques (log reduction) Disinfection Repeats No. of challenged org. (log cfu/ pacifier) A No. of surviving org. (log cfu/pacifier) b Log reduction a-b Tap water 1 2 5.62 5.19 2.76 3.16 2.85 2.03 3 5.43 2.06 3.36 Mean±SD 2.75 Boiling 1 5.62 0 5.62 2 5.19 0 5.19 3 5.43 0 5.43 Mean±SD 5.41 Milton 1 5.62 1.86 3.76 2 5.19 2.01 3.18 3 5.43 1.66 3.77 Mean±SD 3.57 Microwave 1 5.62 0 5.62 2 5.19 0 5.19 3 5.43 0 5.43 Mean±SD Chlorhexidine 1 5.62 0 5.41 5.62 2 5.19 0 5.19 3 5.43 0 5.43 Mean ±SD 5.41 Inoculum: Mean±SD were 5.41 4.4 Disinfection of silicon pacifiers artificially contaminated with Staphylococcus aureus The results are described in Table 4.4 and Table 4.4a. Boiling, Milton, microwave and chlorhexidine were effective in eliminating S. aureus from artificially contaminated silicon pacifiers. However tap water rinsing was ineffective giving % kill results of 99.80 or log reduction of only 2.8. Table 4.4: Disinfection of silicon pacifiers artificially contaminated with Staphylococcus aureus using various disinfection techniques Disinfection Repeats No. of challenged org. (cfu/ pacifier) A No. of surviving org. (cfu/pacifier) b No. org. killed (cfu/ pacifier) a-b % killed a-b a X100 Tap water 1 2 32550000 80100000 34960 57280 32515040 80042720 99.98 99.93 3 21150000 107100 21042900 99.49 Mean±SD 99.80 ± 0.27 Boiling 1 32550000 0 32550000 100 2 80100000 0 80100000 100 Mean±SD 3 21150000 0 21150000 100 100 ± 0 Milton 1 32550000 0 32550000 100 2 80100000 0 80100000 100 3 21150000 0 21150000 100 Mean±SD 100 ± 0 Microwave 1 32550000 0 32550000 100 2 80100000 0 80100000 100 3 21150000 0 21150000 100 Mean±SD 100 ± 0 Chlorhexidine 1 32550000 0 32550000 100 2 80100000 0 80100000 100 3 21150000 0 21150000 100 Mean±SD 100 ± 0 Inoculum: Mean±SD were 44600000 Table 4.4a: Disinfection of silicon pacifiers artificially contaminated with Staphylococcus aureus using various disinfection techniques (log reduction) Disinfection Repeats No. of challenged org. (log cfu/

pacifier) a No. of surviving org. (log cfu/pacifier) b Log reduction a-b Tap water 1 7.51 4.54 2.97 2 7.90 4.76 3.14 3 7.33 5.03 2.3 Mean±SD 2.8 Boiling 1 7.51 0 7.51 2 7.90 0 7.90 3 7.33 0 7.33 Mean±SD 7.58 Milton 1 7.51 0 7.51 2 7.90 0 7.90 3 7.33 0 7.33 Mean±SD 7.58 Microwave 1 7.51 0 7.51 2 7.90 0 7.90 3 7.33 0 7.33 Mean±SD 7.58 Chlorhexidine 1 7.51 0 7.51 2 7.90 0 7.90 3 7.33 0 7.33 Mean±SD 7.58 Inoculum: Mean±SD were 7.58 4.5 Disinfection of silicon pacifiers artificially contaminated with Escherichia coli Table 4.5 and Table 4.5a shows that E. coli contaminated silicon pacifiers were difficult to decontaminate because the % kill for tap water, Milton and chlorhexidine were 98.66, 99.99 and 99.99 (log reduction of 2.28, 7.31 and 6.57) respectively. However, boiling and microwave radiation was once again effective in achieving a complete kill (100%). Table 4.5: Disinfection of silicon pacifiers artificially contaminated with Escherichia coli using various disinfection techniques Disinfection Repeats No. of challenged org. (cfu/ pacifier) A No. of surviving org. (cfu/pacifier) b No. org. killed by (cfu/ pacifier) a-b % killed a-b a X100 Tap water 1 109200000 150480 109049520 99.86 2 48450000 146850 48303150 99.70 3 70650000 2521600 68128400 96.43 Mean±SD 98.66 ± 1.94 Boiling 1 109200000 0 109200000 100 2 48450000 0 48450000 100 Mean±SD 3 70650000 0 70650000 100 100 ± 0 Milton 1 109200000 0 109200000 100 2 48450000 0 48450000 100 Mean±SD 3 70650000 44 70649956 99.99 99.99 ± 0.01 Microwave 1 109200000 0 109200000 100 2 48450000 0 48450000 100 3 70650000 0 70650000 100 Mean±SD Chlorhexidine 1 109200000 0 109200000 100 ± 0 100 2 48450000 0 48450000 100 3 70650000 7500 70642500 99.99 Mean±SD 99.99 ± 0.01 Inoculum: Mean±SD were 76100000 Table 4.5a: Disinfection of silicon pacifiers artificially contaminated with Escherichia coli using various disinfection techniques (log reduction) Disinfection Tap water Repeats 1 No. of challenged org. (log cfu/ pacifier) A 8.04 No. of surviving org. (log cfu/pacifier) b 5.18 Log reduction a-b 2.86 2 7.69 5.17 2.52 3 7.85 6.40 1.45 Mean±SD 2.28 Boiling 1 8.04 0 8.04 2 7.69 0 7.69 3 7.85 0 7.85 Mean±SD 7.86 Milton 1 2 8.04 7.69 0 0 8.04 7.69 3 7.85 1.64 6.21 Mean±SD 7.31 Microwave 1 8.04 0 8.04 2 7.69 0 7.69 Mean±SD 3 7.85 0 7.85 7.86 Chlorhexidine 1 8.04 0 8.04 2 7.69 0 7.69 3 7.85 3.88 3.97 Mean±SD 6.57 Inoculum: Mean±SD were 7.86 4.6 Disinfection of silicon pacifiers artificially contaminated with Candida albicans The results in Table 4.6 and Table 4.6a shows that boiling, microwave radiation and chlorhexidine were successful in eliminating C. albicans from silicon pacifiers. Tap water and Milton were ineffective in eliminating C. albicans from silicon pacifiers (% kill = 99.96 and 99.94 or log reduction of 2.75 and 4.43 respectively). Table 4.6: Disinfection of silicon pacifiers artificially contaminated with Candida albicans using various disinfection techniques Disinfection Repeats No. of challenged org. (cfu/ pacifier) A No. of surviving org. (cfu/pacifier) b No. org. killed (cfu/ pacifier) a-b % killed a-b a X100 Tap water 1 485500 2618 482882 99.46 2 1597500 4949 1592551 99.69 3 1402500 483 1402017 99.97 Mean±SD 99.96 ± 0.03 Boiling 1 485500 0 485500 100 2 1597500 0 1597500 100 3 1402500 0 1402500 100 Mean±SD 100 ± 0 Milton 1 485500 897 484603 99.82 2 1597500 0 1597500 100 3 1402500 63 1402437 99.99 Mean±SD 99.94 ± 0.1 Microwave 1 2 485500 1597500 0 0 485500 1597500 100 100 3 1402500 0 1402500 100 Mean±SD 100 ± 0 Chlorhexidine 1 485500 0 485500 100 2 1597500 0 1597500 100 3 1402500 0 1402500 100 Mean±SD 100 ± 0 Inoculum: Mean±SD were 1161833 Table 4.6a: Disinfection of silicon pacifiers artificially contaminated with Candida albicans using various disinfection techniques (log reduction) Disinfection Repeats No. of challenged org. (log cfu/ pacifier) A No. of surviving org. (log cfu/pacifier) b Log reduction a-b Tap water 1 5.69 3.42 2.27 2 6.20 3.69 2.51 3 6.15 2.68 3.46 Mean±SD 2.75 Boiling 1 5.69 0 5.69 2 6.20 0 6.20 3 6.15 0 6.15 Mean±SD 6.01 Milton 1 5.69 2.95 2.74 2 6.20 0 6.20 3 6.15 1.80 4.35 Mean±SD 4.43 Microwave 1 5.69 0 5.69 2 6.20 0 6.20 3 6.15 0 6.15 Mean±SD 6.01 Chlorhexidine 1 5.69 0 5.69 2 6.20 0 6.20 3 6.15 0 6.15 Mean±SD 6.01 Inoculum: Mean±SD were 6.01 4.7 Summary results of disinfection of artificially contaminated pacifiers Table 4.7 and Table 4.7a shows the summary of results obtained in this study. Tap water consistently proved to be ineffective in disinfecting pacifiers. When the results of all the test organisms are combined, purely from the observation, boiling and microwave radiation proved to be the most effective methods. Milton was effective only 33.33% (1/3 organism) of the time whereas chlorhexidine showed better efficacy (66.66% of time – 2/3 organisms) than Milton. Table 4.7: Summary results of disinfection of artificially contaminated pacifiers (% kill) Test organism Type of pacifier No. of challenged organisms (Mean cfu/pacifier) Tap water Boiling water Mean ± SD % killed (n = 3) Milton Microwave Chlorhexidine (CHX) S. aureus latex 3795000 99.34 ± 0.73 100 ± 0 100 ± 0 100 ± 0 100 ± 0 silicon 44600000 99.80 ± 0.27 100 ± 0 100 ± 0 100 ± 0 100 ± 0 E. coli latex 34483333 99.87 ± 0.04 100 ± 0 99.99 ± 0.01 100 ± 0 99.99 ± 0.01 silicon 76100000 98.66 ± 1.94 100 ± 0

99.99 ± 0.01 100 ± 0 99.99 ± 0.01 C. albicans latex 278600 99.63 ± 0.48 100 ± 0 99.96 ± 0.03 100 ± 0 100 ± 0 silicon 1161833 99.96 ± 0.03 100 ± 0 99.94 ± 0.1 100 ± 0 100 ± 0 Table 4.7a: Summary results of disinfection of artificially contaminated pacifiers (log reduction). Test organism Type of pacifier No. of challenged organisms (Mean log cfu/pacifier) Tap water Boiling water Mean ± log reduction Microwave Milton (n = 3) Chlorhexidine (CHX) S. aureus Latex 6.56 2.35 6.56 6.56 6.56 6.56 Silicon 7.58 2.8 7.58 7.58 7.58 7.58 E. coli Latex 7.54 2.91 7.54 6.93 7.54 6.48 Silicon 7.86 2.28 7.86 7.31 7.86 6.57 C. ablicans Latex 5.41 2.75 5.41 3.57 5.41 5.41 Silicon 6.01 2.75 6.01 4.43 6.01 6.01 4.8 Comparison of disinfectants and statistical analysis Percentage-kill of disinfectants were compared using Kruskal Wallis nonparametric rank tests between the types of pacifiers, types of test organisms and the disinfection procedure. When the disinfection of latex and silicon pacifiers were compared, the results showed that there was no significant difference in the % kill with a p value of 0.85 (Figure 4.1). This suggests that the type of pacifier has no effect on the disinfection method. Figure 4.1 Comparison of different methods in the disinfection of latex and silicon pacifiers Comparison of latex and silicon (n=9) P=0.85 When the disinfection methods in decontamination of pacifiers contaminated with S. aureus, E. coli and C. albicans were compared (Figure 4.2), the results showed that there was no significant difference (p=0.46). This suggest that there was no difference in the results of test organisms. Figure 4.2 Comparison of different methods in the disinfection of pacifiers contaminated with S. aureus (SA), E. coli (EC) and C. albicans (CA) P=0.46 Comparison of organisms (n=6) Results in Figure 4.1 and 4.2 suggests that types of pacifiers and the test organisms behaved similarly and there was no significant difference in the results of disinfection. Therfore, all the results were pooled together to compare the methods of disinfection. When all the results for each of the disinfection methods were pooled together (latex – SA x3, EC x3,CA x3 and silicon – SA x3, EC x3,CA x3) and compared to one another (Figure 4.3), tap water significantly differed from the rest of the methods (P=0.01 and <0.01). However, there was no significant difference in the performance of the disinfection methods such as boiling, Milton, microwave and chlorhexidine. Figure 4.3 Comparison of different methods in the disinfection of pacifiers. 5 Discussion Pacifiers are non-nutritive objects designed for infants around 2 weeks of age to 5 years. They are used to pacify infants when they are crying due to discomfort or feeling insecure. In addition, they are used to increase oromotor coordination and muscular strength in feeding abilities, and to wean them off the breast feeding (Sexton and Natale, 2009; Poore et al, 2008). When it is placed in the oral cavity, the pacifier is in direct contact with the oral saliva and its oral micro flora. These microorganisms adhere and colonise the surfaces of pacifier (Brook and Gober, 1997, Mattos–Graner et al, 2001; Comina et al, 2006) While studies related to pacifier contamination can be found in the literature not much is published on the available cleaning methods and their effectiveness. Pacifiers are made of silicon or latex which shape resembles that of a lactating mother's nipple. Due to their difference in texture, surface structure and durability the colonization with bacteria may differ. Therefore this study investigated the efficacy of home disinfection procedures including commercially available chemicals in the disinfection of latex and silicon pacifiers. 5.1 Disinfection of latex and silicon pacifiers When the % kill results were compared between the two types of pacifiers, no statistical difference was found (Figure 4.1, Table 4.7). For the latex and silicon pacifiers boiling and microwave radiation were adequate. Similarly disinfection with water rinse, Milton and chlorhexidine was inadequate. Although the latex pacifiers are known to get worn out easily and harbour biofilms of microorganisms compared to the silicone pacifiers (da Siliveira et al, 2009; Comina et al, 2006), the test disinfection procedures provided similar results. The second important aspect to consider is the formation of bacterial biofilm on the pacifiers. A biofilm formation is much more easily established on rough surface therefore more commonly founded on old used latex pacifiers. Mature biofilms containing bacteria and slime layer have been found on old used latex pacifiers, whereas silicone pacifiers had a much lower level of slime contaminated with S. aureus and C. albicans (Comina et al, 2006). Biofilms are difficult to remove and a mature biofilm is more resistant to disinfection procedures. This suggests that the test disinfection procedures may not be as efficient in the disinfection of old used pacifiers. Therefore pacifiers should be disinfected regularly from the time they are new to prevent the establishment of a biofilm. This was seen in Sio et al, 1987, were the presents of C. albicans were investigated on used pacifiers. They found the use of silicon pacifiers was associated with a lower rate of C. albicans when compared to latex pacifiers. Also over time, used pacifiers develop a biofilm. This biofilm formation acts as a protective layer for the bacteria in a structural complex and therefore become progressively more resistant to cleaning methods. It is

known that the presence of a biofilm impairs antimicrobial agents penetrating the full depth of the biofilm, thereby retarding diffusion. However, these test pacifiers were new and they did not have micro cracks and irregular surfaces that can shelter bacteria from the disinfection which explains the similar results. The results also showed that rinsing with tape water certainly will not allow complete removal of bacteria but solution and heat based disinfection would be effective. The results also showed that heat based methods such as boiling and microwave are the most effective methods of disinfection compared to the solutions containing antimicrobial compounds (Milton and chlorhexidine) which will be discussed in the disinfection section. 5.2 Disinfection of pacifiers contaminated with different types of bacteria When the % kill results were compared between the contaminants, no significant difference was found which suggest that the methods were either adequate or inadequate for all three test organisms (Figure 4.2, Table 4.7). However, close observation of the results showed that S. aureus was the easiest to kill because most methods were adequate in killing these bacteria. E. coli was the most difficult organism showing resilience to Milton and chlorhexidine. Boiling and Microwave (steam based) proved to be the best disinfection procedure for all the test contaminants. Clearly, tap water rinsing eliminated the least amount of bacteria from the pacifiers irrespective of the type of pacifier and bacteria containment. Resilience of E. coli to the disinfection procedures is of concern because they are often associated with diarrhoea (86% of infants) which is common in pacifier users (Mathur et al, 1990). Festini et al, (2012) also showed that 62% of infants who used pacifier had at least one episode of diarrhoea in their first year of life. If the disinfection procedures are not adequate, these faecal contaminant can be transmitted from one infant to the other particularly in the day care centres. Although widely used disinfectant chlorhexidine which was included as a positive control reduced 6 to 7 logs of E. coli (Table 4.7a), it failed to completely kill them. This is also of concern with regards to hand washing antiseptic soaps which generally contains this compound. C. albicans is known to colonise pacifiers (Comina et al, 2006; da Silviera et al, 2009) which explains the high Candida carriage and potential reservoir for the risk of infection in the infants who use pacifiers (Darwazeh et al, 1995). Although Milton reduced 99.94-99.96% of C. albicans, the log reduction was only 3.57 to 4.43 (Table 4.7a) meaning that the pacifier becomes contaminated with high number of C. albicans, there will be considerable number of organisms present even after the use of this disinfectant. 5.3 Comparison of disinfection procedures When the efficacy of the disinfection methods were compared (Figure 4.3, Table 4.7), the results showed that boiling, Milton, microwave radiation and chlorhexidine were significantly better than tap water rinse ($p=<0.01$). For rinsing, the pacifier was agitated in an upwards and downwards motion for 2 minutes in tap water. This was to mimic a rinsing action. Not much has been done to investigate the effectiveness of rinsing with tap water to disinfect pacifiers or to clean surfaces. However rinsing is a cheap and easy cleaning method that is possibly carried out by many individuals from a lower socioeconomic background. Rinsing does reduce the number of bacteria (lowest reduction – log 2.28 of E. coli from silicone pacifier). However, water has no antimicrobial action and rinsing with tap water is purely an operator dependent mechanical action. Furthermore, rinsing with water will remove the planktonic form of bacteria and may fail to remove biofilm of bacteria. Similar results were reported by Camele et al (2012) with toothbrushes and pacifier disinfection although the test organisms were cariogenic bacteria S. mutans. They reported that rinsing with water was less effective than chlorhexidine and microwave radiation. Placing a pacifier in boiling water for 5 minutes is also an easy and cheap alternative and the results showed that it was effective regardless of the type of pacifier and the contaminants. It is a simple, easy, practical, and economical method and therefore used in many developing countries to sterilize metal instruments. For example, in Khartoum, Sudan 2% of dentists and in Lagos, Nigeria 19.3% of dentists used boiling as a method of sterilization (Elkarim et al, 2004, Sofola and Savage, 2003). A recent survey in India showed that up to 70.2% of dentists use boiling as a sterilization medium (Singh et al, 2012). Simple boiling for 30 minutes is known to kill all vegetative form of bacteria but it will only kill some spores. However, the aim of disinfection of pacifier is to kill all the pathogens and it is not necessary to have a sterile pacifier. In food industries submerging oranges in boiling water for 30 seconds before squeezing juice for bottling have been suggested to reduce the number of faecal contaminants such as E. coli (Bagci and Temiz, 2011). In our study, not only E. coli but other pathogens such as S. aureus and C. albicans were completely eliminated after 5 minutes. Therefore boiling can be used as an effective method of disinfection. The third method carried out in this study was with Milton. Milton's composition is 1.0 %

1sodium hypochlorite and 16.5 % sodium chloride. The results revealed that Milton

completely eliminated S. aureus. Whereas failed to kill E. coli and C. albicans. Hecker et al 2012 suggested the effect of sodium hypochlorite in the endodontic treatment depends on the duration of contact. Corcoran et al 2014 demonstrated that although sodium hypochlorite can reduce Salmonella enterica, a food poison causing pathogen it could not eradicate an early biofilm or a mature biofilm even after a 90 minute contact time. Results of present study (figure 4.7 and figure 4.7a) also showed that sodium chloride/sodium hypochlorite containing product Milton (66.66% of test samples) was less effective when compared to boiling, microwave and chlorhexidine with some surviving E.coli and C. albicans on both latex and silicon pacifiers. Further studies need to be carried out to investigate the effective concentration and the exposure time of Milton. The use of a microwave sterilization unit to clean teats, pacifiers, milk bottles and teething rings has become a popular method in recent years. Microwave irradiation is claimed to be a simple, effective and inexpensive (if one already has a microwave), disinfection method. One hundred percent kill was obtaining with this method for all three organisms on both latex and silicon pacifier. It is important to note that in this study only one parameter (8 minutes at 900 watts) was tested. This result cannot be generalised to other microwaves with different parameters. These units claim to keep items sterilized up to 24 hours provided that the unit cover has not been opened. Also by placing the pacifier in the unit with water the organisms were disinfected by both irradiation and steam. The uses of a microwave to disinfect medical and dental items have been of interest the in health field. For example Rosaspina et al, (1994) demonstrated that 90 seconds of microwave exposure provided effective sterilization of surgical scalpel blades which were contaminated with C. albicans. Their study revealed that irradiation induced a morphological modification and eventually rupture of the cell after 9 minutes. Bhasin et al, (2013) revealed that 5 minute exposure of irradiation can eliminate C. albicans and P. aeruginosa, while elimination of S. aureus took longer (7 minutes). Many studies have found microwave irradiation to be effective in disinfecting used dentures, dental impression material and dental stone (Ribeiro et al, 2009, Silva et al, 2006, Neppelenbroek et al, 2006, Yildirim- Bicer et al, 2014). Finally pacifiers were submerged in chlorhexidine for 2 minutes. This disinfection solution contained 2 mg/ ml of chlorhexidine gluconate with 5.4% m/v ethanol as a preservative. Chlorhexidine eliminated S. aureus and C. albicans completely from both latex and silicon pacifiers. However for E. coli, the results were 99.99% effective on both latex and silicon pacifiers. Chlorhexidine is an antiseptic agent with a broad – spectrum antimicrobial activity. Chlorhexidine destroys bacteria by breaking their membranes and inducing cytoplasmic precipitation (Gomes et al, 2005, Cheung et al, 2012). Due to its well established efficacy, this chemical was used as a positive control. The efficacy of chlorhexidine may have been organism and contact time dependent. Streptococcus mutans can be eliminated from pacifiers (Nelson- Filho et al, 2011, Chamele et al, 2012) whereas chlorhexidine may fail to completely eliminate other bacteria (Sato et al, 2005). In addition, gram negative bacilli are known to have a low susceptibility to chlorhexidine (Brown et al, 1989). Oral cavities of cancer patients are known to be colonised with gram negative bacteria particularly enteric pathogens including E. coli after the continuous use of chlorhexidine mouth rinse (Brown et al, 1989, Raybould et al, 1994). For this reason E. coli may have survived on those pacifiers even after the use of chlorhexidine. 5.4 Comparison between different disinfection procedures All the disinfection methods used in this study including water reduced the number of test organisms regardless of the material construct of the pacifier. However, disinfection with a chemical or heat proved to be superior then water alone. The purpose of disinfection is the removal of pathogens and reduction of number of bacteria. Pacifiers do not have to be sterile however some of the methods used here showed complete elimination of test organisms (microwave and boiling). None of the test organism was sporulating bacteria therefore this discussion only includes vegetative form of bacteria. It is clear that heat based disinfection was better than chemical disinfection. Both boiling and the use of microwave are relatively cheap, easy to use and most people have the required equipment in there house. Although chemical (Milton and chlorhexidine) reduced the number of organisms, they did not show complete elimination particularly of pathogens such as E. coli (Milton and chlorhexidine) and C. albicans (Milton).

Montagner et al, (2009) evaluated the antifungal action of chlorhexidine and hypochlorite on acrylic resin specimens, contaminated with C. albicans. They observed that sodium hypochlorite was more efficacious disinfectants against C. albicans than 2.0 % chlorhexidine solution. Interestingly in our study the results revealed that Milton (sodium hypochlorite) did not eliminate C. albicans but 2.0 % chlorhexidine was effective. Milton is widely used and the manufacturer claims that it eliminates C. albicans. But the results in this study failed to show it. In addition, it is an additional cost to the consumer. Chlorhexidine is generally not used for the disinfection of pacifiers for many reasons. It is costly and inadequate for the enteric pathogens as also shown here. Furthermore, it has unpleasant taste, sometime cause temporary numbness in the oral cavity, alter the taste and stain teeth. 6 Limitations, Future research and Conclusions 6.1 Limitations ? The sucking motion of the tongue creates friction on the pacifier creating micro cracks which allows better bacterial attachment. The presence of saliva may also create a better bacterial attachment. In this study results were based on smooth unused pacifiers. The results reported here on the vegetative form of planktonic bacteria rather than the biofilm. ? Mimicking and creating micro cracks was considered. However to standardise these cracks posed a challenge. ? The pacifiers were placed into the solution of contaminants for 2 minutes and no rubbing was involved which would be different from the used pacifiers. 6.2 ? ? ? ? 6.3 Future research The effective exposure time and the concentration of Milton needs further analysis. Use of chlorhexidine and the limitation particularly the ineffectiveness against gram negative bacteria can be studied. Results of microwave sterilizer (steam based) can be compared to the method where the pacifier can be placed in microwave in water (boiling based). This may eliminate the cost of microwavable steamer. Further studies needs to be carried out to assess the efficacy of these methods on used pacifiers which will have biofilms of bacteria that are difficult to kill. Conclusions Effective cleaning and disinfection of pacifiers remains an important concern. While one is aware that pacifiers do carry and acts as a reservoir for bacterial growth, the usage of these devices are commonly seen. This study has shown that all the test intervention including rinsing with water can reduce the number of contaminants. However the use of disinfectant solutions such as hypochlorite-based commercially available product Milton and chlorhexidine as well as heat-based methods boiling and the use of microwave can significantly reduce the test microorganisms. Among these methods, boiling and the use of microwave proved to be the best in killing common pathogens S. aureus, E. coli and C. albicans completely. These methods are cost effective, readily available and easy to use. Education to the care givers in the day care centres and mothers with regards to the contamination and decontamination of pacifiers is important. It may reduce the transmission of pathogens and hence prevent and reduce the onset of infection among the pacifier using infants. 7 Appendix 7.1 Human Research Ethics Committee Wavier form (Attached on next page) 7. 2 Media and reagents Blood Agar 39 g Columbai agar (Oxoid ltd, UK) 1000 ml Distilled water 5g Sterile defibrinated blood Columbia agar base was suspended in 1000 ml distilled water. It was dissolved completely by bringing to boil. It was sterilized by autoclaving at 15 lb and 1210C for 10 minutes. Allowed to cool to 500C and 5 % sterile defibrinated blood was added. It was poured into plates and allowed to set. Plates were labeled and refrigerated until use. Sabouraud Agar 65 g Sabouraud Agar 1000 ml Distilled water Sabouraud agar was suspended in 500 ml distilled water. The other 500 ml of distil water was boiled. Both were mixed and boiled. Agar was aliquoted, sterilized at 1210C, 15 lb pressure for 20 minutes, cooled to 450C and plates were poured. Phosphate Buffered Saline (PBS) 4.2 g Sodium chloride 0.078 g Sodium dihydrogen phosphate 0.64 g Sodium hydrogen phosphate 500 ml Distilled water Solids were suspended and autoclave at 151b, 1210C for 15 minutes and dispensed. 7.3 Statistical analysis ---------- name: <unnamed> log: /Users/macbookpro/Documents/Stata/Pacifier.log log type: text opened on: 17 Nov 2014, 14:13:05 . use "Pacifier.dta" . . *1 DESCRIPTIVES . . tab type Type | Freq. Percent Cum. ------------+----------------------------------- Silicon | 45 50.00 50.00 Latex | 45 50.00 100.00 ------------+----------------------------------- Total | 90 100.00 . tab org Org. | Freq. Percent Cum. ------------+----------------------------------- S. aureus | 30 33.33 33.33 E. coli | 30 33.33 66.67 C. albicans | 30 33.33 100.00 ------------+----------------------------------- Total | 90 100.00 . tab method Method | Freq. Percent Cum. ------------+----------------------------------- Tap water | 18 20.00 20.00 Boiling | 18 20.00 40.00 Milton | 18 20.00 60.00 Microwave | 18 20.00 80.00 Chlorhexidine | 18 20.00 100.00 ------------+----------------------------------- Total | 90 100.00 . . sum kill, detail % kill ---------- Percentiles Smallest 1% 96.43 96.43 5% 99.49 98.5 10% 99.79 99.08 Obs 90 25% 99.99 99.46 Sum of Wgt. 90 50% 100 Mean 99.89667 Largest

Std. Dev. .4223423 75% 100 100 90% 100 100 Variance .178373 95% 100 100 Skewness -6.753457 99% 100 100 Kurtosis 53.12129 . . *2.Does type of material have any influence on the % kill? . . table type, c(mean kill sd kill) ---------- Type | mean(kill) sd(kill) ----------+---------- Silicon | 99.8733 .5397643 Latex | 99.92 .2614208 ---------- . . kwallis kill, by(type) Kruskal-Wallis equality-of-populations rank test +----------+ | type | Obs | Rank Sum | |---------+-----+----------| | Silicon | 45 | 2066.00 | | Latex | 45 | 2029.00 | +----------+ chi-squared = 0.022 with 1 d.f. probability = 0.8813 chi-squared with ties = 0.034 with 1 d.f. probability = 0.8538 . . *NO SIGNIFICANT EFFECT OF TYPE ON %KILL . . . *3. Does organsism have any influence on the % kill? . table org, c(mean kill sd kill) ---------- Org. | mean(kill) sd(kill) ----------+---------- S. aureus | 99.9137 .2883902 E. coli | 99.8523 .6499903 C. albicans | 99.924 .1966195 ---------- . . kwallis kill, by(org) Kruskal-Wallis equality-of-populations rank test +----------+ | org | Obs | Rank Sum | |----------+-----+----------| | S. aureus | 30 | 1477.50 | | E. coli | 30 | 1339.50 | | C. albicans | 30 | 1278.00 | +----------+ chi-squared = 1.020 with 2 d.f. probability = 0.6006 chi-squared with ties = 1.552 with 2 d.f. probability = 0.4602 . . *NO SIGNIFICANT EFFECT OF ORGANISMS ON %KILL . . . *4 Does treatment have different effects on the % kill? . table method, c(mean kill sd kill) ---------- Method | mean(kill) sd(kill) ----------+---------- Tap water | 99.5022 .8519013 Boiling | 100 0 Milton | 99.9822 .0438655 Microwave | 100 0 Chlorhexidine | 99.9989 .0032345 ---------- . . kwallis kill, by(method) Kruskal-Wallis equality-of-populations rank test +----------+ | method | Obs | Rank Sum | |----------+-----+----------| | Tap water | 18 | 184.50 | | Boiling | 18 | 1062.00 | | Milton | 18 | 792.50 | | Microwave | 18 | 1062.00 | | Chlorhexidine | 18 | 994.00 | +----------+ chi-squared = 44.934 with 4 d.f. probability = 0.0001 chi-squared with ties = 68.411 with 4 d.f. probability = 0.0001 . . *SIGNIFICANT EFFECT OF TREATMENT METHOD ON %KILL, WE NEED TO FIND THE EFFECTIVE > METHODS . . . *5 Which treatment is significanly better than water (w)? . oneway kill method, tab sch | Summary of % kill Method | Mean Std. Dev. Freq. ----------+---------- Tap water | 99.502222 .85190132 18 Boiling | 100 0 18 Milton | 99.982222 .04386547 18 Microwave | 100 0 18 Chlorhexi | 99.998889 .0032345 18 ----------+---------- Total | 99.896667 .42234228 90 Analysis of Variance Source SS df MS F Prob > F ---------- Between groups 3.50479846 4 .876199614 6.02 0.0003 Within groups 12.3703985 85 .1455341 ---------- Total 15.875197 89 .178373 Bartlett's test for equal variances: chi2(2) = 228.4909 Prob>chi2 = 0.000 note: Bartlett's test performed on cells with positive variance: 2 multiple-observation cells not used Comparison of % kill by Method (Scheffe) Row Mean-| Col Mean | Tap wate Boiling Milton Microwav ---------+---------- Boiling | .497778 | 0.007 | Milton | .48 -.017778 | 0.010 1.000 | Microwav | .497778 0 .017778 | 0.007 1.000 1.000 | Chlorhex | .496666 -.001111 .016666 -.001111 | 0.007 1.000 1.000 1.000 . . *ALL TREATMENT BETTER THAN WATER STATISTICALLY. . *SOME TREATMENTS ARE NOT SUPERIOR TO EACH OTHER SUCH AS MILTON AND BOILING WATER . . . log close name: <unnamed> log: /Users/macbookpro/Documents/Stata/Pacifier.log log type: text closed on: 17 Nov 2014, 14:13:05 ----------

7.4 Plagiarism declaration 7.5 Plagiarism report generated on Turnitin 8 References: ? Adair SM, Milano M, Duskhu JC. Evalulation of the effects of orthodontic pacifiers on the primary dentition of 24 to 59 month old children: preliminary study. Pediatric Dentistry 1992; 14(1): 13-18. ? Aliprandini P, Ferreira FB, Bertol LS, Kindlein Junior W. Comparison of design, materials selection and characterization of pacifiers produced in Brazil. Australasian Medical Journal 2011; 4(2): 76-80. ? American Academy of Pediatrics. The changing concept of sudden infant death syndrome. Pediatrics 2005; 116(5): 1245–1255. ? Arias E, MacDorman MF, Strobino DM, Guyer B. Annual summary of vital statistics- 2002. Pediatrics 2003; 112: 1215–1230. ? Arvedson J, Clark H, Lazarus C, Schooling T, Frymark T. Evidence – based systematic review; effects of oral motor interventions on feeding and swallowing in preterm infants. American Journal of Speech-Language Pathology 2010; 19(4): 321–340. ? Bagci U, Temiz A. Microbiological quality of fresh – squeezed orange juice and efficacy of fruit surface decontamination methods in microbiological quality. Journal of food Protection 2011; 74(8): 1238-1244. ? Bagg J, MacFarlane TW, Poxton IR, Miller CH, Smith AJ. Essentials of Microbiology for Dental Students. Oxford Medical Publications. 1999. ? ? ? ? ? ? Bhasin A, Vinod V, BhasinV, Mathew X, Sajjan S, Ahmed ST. Evaluation of effectiveness of microwave irradiation for

disinfection of silicone elastomeric impression material. Journal of Indian Prosthodontic Society 2013; 13 (2); 89-94. Bonesvoll P. Oral pharmacology of chlorhexidine. Journal of Clinical Periodontology 1977; 4: 49-65. Bork K, Hoede N, Korting GW, Burgdorf WHC, Young SK. Diseases of the oral mucosa and the lips. W. B. Saunders: Philadelphia. 1993. Brexc M, Brownstone E, MacDonald L. Efficacy of Listerine, Merdial and chlorhexidine mouth rinses as supplements to regular tooth-cleaning measures. Journal of Clinical Periodontology 1992; 19: 202-207. Brooks GF, Butel JS, Morse SA. Jawetz, Melnick and Adelberg's Medical Microbiology 22nd Edition. McGraw- Hill Companies, 2001. Brook I, Gober AE. Bacterial colonization of pacifiers of infants with acute otitis media. The Journal of Laryngology and Otology 1997; 111: 614-615. ? Brown AT, Sims RE, Raybould TP, Lillich TT, Henslee PJ, Ferretti GA. Oral gram – negative bacilli in bone marrow transplant patients given chlorhexidine rinses. Journal of Dental Research 1989; 68(7): 1199–1204. ? Bukowski M, Wladyka B, Dubin G. Exfoliative Toxins of Staphylococcus aureus. Toxins 2010; 2(5): 1148-1165. ? ? ? ? ? Chamele J, Bhat C, Saraf T, Jadhav A, Beg A, Jagtap C, Ubeja R, Patil P. Efficacy of microwaves and chlorhexidine for disinfection of pacifiers and toothbrushes: an in vitro study. The Journal of Contemporary Dental Practice 2012; 13(5): 690-694. Cheung HY, Wong MM, Cheung SH, Liang LY, Lam YW, Chiu SK. Differential actions of chlorhexidine on the cell wall of Bacillus subtilis and Escherichia coli. Public library Science 2012; 7(5): e36659. doi: 10.1371/journal.pone.0036659. Cole AM, Tahk S, Oren A, Yoshioka D, Kim Y-H, Park A, Ganz T. Determinants of Staphylococcus aureus nasal carriage. Clinical Vaccine Immunology 2001; 8(6): 1064–1069. Comina E, Marion K, Renaud FN, Dore J, Bergeron E, Freney J. Pacifiers: a microbial reservoir. Nursing Health Science 2006; 8(4): 216–223. Corcoran M, Morris D, De Lappe N, O'connor J, Lalor P, Dockery P, Cormican M. Commonly used disinfections fail to eradicate Salmonella enteric biofilms from food contact surface materials. Applied and Environmental Microbiology 2014; 80(4): 1507- 1514. ? Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilm: a common cause of persistent infection. Science 1999; 284: 1318 -1322. ? Darwazeh AM, al-Bashir A. Oral candidial flora in healthy infants. Journal of Oral Pathology and Medicine 1995; 24(8): 361-364. ? Da Silva CH, Paranhos Hde F. Efficacy of biofilm disclosing agent and of three brushes in the control of complete denture cleaning. Journal of Applied Oral Science 2006; 14(6): 454- 459. ? Da Silveira LC, Charone S, Maia LC, Soares RM, Portela MB. Biofilm formation by Candida species on silicone surfaces and latex pacifier nipples: an in vitro study. The Journal of Clinical Pediatric Dentistry 2009; 33(3): 235-340. ? Elkarim IA, Abdulla ZA, Yahia NA, Al Qudah A, Ibrahim YE. Basic infection control procedures in dental practice in Khartoum – Sudan. International Dental Journal 2004; 54(6): 413-417. ? Evans Jr D, Evans DG, Escherichia coli. Medical Microbiology, 4th edition, The University of Texas, Medical Branch at Galveston. 2007. ? Festini F, Giusti F, Paoletti E, Biancalani L, Poggi GM. Is early use of pacifier risk factor for diarrhea? Minerva Pediatrics 2012; 64(3): 303-305. ? Gehlbach SH, MacCormack JN, Drake BM, Thompson WV. Spread of disease by fecal- oral route in day nurseries. Health Service Reports 1973; 88: 320-322. ? Gillespie S and Bamford K. Medical Microbology & Infection at a Glance, 3rd edition. Blackwell Publishing, 2007. ? Gjermo P, Baastad KL, Rolla G. The plaque inhibiting capacity of 11 antimicrobial components. Journal of Periodontal research 1970; 5: 102-109. ? Gomes BPFA, Vianna ME, Matsumoto Cu, Rossi Vde P, Zaia AA, Ferraz CC, Souza Filho FJ. Disinfection of gutta – percha cones with chlorhexidine and sodium hypochlorite. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics 2005; 100: 512–517. ? Greenstein G, Berman C, Jaffin R. Chlorhexidine a adjunct to periodontal therapy. Journal of Periodontology 1986; 57: 370-377. ? Grossman E, Meckel AH, Isaacs RL, Ferretti GA, Sturzenberger OP, Bollmer BW, Moore DJ, Lijana RC, Manhart MD. A clinical comparison of antibacterial mouthrinses: effects of chlorhexidine, phenolics and sanguinarine on dental plaque and gingivitis. Journal of Periodontology 1989; 60(8): 435-340. ? Grozdanov L, Raasch C, Schulze J, Sonnenborn U, Gottschalk G, Hacker J, Dobrindt U. Analysis of the genome structure of the nonpathogenic probiotic Escherichia coli strain Nissle 1917. Journal of Bacteriology 2004; 186: 5432-5441. ? Hanafin S, Griffiths P. Does pacifier use cause ear infection in young children? British Journal of Community Nursing 2002; 7(4):206, 208-11. ? Hauck FR, Omojokun OO, Siadaty MS. Do pacifiers reduce the risk of sudden infant death syndrome? Pediatrics 2005; 116(5): 716–723. ? Hecker S, Hiller KA, Galler KM, Erb S, Mader T, Schmalz G. Establishment of an optimized ex vivo system for artificial root canal infection evaluated by use of sodium hypochlorite and the photodynamic therapy. International Endodontic Journal 2013; 46 (5): 449–457. ? Howard CR, Howard FM, Lanphear B, Eberly S, deBlieck EA, Oakes D, Lawrence RA. Randomized clinical trial of pacifier use

and bottle-feeding or cupfeeding and their effects on breastfeeding. Pediatrics 2003; 111(3): 511-518. ? Hudault S, Guignot J, Servin AL. Escherichia coli strains colonizing the gastrointestinal tract protect germ-free mice against Salmonella triphimurium infection. Gut 2001; 49(1):47-55. ? Jenik AG, Vain N. The pacifier debate. Early human development 2009; 85(10): 89-91. ? Johnson BR and Remeikis NA. Effective shelf – life of prepared sodium hypochlorite solution. Journal of Endodontic 1993; 19(11): 40–43. ? Kamada N, Inoue N, Hisamatsu T, Okamoto S, Matsuoka K, Sato T, Chinen H, Hong KS, Yamada T, Suzuki T, Watanabe N, Tsuchimoto K, Hibi T. Nonpathogenic Escherichia coli strain Nissle 1917 prevents murine acute and chronic colitis. Inflammatory Bowel Diseases 2005; 11:455-463. ? Kaufman GE, Cimo S, Miller LW, Blass EM. An evaluation of the effects of sucrose on neonatal pain with 2 commonly used circumcision methods. American Journal of Obstetrics and Gynaecology 2002; 28(3): 564–568. ? Kluytmans J, van Belkum A, Verbrugh H. Nasal carriage of Staphylococcus aureus: epidemiology, underlying mechanisms, and associated risks. Clinical Microbiology Reviews 1997; 10(3): 505–520. ? Lieberthal AS, Carroll AE, Chonmaitree T, Ganiats TG, Hoberman A, Jackson MA, Joffe MD, Miller DT, Rosenfeld RM, Sevilla XD, Schwartz RH, Thomas PA, Tunkel DE. The Diagnosis and Management of Acute Otitis Media. Pediatrics 2013; 131: 964. ? Loe H, Schiott CR. The effect of mouthrinses and topical application of chlorhexidine on the development of dental plaque and gingivitis in man. Journal of Periodontology 1970; 5: 79-83. ? Mainstone A. Domestic hazard analysis of infant feeding utensiles. British Journal of Midwifery 2004; 12(6): 368-372. ? Marrie TJ, Nelligan J, Costerton JW. A scanning and transmission electron microscopic study of an infected endocardial pacemaker lead. Circulation 1982; 66(6): 1339-1341. ? Mathur GP, Mathur S, Khanduja GS. Non – nutritive suckling and use of pacifiers. Indian Pediatrics 1990; 27(11): 1187-1189. ? Mattos-Graner RO, de Moraes AB, Rontani RM, Birman EG. Relation of oral yeast infection in Brazilian infants and use of a pacifier. ASDC Journal of Dentistry for Children 2001; 68(1): 33-36,10. ? Mernelius S, Löfgren S, Lindgren PE, Blomberg M, Olhager E, Gunnervik C, Lenrick R, Thrane MT, Isaksson B, Matussek A. The effect of improved compliance with hygiene guidelines on transmission of Staphylococcus aureus to newborn infants: The Swedish Hygiene Intervention and Transmission of S. aureus study. American Journal of Infection Control 2013;41(7): 585-590. ? Mims C, Dockrell HM, Goering RV, Roitt I, Wakelin D, Zuckerman M. Medical Microbiology Third Edition. Elsevier Limited, 2004. ? Mitchell EA, Blair PS, L'Hoir MP. Should pacifiers be recommended to prevent sudden infant death syndrome? Pediatrics 2006; 117: 1755-1758. ? Mohammadi Z, Abbott PV. The properties and applications of chlorhexidine in endodontics. International Endodontic Journal 2009; 42: 288-302. ? Montagner H, Montagner F, Braun KO, Peres PE, Gomes BP. In vitro antifungal action of different substances over microwave-cured acrylic resins. Journal of Applied oral Science 2009; 17: 432–435. ? Nam SH, Seo YM, Kim MG. Bisphenol A migration from polycarbonate baby bottle with repeated use. Chemosphere 2010; 79(9): 949-952. ? Nelson-Filho P, da Silva LA, Ds Silva RA, da Silva LL, Ferreira PD, Ito IY. Efficacy of microwaves and chlorhexidine on the disinfection of pacifiers and toothbrushes: an in vitro study. Pediatics Dentistry 2011; 33(1): 10-13. ? Neppelenbroek KH, Pavarina AC, Spolidorio DM, Vergani CE, Mima EG, Machado AL. effectiveness of microwave sterilization on three hard chairside reline resins. International Journal of Prosthodontics 2003; 16(6): 616-620. ? Newman and Nisengard. Oral Microbiology and Immunology. W.B Saunders: Philadelphia.1988. ? Niemela M, Pihakari O, Pokka T, Uhari M, Uhari M. Pacifier as a risk factor for acute otitis media: A randomized, controlled trial of parental counseling. Pediatrics 2000; 106: 483-488. ? Niemela M, Uhari M, Mottonen MA. Pacifier increases the risk of recurrent acute otitis media in children in daycare centers. Pediatrics 1995; 96:884-888. ? Nordqvist C. What is E.coli? Medical News Today.2013 ? Ollila P, Niemela M, Uhari M, Larmas M. Prolonged pacifier-sucking and use of nursing bottle at night: possible risk factors for dental caries in children. Acta Odontologica Scandinavica 1998; 56(4): 233-237. ? Peressini S. Pacifier use and early childhood caries: An evidence – based study of the literature. Journal of Canadian Dental Association 2003; 69(1): 16-9. ? Poore M, Zimmerman E, Barlow SM, Wang J, Gu F. Patterned orocutaneous therapy improves sucking and oral feeding in preterm infants. Acta Paediatrica 2008; 97(7): 920- 927. ? Poyak J. Effects of pacifiers on early oral development. International Journal of Orthodontics (Milwaukee, WiS) 2006; 17(4): 13-16. ? Raybould TP, Carpenter AD, Ferretii GA, Brown AT, Lillich TT, Henslee J. Emergence of gram- negative bacilli in the mouths of bone marrow transplant recipients suing chlorhexidine mouthrinse. Oncology Nursing Forum 1994; 21(4): 691-696. ? Redmond EC, Griffith CJ, Riley S. Contamination of bottles used for feeding reconstituted powdered infant formula and implications for public health. Perspectives Public

Health 2009; 129(2): 85-94. ? Reid G, Howard J, Gan BS. Can bacteria interference prevent infection? Trends Microbiology 2001; 9: 424-428. ? Ribeiro DG, Pavarina AC, Dovigo LN, Palomari Spolidorio DM, Giampaolo ET, Vergani CE. Denture disinfection by microwave irradiation: a randomized clinical study. Journal of Dentistry 2009 Sep; 37(9): 666 -672. ? Rolla G, Loe H, Schiott CR. The affienity of chlorhexidine for hydroxyapatite and salivary mucins. Journal of periodontal Res 1970; 5: 90-95. ? Rosaspina S, Salvatorelli G, Anzanel D, Bovolenta R. Effect of microwave radiation on Candida albicans. Microbiology 1994; 78(314): 55 -59. ? Rovers MM, Numans ME, Langenbach E, Grobbee DE, Verheij TJM, Schilder AGM. Is pacifier use a risk factor for acute otitis media? A dynamic cohort study. Family Practice 2008; 25: 233-236. ? Rutala WA, Weber DJ. Infection control; the role of disinfection and sterilization. Journal of Hospital Infections 1999; 43: 43-55. ? Rutala WA, Weber DJ. Uses of inorganic hypochlorite (bleach) in health – care facilities. Clinical Microbiology 1997; 10 (4): 597 – 610. ? Rweyendela IH, Patel M, Owen CP. Disinfection of irreversible hydrocolloid impression material with chlorinated compounds. South African Dental Journal 2009; 64(5): 208, 210-212. ? Sato S, Pedrazzi V, Guimaraes Lara EH, Panzeri H, Ferreira de Albuquerque R Jr, Ito IY. Antimicrobial spray for toothbrush disinfection: an in vivo evaluation. Quintessence International 2005; 36(10): 812-816. ? Sexton S, Natale R. Risks and Benefits of Pacifiers. American Family Physician 2009; 79(8): 681-685. ? Silva M, Consani R, Sardi J, Mesquita M, Macêdo A, Takahashi J. Microwave irradiation as an alternative method for disinfection of denture base acrylic resins. Minerva Stomatology 2013; 62(1-2): 23-29. ? Singh BP, Khan SA, Agrawal N, Siddharth R, Kumar L. Current biomedical waste management practice and cross – infection control procedures of dentist in India. International Dental journal 2012; 62 (3): 111 -116. ? Sio JO, Minwallla FK, George RH, Booth IW. Oral Candida: is dummy carriage the culprit? Archives of disease in childhood 1987; 62(4): 406– 408. ? Sofola OO,Savage KO. Assessment of the compliance of Nigerian dentists with infection control: a preliminary study. Infection Control and Hospital Epidemiology 2003; 24(10): 737–740. ? Sussman M. Escherichia coli: mechanisms of virulence. Department of Microbiology, Medical School, Newcastle upon Tyne; Cambribridge university press:1997. ? Todar K. Staphylococcus aureus and Staphylococcal Disease.Text Book of Bacteriology. Madison, Wisconsin 2012. ? Tomasi E, Victora CG, Post PR, Olinto MT, Béhague D.The use of pacifiers in children: fecal contamination and association with diarrhea. Revista de Saúde Pública 1994; 28(5): 373-379. ? Victora CG, Tomasi E, Olinto MT, Barros FC. Use of pacifiers and breastfeeding duration. Lancet 1993; 341(8842): 404-406. ? Yildirim – Bicer AZ, Peker I, Akca G, Celik I. In vitro antifungal evaluation of seven different disinfectants on acrylic resins. BioMed Research International 2014; 2014:519098. doi: 10.1155/2014/519098 ? Yonezu T, Yakushiji M. Longitudinal study on influence of prolonged non-nutritive sucking habits on dental caries in Japanese children from 1.5 to 3 years of age. The Bulletin of Tokyo Dental College 2008; 49(2): 59-63. ? Zempsky WT, Cravero JP. Relief of pain and anxiety in pediatric patients in emergency medical systems. Pediatrics 2004; 114(5): 1348-1356. ? Zimmer S, Barthel CR, Ljubicic R, Bizhang M, Raab WH. Efficacy of a novel pacifier in the prevention of anterior open bite. American Academy of Pediatric Dentistry 2011; 33(1): 52-5. 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74