In vitro efficacy of different solutions in the disinfection of silicone pacifiers

SADJ May 2017, Vol 72 no 4 p158 - p161

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SUMMARY

Introduction: Pacifiers used by toddlers and young children are susceptible to contamination by microorganisms. Effective methods of disinfection are required to prevent oral infections. There are limited studies on pacifier disinfection in South Africa.

Aim: To investigate the efficacy of four pacifier disinfecting agents.

Methods: Two groups of 80 pacifiers each were contaminated with a standardized suspension of either *Staphylococcus epidermidis* or *Candida parapsilosis*. Each group was subdivided into four sub-groups, and severally disinfected with 0.12% GUM® Paroex™ Chlorhexidine Gluconate Oral Rinse (chlorhexidine), Brushtox®, apple cider vinegar and sterile distilled water (control), followed by microbiological analysis. Data were analyzed using the Kruskal Wallis Anova test.

Results: Chlorhexidine and Brushtox® were statistically similar in eliminating the fungi from pacifiers ($p>0.05$). Statistically significant differences were noted between the effects of chlorhexidine and Brushtox® compared with that of apple cider vinegar for both test organisms ($p=0.0001$). Statistically significant differences were observed in the efficacies of disinfection between chlorhexidine, Brushtox®, apple cider vinegar and sterile distilled water ($p=0.00089$).

Conclusion: Chlorhexidine and Brushtox® were equally effective in eliminating both *S. epidermidis* and *C. parapsilosis* from silicone pacifiers. Apple cider vinegar was the least effective disinfecting solution.

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ACRONYMS

PBS: Phosphate buffered saline
SDA: Sabouraud dextrose agar

Key words: Pacifier, *Candida parapsilosis*, *Staphylococcus epidermidis*, 0.12% GUM® Paroex™ Chlorhexidine Gluconate Oral Rinse, Brushtox®, Apple cider vinegar.

INTRODUCTION

Pacifiers are used to calm crying babies.[1] However, prolonged contact between the pacifier nipple and the oral microflora leads to the development of microbial biofilms.[2] In addition, children usually drop their pacifiers to the floor, exposing them to a wide range of microorganisms.[2] Children who suck pacifiers over a long period of time are deemed more likely to develop dental caries and periodontal disease.[3]

The use of pacifiers has been associated with inflammation of the middle ear in children,[4] childhood caries,[5] viral[6] and yeast[1] infections. The advantages of using pacifiers include improving sleep[7] and reducing the risk of sudden death in the first six months of life.[8] In addition, pacifier use helps in controlling pain from minor procedures, especially in infants up to six months of age.[9]

Candida parapsilosis is one of the fungi most frequently isolated from human hands.[10] The organism is a well-known cause of nosocomial bloodstream infections in children and is associated with a 10% mortality rate and 17–50% of fungemia in children.[11] A study by Silva *et al.* examined epithelial infection with *C. parapsilosis* on reconstituted human oral epithelium.[12] The organism was found to induce significant tissue damage to the oral epithelium.[12]

C. parapsilosis can form biofilms on pacifiers and has been shown to be the most frequently-isolated organism from pacifiers after *Staphylococcus* genera.[2] Isolates of *C. parapsilosis* have been found to form biofilms on both latex and silicone pacifiers, with the latter more resistant to this colonization.[13]

Staphylococci have been reported to colonize the oral cavity and dental plaque,[14] which may serve as

potential reservoirs for transmission to other body sites.[15] *Staphylococcus epidermidis* was the second most prevalent bacterium in the saliva of hospitalized individuals.[15] This organism was previously regarded as a harmless commensal microorganism on the human skin; however it has been reported as an important opportunistic pathogen most prevalent in the periodontal pocket and oral cavity in patients with chronic periodontitis.[16] *S. epidermidis* has also been reported as a predominant agent associated with Infective Endocarditis.[17]

When the microbial contamination of pacifiers was assessed, *Staphylococcus* was found to be the dominant genera present, with *S. epidermidis* being one of the species isolated.[2] Aerobic microorganisms, including small quantities of *S. epidermidis*, have been identified on the surface of the pacifiers of children with acute otitis media,[18] and also on previously used pacifiers.[19]

Used pacifiers can retain various kinds of microorganisms.[1,4-6] However, there are limited studies worldwide on pacifier disinfection methods and on their efficacy in reducing contamination. Pacifiers confer many advantages and the aim should not be to discourage their use, but rather to ensure that they are rendered disinfected and safe to use. None of the previous studies have tested the efficacy of apple cider vinegar in the disinfection of pacifiers. Apple cider vinegar is a solution of acetic acid produced by fermentation of apples and has easy application, low cost and low toxicity.

The aim of this study was to investigate the efficacy of 0.12% GUM® Paroex™ Chlorhexidine Gluconate Oral Rinse solution, Brushtox® and apple cider vinegar for the disinfection of pacifiers contaminated with *S. epidermidis* and *C. parapsilosis*.

MATERIALS AND METHODS

Study population and methodology

The study was conducted at the University of the Witwatersrand, Johannesburg, South Africa, approval having been obtained from the Human Research Ethics Committee (Medical) (Certificate W-CJ-130916-2). *C. parapsilosis* (ATCC 22019) and *S. epidermidis* (ATCC 12228) were used in the study. One hundred and sixty new silicone pacifiers (Golden Baby, CKT Tek Co. Ltd, New Taipei, Taiwan) were used, divided into two groups of 80. The two groups were soaked, respectively, in standardised inocula (150x106cfu/ml) of either *C. parapsilosis* and *S. epidermidis* for 5 minutes. The contaminated pacifiers were randomly divided into four sub-groups of 20 each. These were severally sprayed on three occasions with 0.12% GUM® Paroex™ Chlorhexidine Gluconate Oral Rinse solution (Sunstar Americas, Inc, Ontario, Canada), or with Brushtox® (Dentox Ltd., Warwick, England), or 5% apple cider vinegar (Instant Trading Co. (Pty) Ltd, Durban, South Africa) or sterile distilled water.

Microbiological evaluation

Aseptic conditions were followed in transferring and suspending the contaminated pacifiers in 20ml of sterile phosphate buffered saline (PBS) for two minutes, followed by vortexing for microbial cell detachment. Dilutions of 10-1, 10-2 and 10-3 were prepared in sterile PBS from the initial suspension, and 0.1 ml of each dilution was plated on 5% blood agar to recover *S. epidermidis* and on Sabouraud Dextrose agar (SDA) for *C. parapsilosis* recovery. The SDA and 5% blood agar plates were incubated at 37°C for 48 hours. On conclusion, the number of colonies in each plate represented a measure of the presence of organisms.

Data analysis

Data were analysed using the Kruskal-Wallis ANOVA test. Statistical significance was set at the 5% significance level.

RESULTS

The 0.12 GUM® Paroex™ Chlorhexidine Gluconate Oral Rinse solution, Brushtox® and apple cider vinegar removed *S. epidermidis* from, respectively, 70%, 50% and 65% of the pacifiers. The Brushtox® and chlorhexidine were both successful in removing *C. parapsilosis* from all of the pacifiers. All pacifiers contaminated with *C. parapsilosis* and treated with apple cider vinegar remained contaminated (Figure 1). The control sample, treated with sterile water, remained contaminated.

The chlorhexidine and Brushtox® were statistically similar in the elimination of *C. parapsilosis* and *S. epidermidis* from pacifiers ($p>0.05$). There were statistically significant differences when chlorhexidine and Brushtox® were compared with apple cider vinegar in their relative efficacy in disinfecting pacifiers contaminated with *S. epidermidis* and *C. parapsilosis* ($p=0.0001$). Statistically significant differences were observed in disinfection efficacy between all the solutions when the control, sterile distilled water, was included in the analysis. (Table 1).

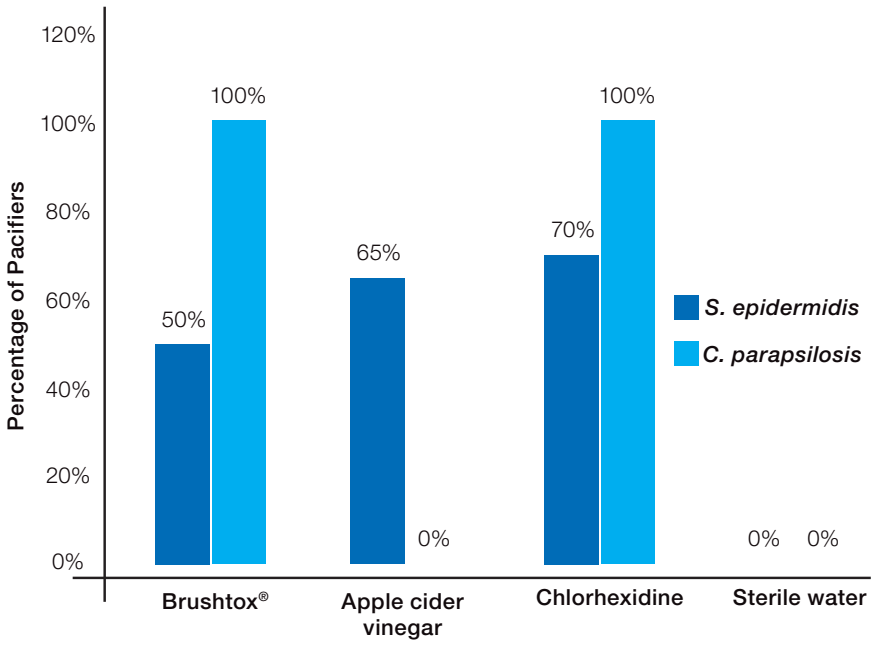

Figure 1: Proportion of pacifiers without contamination after disinfection procedures

Table 1: Statistical assessment of colony forming units for *S. epidermidis* and *C. parapsilosis* after disinfection with different methods (Kruskal-Wallis ANOVA)

Organism tested	Solution	N	Mean	Median	Minimum	Maximum	Std. Dev	Statistical Significance (p value)
S. epidermidis	Water	20	427950.0	230000.0	2000.000	1780000	514587.7	Water Chlorhexidine Brushtox® Apple cider vinegar **(p=0.00089)**
	Chlorhexidine	20	145.0	0.0	0.0	1000	298.2	
	Brushtox®	20	640.0	0.0	0.0	3400	1031.8	
	Apple cider vinegar	20	2760.0	600.0	0.0	36000	7910.2	Apple cider vinegar Brushtox® Chlorhexidine **(p<0.05)**
C. parapsilosis	Water	20	34050.0	30000.0	4000.0	100000.0	23538.27	
	Chlorhexidine	20	0.0	0.0	0.0	0.0	0.0	
	Brushtox®	20	0.0	0.0	0.0	0.0	0.0	Brushtox® Chlorhexidine **(p>0.05)**
	Apple cider vinegar	20	1495.0	1550.0	200.0	3500.0	1009.16	

DISCUSSION

Pacifiers become contaminated with various microorganisms which may lead to systemic and oral diseases.[5,11,16] However, currently little attention has been given to the disinfection of pacifiers.[20-23]

In this study the efficacy of 0.12% GUM® Paroex™ Chlorhexidine Gluconate Oral Rinse, Brushtox® and apple cider vinegar were investigated for the disinfection of pacifiers contaminated with *S. epidermidis* and *C. parapsilosis*.

S. epidermidis has been detected on pacifiers and the current study confirms previous reports that *S. epidermidis* can adhere to silicone pacifiers.[18,19] However studies on removal of this organism from pacifiers are not documented, hence comparisons are not possible. In the study under report, chlorhexidine was as effective as Brushtox® in removing *S. epidermidis* from pacifiers. This finding is in accord with a recent study, in which it was shown that chlorhexidine gluconate was effective in removing *S. epidermidis* from toothbrushes used by kindergarten children.[24] In contrast, a recent study reported *S. epidermidis* strains that were resistant to chlorhexidine.[25]

The chemical was found to be effective in also removing *Streptococcus mutans* from silicone pacifiers.[21,22] These results highlight reasons for concern, since this organism is associated with chronic periodontitis[16] and infective endocarditis.[17] Chlorhexidine is considered the gold standard rinsing agent for controlling dental plaque,[26] and the current study and above-mentioned studies confirm its efficacy also in the disinfection of pacifiers.

Studies on the use of disinfection methods for the removal of *C. parapsilosis* from pacifiers are also not documented. In attempting to address this gap, this study found that the 0.12% GUM® Paroex™ Chlorhexidine Gluconate Oral Rinse solution and Brushtox® were equally effective in removing *C. parapsilosis* from pacifiers. This finding differs with a recent *in vivo* study where Brushtox® was less effective than 0.12% chlorhexidine for the disinfection of pacifiers contaminated with *Streptococcus mutans*.[27] A study by Nelson-Filho *et al*., also reported Brushtox® to have lower efficacy when compared with Periogard chlorhexidine solution against the formation of *S. mutans* colonies on the toothbrush bristles.[23] Brushtox® is an antiseptic toothbrush cleanser for disinfecting contaminated toothbrushes[28] and has now been shown to also be effective in disinfecting silicone pacifiers. Our results are in agreement with previous studies, where *C. parapsilosis* was found capable of adhering to silicone pacifiers.[2,13]

This is another cause for concern as this organism can cause fungemia in children[11] and induce significant tissue damage on the oral epithelium.[12]

Apple cider vinegar is a solution of acetic acid produced by fermentation of apples. This solution is cheap as compared with 0.12% GUM® Paroex™ Chlorhexidine Gluconate Oral Rinse and Brushtox®, and is widely available in South African markets. The current study shows apple cider vinegar to be the least effective solution when compared with 0.12% GUM® Paroex™ Chlorhexidine Gluconate Oral Rinse and Brushtox® for eliminating both *C. parapsilosis* and *S. epidermidis*. This finding is in contrast to that of a study by Mota *et al*., in which apple cider vinegar exhibited fungicidal and fungistatic properties against *Candida* species involved in denture stomatitis.[29] It has also been demonstrated that apple cider vinegar can significantly reduce the number of organisms in root canals contaminated *in vitro* with *Enterococcus faecalis*, an organism that infects dentin tubules.[30]

All pacifiers treated with sterile water exhibited a high number of *C. parapsilosis* and *S. epidermidis* colonies. This study corroborates findings of previous studies where sterile water was found to be not at all effective in eliminating pacifier contamination.[1,2,21,23,31]

The current study used new pacifiers, which may be a limitation, as used, worn out pacifiers may have provided more appropriate environments for microorganisms to flourish.

CONCLUSIONS

Brushtox® was as effective as the 0.12% GUM® Paroex™ Chlorhexidine Gluconate Oral Rinse solution in eliminating *C. parapsilosis* and *S. epidermidis* from silicone pacifiers, whilst apple cider vinegar was the least effective disinfecting solution.
There is considerable importance in the effective disinfection of pacifiers.

Conflict of interest: None declared.

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