Assessing the Efficacy of Recommended Antiseptics for Killing Bacterial Growth in Neonatal Blue Bulb Syringes: Addressing a Clinical Issue

Linda Angell Hanson

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Assessing the Efficacy of Recommended Antiseptics for Killing Bacterial Growth in Neonatal Blue Bulb Syringes: Addressing a Clinical Issue

by

Linda Angell Hanson

An Honors Capstone submitted in partial fulfillment of the requirements for the Honors Diploma
to
The Honors College of The University of Alabama in Huntsville

October 30, 2017

Honors Capstone Director: Dr. Pamela V. O’Neal
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Student (signature) Date

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Department Chair (signature) Date

Honors College Dean (signature) Date

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Linda Angell Hanson

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10/24/17

Date
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This research would not have been possible without the guidance and assistance from Pamela V. O’Neal, PhD, RN, Ellise Adams, PhD, CNM, and Joseph G. Leahy, PhD. Under their guidance and tutelage, I have learned far more than I ever thought possible. Lana Harwell (Biology), John Alexander Moore (Biology), and Tanuj Alapati (Randolph School) were invaluable in the laboratory and offered more assistance and support with this research than I deserved. The UAH Research and Creative Experience for Undergraduates (RCEU) provided the initial funding for this research and served as the seed for a much larger exploration.

This thesis is dedicated to my husband, John M. Hanson, in recognition of his unfailing support of my desire to return to school to study Nursing. His pursuit of scholarly excellence has been an inspiration throughout our marriage and served as a model for my scholarly inquiry. This thesis is further dedicated to my daughters Kathleen M. Hanson, Jennifer A. Hanson, Kristin H. Blackerby, and Emily M. Hanson, and to my son-in-law William T. Blackerby. You have all inspired me with your pursuit of scholarly excellence and inquiry in your academic careers. Your relentless enthusiasm and pride has kept me moving forward each day as I pursued this degree and research.

This thesis is dedicated to the memory of Dr. Joseph G. Leahy, whose life served as a shining example of forgiveness, scholarly excellence, and tenacity. I am a better person for knowing and working with him.

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Abstract

**Background:** Blue bulb syringes (BBSs) have long been used to remove oral and nasal secretions from newborns to promote airway clearance. The BBS is provided to parents at hospital discharge and can be purchased by parents for home use in removing secretions during times of respiratory illness. Consequently, the BBS is a multi-use device. Current protocol recommends rinsing in warm, soapy water to clean the BBS between uses. No research studies have identified the efficacy of cleaning methods for killing bacteria growing in secretions inside the BBS. This experimental pilot study identifies antiseptics effective in killing bacterial growth within a BBS.

**Methods:** Using clinical isolates of *Escherichia coli* from BBSs collected at a large urban hospital, disinfection experiments, along with a control, were conducted using several antiseptics. Intervention consisted of application of specific concentrations of each antiseptic to the isolate. Antiseptics tested included dish detergents with Triclosan or l-lactic acid as their active ingredient, hydrogen peroxide, povidone-iodine, and chlorhexidine gluconate mouthwash.

**Results:** Five antiseptics were tested. Two, Triclosan and hydrogen peroxide, were ineffective in killing bacteria in the BBS within one minute. Failure of Triclosan to effectively kill bacteria in the BBS suggests that reconsideration of current protocol is critical. L-lactic acid, povidone-iodine, and chlorhexidine gluconate each achieved a 2-log kill, meaning 99 percent of existing bacteria were killed, in under one minute using concentrations at least three times the minimum inhibitory concentration.

**Discussion:** Several low-cost, widely-available options exist for effective disinfection of the BBS; these products should be recommended for effective bactericidal outcomes.
**Introduction**

The Germ Theory of Disease, which was developed by Pasteur, Lister, Koch and Henle from 1840 to the 1860s, suggests that specific microscopic organisms, referred to as germs or microbes, cause specific and distinct diseases and infections (Burton, 1983, p. 9). Further, Abdellah’s Theory of 21 Problems in Nursing proposes that nurses have a duty to promote safety, at least in part, by taking action in patient care to prevent disease (Tomey & Alligood, 2002, p. 114).

Oronasopharyngeal suctioning (ONPS) devices such as blue bulb syringes have been a traditional intervention used on newborns in the period immediately after delivery. Numerous studies (Aguilar & Vain, 2011, Chadha, 2010, Cordero & Hon, 1971, Jaques & Kennea, 2012, and Vain et al., 2004) suggest that suctioning may cause apnea and/or bradycardia. Studies by Waltman, Brewer, Rogers and May (2004), Gungor et al. (2005) and Gungor et al. (2006) suggest that ONPS intervention may also cause infection as a result of repeated use of the suctioning device. Since these devices are frequently sent home with parents upon discharge, repeated use of the device may occur at home as well. Therefore, it seems important that these devices should be disinfected between uses to prevent infection from occurring.

In the field of Microbiology, an antiseptic is defined as “a chemical disinfectant that is safe for use on living tissues” and a disinfectant is defined as “a chemical agent used to destroy pathogens on or within nonliving materials” (Burton, 1983, p. 94). However, most of the literature and, in fact, these definitions themselves use the terms antiseptics and disinfectants interchangeably. For purposes of this study, a disinfectant is intended for use on surfaces which may only come into casual contact with human tissues (primarily skin, after the disinfectant has dried), while an antiseptic is intended for use on more delicate human tissues such as oral or
respiratory membranes. A disinfectant in this study which is described as a human-safe disinfectant includes ones that, while they would not normally be used on delicate tissues at full concentration, may come into contact without harmful effect provided that they are diluted. Bacterial load is the concentration of bacteria in relation to surface area.

Antiseptics such as hydrogen peroxide and alcohol have been shown to be effective in eradicating microbial growth on hospital privacy curtains (Sood, Huber, Dam, Zenilman, & Riedel, 2014). Cheng, Boost, and Chung (2011) studied the effectiveness of disinfecting wipes on hospital surfaces with which patients frequently come into contact during their course of treatment, and found them to be effective in reducing bacterial load in the patient environment. Singh et al. (2012) evaluated the efficacy of locally available disinfectants on surfaces and infectious microbiological hospital waste. They found that while all tested disinfectants initially killed the majority of microbial growth, newer quaternary ammonium and aldehyde products were more effective for heavy contamination and long term disinfection. Li, Ai, Li, Zheng and Jie (2015) studied the use of oral antiseptics to prevent pneumonia in patients with mechanical ventilators to assist in breathing and found them to be effective in preventing ventilator-assisted pneumonia.

While each of these studies have looked at eradicating known pathogenic microbes in the hospital setting, and have contributed to greater knowledge in microbial eradication, none of their results can be generalized to blue bulb syringe disinfection. One study used disinfectants that, while safe on surfaces, are not safe for human ingestion or contact with delicate tissues (Singh et al., 2012). Two others by Sood et al. (2014) and Cheng et al. (2011), although both used antiseptics or human-safe disinfectants, tested hospital surfaces, not equipment inserted into a patient in a manner similar to that in which a blue bulb syringe is inserted. Li et al (2015)
tested oral antiseptics on equipment that is typically inserted into a patient’s trachea, but tested only adults. It would appear that no one has tested disinfectants for use on equipment inserted into children which comes into contact with delicate oral and respiratory membranes in the manner of a blue bulb syringe.

Kerur, Bhat, Harish, Habeebullah and Kumar (2006) evaluated the role of maternal genital bacteria and baby’s surface colonization in early onset neonatal sepsis and found correlation between maternal genital bacteria, baby’s surface colonization and neonatal sepsis, particularly when membranes were ruptured more than 24 hours prior to delivery or in low birth weight (LBW) or very low birth weight (VLBW) infants.

*Escherichia coli* (*E. coli*) was selected as the bacterium tested. Bizzarro et al. (2015) found that *E. coli* is the most common cause of early-onset sepsis in newborns, representing a changeover from Group B *Streptococcus* (GBS) as the leading cause. May, Daley, Donath, and Isaacs (2005) found that, with routine screening and treatment of GBS, *E. coli* is the leading cause of Early Onset Neonatal Bacterial Meningitis (EONBM). Voller and Myers (2016) found that, since the implementation of intrapartum antibiotic treatment of GBS, *E. coli* has emerged as the leading cause of early onset sepsis if preterm neonates. In a study of neonates of any gestational age who were diagnosed with clinical sepsis within the first 72 hours of life, Silva-Junior et al. (2016) found that *E. coli* was the most commonly-occurring bacterium found in blood samples.

The null hypothesis is that application of specific concentrations of selected antiseptics will show no difference in colony counts and will not effectively kill existing *Escherichia coli*.
Review of Literature

The review of literature was conducted using the Cumulative Index to Nursing and Allied Health Literature (CINAHL) and MEDLINE (PubMed) databases, along with the Cochrane Database of Systematic reviews, using the keywords MRSA eradication, MRSA antiseptic, hospital disinfectants, blue bulb suction and oronasopharyngeal. Because so few studies have been conducted on disinfection of suctioning devices, exclusion of keywords was not necessary to limit search results. In addition, Google Scholar and the UAH Library catalog were searched for Germ Theory and Abdellah’s 21 Theories of Nursing, using these as keywords.

Of the articles that were available, virtually all articles which included both disinfectants which were not human-safe and surfaces were excluded from consideration. Articles were included if they were primary sources, were interventional studies to eradicate microbial growth, and the investigators had studied antiseptics or human-safe disinfectants or equipment that would come into contact with delicate tissues. Although Singh et al. (2012) would have been excluded since it included primarily disinfectants which were not human-safe and involved only hospital surfaces, it was included in the review of literature due to its detailed discussion of methodology and variety of disinfectants and bacteria tested; it was far more comprehensive in nature than the other studies.

Singh et al. (2012) studied the effectiveness of several disinfectants that are routinely used in hospitals in India. They investigated both the most commonly used disinfectants and drug-resistant species of several clinical and standard isolates that are currently a common cause of hospital-acquired bacterial infections. Their study showed that while all of the disinfectants tested were initially effective in killing microbial growth, the quaternary ammonium compound DesNet and Bacilloid provided the most effective disinfection across the tested species, and also
provided the best long-term disinfection, in that species re-growth took longer than with other 
disinfectants. The methodology in this study reflected sound laboratory practice with tests 
carried out in triplicate to assure accuracy of results. Only hospital surfaces such as floors, walls, 
instrument tables and trolleys were tested; they did not test equipment used on, or inserted into, 
humans. Only disinfectants were tested; antiseptics were not. Disinfectants are intended for use 
on surfaces, and therefore do not have to be human-safe, much less human-consumptive. 
Antiseptics are intended for use on humans. Since only disinfectants were studied, it is not clear 
that they could be used on items that will come into contact with delicate patient tissues. Only 
bacterial growth was tested; fungal, viral and mycobacterial growth was not included in their 
study, so results are not generalizable to these microbial forms.

Sood et al. (2014) investigated the use of antiseptics such as hydrogen peroxide and 
isopropyl alcohol on privacy curtains to eradicate methicillin-resistant Staphylococcus aureus 
(MRSA), vancomycin-resistant Enterococcus (VRE) and Clostridium difficile. Their findings 
suggest that these antiseptics are at least marginally effective in eradicating all three microbes. 
Their use of antiseptics, rather than disinfectants, may be applicable to blue bulb syringes. In 
addition, they tested more than one species of bacteria, so indicated some margin of 
generalizability across species. They tested a single intervention, application of a single 
 disinfectant to the inoculated (contaminated) curtain, and the resultant decrease in live bacteria. 
Therefore, a more direct causal relationship of the intervention may be implied. This study, 
however, included only privacy curtains; items that come into closer human contact, such as 
equipment, were not included in the study. As a result their findings cannot be generalized to 
equipment. In addition, they inoculated the curtains themselves, rather than using curtains from 
rooms of patients known to be colonized with the bacteria in the study. As a result, the bacterial
load on the curtains was likely higher than would normally be found, and their use of small swatches of the curtains meant that the antiseptic application was likely more concentrated than might be encountered in a true hospital setting. Their use of a spray form of the antiseptic in their testing meant that any deleterious effects on users resulting from inhalation of the spray were not taken into effect in the study. As a result, the treatment method to eradicate the bacteria could be more harmful than the presence of the bacteria on the curtains.

Li et al. (2015) investigated the use of oral antiseptics in conjunction with mechanical ventilation, and found that routine oral care with antiseptics such as chlorhexidine and povidone-iodine was effective in reducing ventilator associated pneumonia. This study combined both antiseptics which are intended to come into contact with oral membranes and a piece of equipment intended to be inserted into the body and to come into contact with respiratory membranes. This study also looked at the incidence of disease resulting from use of the device, not just the reduction in bacterial load. They explicitly mention of methods used to ensure the quality of their research; they detail the methods used to test all supplies used. This study was a meta-analysis which looked at seventeen randomized trials, and included 4249 patients. As a result, the meta-analysis may allow broader generalization to other types of equipment. Only adults were tested; the results cannot be generalized to young children or newborns. It seems possible that the timing of the application of the oral antiseptic may have an impact on its effectiveness; a shorter or longer time between applications could have an impact on effectiveness, and this does not appear to have been considered in the studies. Since this study was a meta-analysis, the testing conditions were not the same throughout each of the seventeen studies; different concentrations and forms (gel, liquid) of the antiseptics were used, and the definition of VAP varied between different studies.
Cheng, et al. (2011) studied the effectiveness of disposable and non-disposable disinfectant wipes containing hypochlorite in cleaning the environment of patients known to be colonized with MRSA over a period of 7 days. Their study concluded that, while the wipes were effective in reducing MRSA, the risk of cross infection from reuse of the wipe or contact with the “disinfected” surface was not eliminated. Their study showed very strong correlation (P<.001) between the MRSA load on the bed rail tested and the contamination of the wipe used to disinfect it. This study used clinical specimens; their samples were collected from the hospital environments of eight MRSA-colonized patients, rather than being artificially introduced in a laboratory setting. As a result, the bacterial load on the items test was likely more realistic. This study includes the testing of only one disinfectant and one species of bacteria. Results cannot be assumed to be generalizable to other disinfectants and bacterial species, nor to other microbes such as fungi and viruses. Another weakness was that the disinfectant was not human-safe for contact with delicate tissues, unless it is diluted; it seems logical that it could not be used at full strength on hospital equipment that comes into contact with these tissues. Use of hypochlorite on equipment used on babies is unclear; further testing would be necessary in order to determine if such use were safe.

**Theoretical Framework**

Germ Theory, as put forth in the 1860s by Pasteur and Lister, along with Koch’s Postulates, form the basis for much of our understanding of causality of disease and host-pathogen interaction. Lister’s work led to antiseptic surgical technique, which has been shown to significantly reduce the likelihood of infection and/or death (Wilson, Mizer & Morello, 1979, p. 12). Koch has been credited with showing that specific microbes cause specific diseases such as cholera and tuberculosis (Burton, 1983, p. 16-17), while Pasteur is credited with showing that
heat could kill bacteria (Burton, 1983, p. 8). Germ Theory also led to an understanding of how germs are spread, largely due to investigations in 1840 by Henle (Wilson et al., 1979, p. 12).

Casadevall and Pirofski (2000) examined the terminology used with regard to infectious disease and found that the majority of this terminology derives from Germ Theory. They propose some adjustments to the terminology to eliminate confusion. They define infection as acquisition of a microbe by a host, and infectious disease as the clinical manifestation of damage resulting from host-microbe interaction. A pathogen is a microbe which can cause host damage, or interruption of normal tissue structure and/or function. Since known pathogenic microbes have been isolated from blue bulb syringes in the clinical setting (O’Neal et al., 2017), it would appear that continued use of these syringes, especially without effective disinfection, may cause infection in the newborns on whom they are used.

**Significance to Nursing**

Microbes have been shown to cause disease. Since pathogenic microbes have been identified in clinical isolates taken from blue bulb syringes used on newborns, it is important to determine what techniques should be used to disinfect these syringes and eradicate the microbes growing within them.

If nurses continue to use these syringes, unaware that they contain pathogenic microbes, is that continued use creating a risk of infection and damage to the babies upon whom they are used? Are parents putting their babies at risk, too, since they frequently continue to use them at home?

Until a connection can be made between blue bulb syringe use on and subsequent infection in the same child, we will be unable to answer these questions. Clearly, further
investigation is needed. If that connection is made, having an effective disinfection method already in place will be essential for nurses and parents in providing better health care to babies in their charge.

It appears that there has not been a study which looks specifically at disinfection techniques for use on blue bulb syringes. As a result, additional research is needed in this area. The review of literature includes studies using human-safe antiseptics on other types of equipment, as well as studies which show effective eradication of microbial growth, but with disinfectants that are not human-safe. Most of the antiseptics and disinfectants which have been tested are fairly expensive, are not commercially available, and are likely neither available nor feasible for use in areas of the world which do not have access to high quality medical care, yet have disease.

The study by Li et al (2015), which was completed only very recently, comes the closest to matching the testing needed for blue bulb syringes; it combined a human-safe antiseptic with a piece of equipment that routinely comes into contact with delicate tissues. As a result, low-cost, widely-available, human-safe antiseptics, such as those used by Li et al. (2015), were tested on blue bulb syringes to determine their effectiveness in eradicating microbial growth found in the bulbs.

A potential outcome of new, original research into best practice for disinfection of blue bulb syringes is the need for a new disinfection protocol for healthcare providers and parents. In order to implement this new protocol, hospitals and parents would need to purchase the antiseptics, and healthcare providers and parents would require education in the new protocol.
Barriers to implementation of a new method of disinfection are manifold. The first big hurdle is tradition. Blue bulb suctioning has been a routine intervention on newborns for decades, and the bulbs are routinely given to parents to take home. Whether or not parents routinely clean these syringes is unknown. It may be difficult to overcome attitudes such as if a blue bulb syringe was used on the parent when they were a baby, it was cleaned only sporadically, and the parent survived with no ill effects, why should the parent do any differently with their own child?

Education of healthcare providers and parents is another barrier to implementation; clearly, if a new disinfection protocol is needed, both healthcare providers and parents will require training in the new protocol. This training requirement, in turn, leads to another barrier: cost. The cost of healthcare provider and parent training could be substantial.

In addition to educational costs, there is also the cost of the antiseptics themselves. Many of the newer antiseptics and disinfectants are expensive, and may or may not be commercially-accessible for home use. Cost and accessibility of the antiseptic must also be considered when deciding on a new protocol. This is a particularly important consideration for poorer socioeconomic areas and areas in which regular access to high-quality medical care may be in question.

Methods

Selection of Antiseptics

Criteria for selection of an antiseptic included prior use in the hospital setting or claims that the antiseptic kills bacteria. The antiseptics selected met one or both of these criteria as well as our self-imposed criterion that the antiseptic be inexpensive and widely-available so that
parents would have ready access for home use, and so that any effective antiseptic could be made available at minimal cost to underdeveloped areas of the world.

Five antiseptics were selected for testing. Table 1 summarizes the selected antiseptics and their available formularies.

Table 1. Selected antiseptics and formularies

<table>
<thead>
<tr>
<th>Antiseptic</th>
<th>Availability</th>
<th>Preparation used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triclosan 0.1%</td>
<td>Active ingredient in Great Value® antibacterial dish detergent</td>
<td>50% solution with sterile water as solvent (effectively 0.05%)</td>
</tr>
<tr>
<td>Hydrogen peroxide 3%</td>
<td>Active ingredient in Equate® hydrogen peroxide</td>
<td>Full strength; no dilution</td>
</tr>
<tr>
<td>Povidone-iodine 10%</td>
<td>Active ingredient in Equate® First Aid Antiseptic</td>
<td>Full strength; no dilution</td>
</tr>
<tr>
<td>l-lactic acid 2%</td>
<td>Active ingredient in Palmolive Ultra® Antibacterial Orange Dishwashing Liquid &amp; Hand Soap</td>
<td>75% solution with sterile water as solvent (effectively 1.5%)</td>
</tr>
<tr>
<td>Chlorhexidine gluconate 0.12%</td>
<td>Active ingredient in Peridex® prescription mouthwash</td>
<td>Full strength; no dilution</td>
</tr>
</tbody>
</table>

**Laboratory Protocol**

Experiment protocols in the laboratory typically require each experiment to be performed in triplicate to ensure validity and reliability of results; this requires three sets of supplies and equipment for each experiment.

The terms antiseptic and disinfectant are frequently used interchangeably. Strictly speaking, they have different meanings. An antiseptic is safe for application on human tissues, whereas a disinfectant is typically used on inanimate objects. For purposes of this study, the term antiseptic is used when referring to the chemical added to the bacteria specimen which is intended to kill it. Since a BBS comes into contact with mucosal tissue, only antiseptics have
been tested. The term disinfection applies to the process of attempting to bacteria using either an antiseptic.

This study involved an experimental approach to eradicating bacterial growth in the BBS. Each testing cycle consisted of three identical experiments which tested a specific concentration of a selected antiseptic. Prior to each experiment, a bacterial culture suspension was prepared from BBS samples obtained from a local hospital and identified during an earlier research project (O’Neal et. al, 2017). In that study, the dried accumulated secretions were washed from the original BBS using sterile phosphate buffered saline (PBS) and the resulting suspension was plated for growth. Isolated colonies were sampled and identified via numerous tests. Bacterial isolates were prepared from the now-identified bacteria, and provided the \textit{Escherichia coli} for our disinfection testing.

Two days prior to each experiment, a sample of the isolate was cultured from freezer stock on Mueller-Hinton (MH) agar and incubated overnight at 37°C in a VWR® incubator. The day before each experiment, isolated colonies were selected from the previous day’s culture and were subcultured in 50 mL MH broth at 37°C at 200 rpm in a Lab Line 3527 Orbital Incubator Shaker.

On experiment day one, 100 µL of suspension from the previous day’s subculture was added to 50 mL MH broth to create a suspension which was grown, again at 37°C at 200 rpm, to a specific optical density (at least 0.003). The optical density at 600 nm wavelength of light (\(OD_{600}\)) was measured using a Thermo Scientific™ Spectronic 20D+ spectrophotometer, with a sample of 50 mL of MH broth and 100 µL of sterile water to calibrate. The optical density relates directly to the number of colony-forming units (CFU) per milliliter and ensures consistent concentration of bacteria in the experimental sample (Figure 1). Once the subculture grew to the
minimum optical density, the suspension was centrifuged for 10 minutes at 4°C at 7000 rpm using a Beckman Coulter® model J2-21 centrifuge, the solute was decanted, the packed cells were resuspended in 5 ml fresh Mueller-Hinton broth, and placed on ice for 30 minutes.

The volume of calibrated isolate, $V_1$, added to the experimental sample was determined using the formula

$$C_1 \times V_1 = C_2 \times V_2.$$

$C_1$ represents the desired optical density/concentration ($OD_{600}$) of the calibrated experimental sample (in this case 0.002), while $V_1$ represents the volume of calibrated isolate to be added to the experimental sample. $C_2$ represents the $OD_{600}$ of the available cultured sample, while $V_2$ represents the volume of control sample, in this case 1 ml Mueller-Hinton broth. When the volume of calibrated isolate added to the experimental sample exceeded ten percent (10%) of the experimental sample volume, the equation

$$C_1 \times V_1 = C_2 \times (V_1 + V_2)$$

was used to adjust for the additional volume and ensure that the concentration of the experimental sample did not exceed desired parameters.

Each experiment included an intervention consisting of the application of a specific concentration of the antiseptic being tested. Each antiseptic tested also included a negative control experiment, in which the complete experiment was run, but without adding any antiseptic to the bacterial suspension.

Prior to the application of the antiseptic, a sample of the bacterial suspension was drawn, serial dilutions were completed and were plated and incubated for growth; this became the time 0
sample to which all subsequent samples were compared. The antiseptic was added to the bacterial suspension, and samples were drawn at 1, 2, 3, 4, 5, 10, 15, 20, 25 and 30 minutes. For each of these time points, a sample was drawn from the now-disinfected bacterial suspension and serial dilutions were performed to determine the time required to kill existing bacteria in the suspension. The serial dilutions were plated and incubated overnight for colony growth and observation (Figure 2).

After incubation, the number of CFU in the suspension at each time point was determined by dividing the colony count by the dilution factor. A logarithmic graph of the CFU at time t divided by the CFU at time 0 was created to indicate the ratio of bacteria remaining after disinfection, using the equation \( \log \left( \frac{N_t}{N_0} \right) \). \( N_t \) represents the number of CFU grown from a sample obtained at time t minutes into the experiment, while \( N_0 \) represents the number of CFU grown from the calibrated experimental sample at time 0, before adding any antiseptic. An effective kill is defined as a 2-log kill in under one minute.

The initial experiment, defined as a specific combination of bacteria and antiseptic, was repeated twice more during the testing cycle to ensure reliability and validity of experimental findings.

**Results**

Of the five antiseptics tested, two, Triclosan and hydrogen peroxide, were essentially ineffective in achieving a 2-log kill in under one minute. Triclosan, even at very high concentrations up to five time the MIC, showed little to no bactericidal activity; it was bacteriostatic at all tested concentrations. These findings support the Food and Drug Administrations’s recent ban on Triclosan (FDA, 2016) in household products.
Hydrogen peroxide achieved mixed results; eventually it was bactericidal, but took an unacceptably long nearly four minutes to achieve bactericidal state. Consequently, hydrogen peroxide cannot be said to be a reliable antiseptic for killing \textit{E. coli} growth in a BBS within a desired reasonable timeframe.

Povidone-iodine, l-lactic acid and Chlorhexidine gluconate each proved an effective antiseptic, achieving a 4-log kill, meaning that it killed 99.99\% of bacteria in the calibrated experimental sample, in under sixty seconds, provided concentrations of at least four times MIC were used. Since each achieved a 4-log kill in under a minute, they far exceeded the hoped-for 2-log kill under one minute that indicated efficacious killing of \textit{E. coli} in the BBS. Povidone-iodine achieved a 2-log kill, meaning that more than 99\% of bacteria in the calibrated experimental sample were killed, in an average 26.3 seconds. L-lactic acid achieved a 2-log kill in an average 25.3 seconds, while Chlorhexidine gluconate required an average of only 24.4 seconds to achieve a 2-log kill. Table 2 summarizes the average kill times for each antiseptic at the required minimum concentration to assure a 2-log kill.

Table 2. 2-log kill times

<table>
<thead>
<tr>
<th>Antiseptic</th>
<th>Minimum Inhibitory Concentration (MIC)</th>
<th>Concentration required</th>
<th>2-log kill time (average of 3 experiments)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triclosan 0.1%</td>
<td>64 µg/ml (Assadian et al., 2011)</td>
<td>320 µg/ml (5 * MIC)</td>
<td>&gt;30 minutes</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>2505 µg/ml (Mazzola, Jozala, Novaes, Moriel, &amp; Penna, 2009)</td>
<td>12525 µg/ml (5 * MIC)</td>
<td>5.25 minutes</td>
</tr>
<tr>
<td>Povidone-iodine</td>
<td>1024 µg/ml (Oduwole et al., 2010)</td>
<td>4200 µg/ml (4 * MIC)</td>
<td>26.3 seconds</td>
</tr>
<tr>
<td>l-lactic acid 0.2%</td>
<td>2000 µg/ml</td>
<td>8000 µg/ml (4 * MIC)</td>
<td>25.3 seconds</td>
</tr>
<tr>
<td>Chlorhexidine gluconate 0.12%</td>
<td>71 µg/ml (Mazzola et al., 2009)</td>
<td>355 µg/ml (5 * MIC)</td>
<td>24.4 seconds</td>
</tr>
</tbody>
</table>
Figure 3 summarizes, in graphical form, the comparative effectiveness of the tested antiseptics in killing *E. coli*. The log \( \frac{N_t}{N_0} \) for the control experiment remained near zero, indicating that the absence of an intervention in the form of application of antiseptic resulted in a nearly constant level of bacteria. The log \( \frac{N_t}{N_0} \) for Triclosan also remained near zero, indicating that it did not achieve bactericidal status, but was merely bacteriostatic. Triclosan neither killed nor allowed further bacterial growth; it, too, resulted in a nearly constant level of bacteria. For hydrogen peroxide, the log \( \frac{N_t}{N_0} \) falls below -2, indicating a 2-log kill, approximately 5.25 minutes after application of hydrogen peroxide to the bacterial sample. The log \( \frac{N_t}{N_0} \) for Povidone-iodine, Chlorhexidine gluconate and l-lactic acid all fall below -2 within 26.3 seconds after application, indicating that each of these effectively killed 99 percent of existing *E. coli* bacteria in under 27 seconds.

![Figure 3. Disinfection of *E. coli* V050-A1 in Mueller-Hinton broth with Triclosan, hydrogen peroxide, povidone-iodine, l-lactic acid and Chlorhexidine gluconate](image-url)
Detailed experimental results and graphs for each antiseptic are summarized in Figures 4 through 8, which are included in Appendix B.

**Limitations**

This pilot study was limited in its scope and breadth; therefore, its findings are limited as well. Only *Escherichia coli* was included in this study. Consequently, the findings of this study may not be applicable to other bacteria growing in a BBS. Only bacteria were tested. Fungi, viruses and molds were excluded from this study. A quick Google image search on “blue bulb syringe cut open” yields many photos with evidence of mold and other growth in a BBS. Further study is needed to determine if the same antiseptics which were effective on *E. coli* would be effective on other bacteria, fungi, viruses and/or molds found growing in a BBS.

**Discussion and Implication to Practice and Implementation**

The current recommendation of rinsing in warm, soapy water is clearly an ineffective method for killing *E. coli* growing in a BBS. Triclosan, the most common active ingredient in so-called antibacterial dish detergents, which was recently banned by the FDA for inclusion in household products, never achieved bactericidal status; it was merely bacteriostatic even at the highest concentrations tested. Clearly, a new method of cleaning the BBS, one which adds the potential for killing bacterial growth inside a BBS, is needed.

While hydrogen peroxide did eventually kill the *E. coli*, it is safe to assume that hydrogen peroxide would not be the go-to antiseptic of choice for disinfecting a BBS. It is unrealistic to expect busy parents, healthcare workers and medical missioners to spend ten minutes soaking a BBS in hydrogen peroxide when other options exist which will achieve the same bactericidal status in one tenth the time, and at similar cost.
Povidone-iodine, although not currently approved by the FDA for contact with mucosal tissues for patients in the United States, was an effective antiseptic, killing existing *E. coli* in under 30 seconds at concentrations of at least four times MIC. It is, however, used as a mouthwash to prevent VAP in other countries, including India (Seguin, Tanguy, Laviolle, Tirel & Mallédant, 2006), so could potentially be used on a BBS elsewhere other than the United States. It was the lowest cost bactericidal option for disinfecting a BBS: this warrants further research into whether it would be safe for use on a BBS if it were followed by a sterile water rinse.

L-lactic acid, the active ingredient in Palmolive Ultra® Antibacterial Orange Dish Detergent, was effective in killing *E. coli* growth provided it was used at concentrations of at least four times MIC. Since this detergent is already used to wash dishes, glassware and utensils that come into contact with oral mucosal surfaces while eating, it is reasonable to assume that this antiseptic could be used on a BBS as well, without adverse effect.

Chlorhexidine gluconate is the active ingredient in prescription mouthwashes such as Peridex® and is used in routine oral care in the healthcare setting for prevention of VAP. Consequently, it already has FDA approval for oral mucosal contact.

As nurses, healthcare workers, parents and caregivers continue to suction newborns, infants and young children, care must be taken to assure that bacteria is not reintroduced into the oral and nasal cavities. Three widely available, low cost options exist for assuring that, at a minimum, the most common bacteria found in a BBS syringe after a vaginal delivery is effectively eradicated following a thirty second application of antiseptic.
References


Appendix A

Figure 1. Specimen Preparation (Experiment Phase)

- Grow bacterial suspension to minimum optical density (≥0.003)
- Centrifuge to pack cells and resuspend
- Subculture in MH broth
- Streak for isolation on Mueller-Hinton (MH) agar
- Experiment Day
- Day Prior to Experiment
- Two Days Before Experiment
Figure 2. Experimental Process

1. Add bacterial suspension and sample time 0
2. Add specified concentration of antiseptic
3. Prepare serial dilutions and plate for growth
4. Draw sample of disinfected bacteria
5. Incubate at 37°C
6. Observe plates for growth and colony count
7. Repeat at times 1, 2, 3, 4, 5, 10, 15, 20, 25, 30

Day After Experiment

Experiment Day
Figure 4. Disinfection of *Escherichia coli* in Triclosan
Figure 5. Disinfection of *Escherichia coli* in Hydrogen Peroxide
Figure 6. Disinfection of *Escherichia coli* in Povidone-Iodine
Figure 7. Disinfection of *Escherichia coli* in l-lactic acid
Figure 8. Disinfection of *Escherichia coli* in Chlorhexidine gluconate
Appendix C

IRB Statement

IRB approval for this study was deemed unnecessary since human specimens were not collected for this research.
Appendix D

Dissemination of Scholarly Work and Awards

Honors and Awards

UAH Research Horizons Day, Undergraduate Nursing Award, April 2016 – Development of a new model for laboratory testing to improve efficiency and reduce costs

Alabama Academy of Science Health Service Paper Award, February 2016 – Efficacy of Triclosan and Povidone-Iodine as Disinfectants Against *Escherichia coli* Isolated from Neonatal Oronasopharyngeal Suctioning Devices

Sigma Theta Tau International inductee, Spring 2017

Nominated for UAH College of Nursing Undergraduate Programs Award for Leadership Excellence and consideration for Dean’s Award

Vaught and Heffelfinger Family Memorial Scholarship awardee, 2015-present

Activities and Organizations

Undergraduate Representative, UAH College of Nursing Strategic Management Committee, 2016-2017

2nd Vice President, UAH Association of Nursing Students, 2016-present

Elsevier Peer Support Group, UAH, 2015-present

Misión Médica de San Tomás, medical mission to Colonia Episcopal, Puerto Cortés, Honduras, 2016-present

Finance Manager for annual disaster drill, 2017

Research Experience and Presentations

Member of CLEarance of the AiRways (CLEAR) research team, investigating best practice in oronasopharyngeal suctioning, UAH, 2016-present
KILLING BACTERIAL GROWTH IN A BBS: CLINICAL ISSUE

Research Assistant, CLEAR team, 2017 – AWHONN grant on newborn suctioning practices
Rising Stars in Research and Scholarship Invited Student Poster, Sigma Theta Tau International Biennial Conference, Indianapolis, IN, October 2017 – Recommended Antiseptics for Killing Bacterial Growth in Neonatal Blue Bulb Syringes: Addressing a Clinical Issue
So You Think You Can Research?, UAH, April 2017 – Blue Bulbs, E. coli and Babies, Oh My!
So You Think You Can Research?, UAH, April 2016 – Efficacy of Triclosan and Povidone-Iodine as Disinfectants Against Escherichia coli Isolated from Neonatal Oronasopharyngeal Suctioning Devices
Research and Creative Experience for Undergraduates (RCEU) student research poster presentation, September 2015 – Identification of Effective Disinfectants on Bacterial Growth
Invited poster presenter: Alabama State Nurses Association conference: Abuse, Neglect and Human Trafficking, September 2015
RCEU student research representative, prospective student day, July 2015 – Identification of Effective Disinfectants on Bacterial Growth
RCEU grant for $3200, Summer 2015 – Identification of Effective Disinfectants on Bacterial Growth

Academic Journal Publications

devices used to promote airway clearance in newborns in intrapartum and postpartum units. *American Journal of Infection Control* (In press).

**Certifications and Specialty Coursework**

Basic Life Support

VHA Military Culture: Core Competencies for Health Care Professionals Self-Assessment and Introduction to Military Ethos

VHA Military Culture: Core Competencies for Health Care Professionals Military Organization and Roles

VHA Military Culture: Core Competencies for Health Care Professionals Stressors and Resources

VHA Military Culture: Core Competencies for Health Care Professionals Treatment Resources and Tools

FEMA IS-00100.hcb Introduction to the Incident Command System (ICS 100) for Healthcare/Hospitals

FEMA IS-00200.hca Applying ICS to Healthcare Organizations ICS-200 for Health Care/Hospitals

FEMA IS-00240.b Leadership and Influence

Graduate level independent study on Donabedian Design, Lean Six Sigma, Plan-Do-Study-Act applied in the laboratory setting, 2016
Figure 9. Sigma Theta Tau International Rising Stars of Research and Scholarship Invited Student Poster, Indianapolis, Indiana, October 2017
Figure 10. Blue Bulbs, *E. coli* and Babies, Oh My!, UAH So You Think You Can Research?, April 2017
Assessing the Efficacy of Recommended Antiseptics for Killing Bacterial Growth in Neonatal Blue Bulb Syringes: Addressing a Clinical Issue

Linda A. Hanson, MBA, BSN Honors Student, College of Nursing, Pamela V. O’Neal, PhD, RN, College of Nursing, Ellise Adams, PhD, CNM, College of Nursing, Joseph G. Leahy, PhD, College of Science

Overview
Blue Bulb Syringes (BBSs)
- Used to remove oral and nasal secretions from newborns
- Provided to parents at hospital discharge
- Can be purchased by parents for home use during times of respiratory illness
- Multi-use device
- Current recommendation for cleaning: rinsing in warm, soapy water
- Previous research identified bacterial growth in a BBS
- No research studies have identified the efficacy of cleaning methods for killing bacteria growing in secretions inside the BBS
- Three inexpensive, widely-available antiseptics are effective in killing bacterial growth within a BBS

Methods
Why *Escherichia coli*?
- Most common bacteria (approximately 10%) found in BBS used in vaginal deliveries
- Leading cause of neonatal sepsis in newborns
- Leading cause of Early Onset Neonatal Bacterial Meningitis (EONBM)

Intervention
Application of a specific concentration of selected antiseptic
Experiments run in triplicate to ensure integrity of results
Null hypothesis
Intervention would have no impact on bacterial colony count

Criteria for selection of antiseptic included being inexpensive and widely available:
- Triclosan: active ingredient in Equate® antibacterial dish detergent
- Hydrogen Peroxide: Equate® hydrogen peroxide
- Povidone-Iodine: active ingredient in Equate® antiseptic and Betadine
- L-Lactic Acid: active ingredient in Palmolive® antibacterial dish detergent
- Chlorhexidine Gluconate: active ingredient in Peridex® mouthwash

Results
- Negative control experiment showed that intervention is necessary to achieve bactericidal state
- Triclosan is not an effective antiseptic – supports the recent FDA ban on Triclosan in household products
- Hydrogen Peroxide was bactericidal, but took approximately 4 minutes to achieve a 2-log kill
- Povidone-Iodine achieved a 2-log kill, killing 99% of existing bacteria in 27 seconds
- L-Lactic Acid achieved a 2-log kill, killing 99% of existing bacteria in 26 seconds
- Chlorhexidine Gluconate achieved a 2-log kill, killing 99% of existing bacteria in 25 seconds

Impact on Nursing
- Blue Bulb Syringes have the potential to cause disease in a newborn or young child if reused
- Three antiseptics identified which are more effective than the current recommendation at killing bacterial growth in a BBS
- Identified antiseptics are inexpensive and widely available
- Identified antiseptics can be used in underdeveloped areas of the world
- Effective killing of bacteria in a has potential to break the chain of infection at the mode of transmission

Acknowledgments
Research and Creative Experience for Undergraduates, UAH Laboratory assistants: Tanuj Alapati, Lana Harwell, Alex Moore
The CLEAR Project Team
Marsha Adams, PhD, RN, CNE, ANEF, FAAN, Dean, College of Nursing

Figure 11. Assessing the Efficacy of Recommended Antiseptics for Killing Bacterial Growth in Neonatal Blue Bulb Syringes: Addressing a Clinical Issue, UAH Research Horizons Day, April 2017
Figure 12. Efficacy of Triclosan and Povidone-Iodine as Disinfectants Against *Escherichia coli* Isolated from Neonatal Oronasopharyngeal Suctioning Devices, UAH So You Think You Can Research?, April 2016
KILLING BACTERIAL GROWTH IN A BBS: CLINICAL ISSUE

Research Horizons Day
April 12, 2016

Development of a New Model for Laboratory Testing to Improve Efficiency and Reduce Costs

Linda A. Hanson, MBA, AB, College of Nursing, Pamela V. O’Neal, PhD, RN, College of Nursing

Overview
- Laboratory protocols typically require experiments to be performed in triplicate to ensure validity and reliability of results
- Each round of experiments requires three sets of supplies and equipment
- For our research, a round of experiments consists of a selected clinical isolate of bacteria, along with a selected concentration of antiseptic, completed in triplicate
- New model uses a more targeted approach for selecting the concentrations of antiseptics tested
  - Minimum inhibitory concentration (MIC) - the minimum concentration of antiseptic required to inhibit further bacterial growth is tested
  - A higher concentration is tested
  - If experimental outcome is similar (remains bacteriostatic), a higher concentration is selected
  - If experimental outcome is opposite (becomes bactericidal), an intermediate concentration is selected

Experiment Schedule Using Old Model
- Concentrations tested (in triplicate):
  - Minimum inhibitory concentration (MIC)
  - 2 times MIC
  - 3 times MIC
  - 4 times MIC
  - 5 times MIC
  - Intermediate concentrations to determine point at which antiseptic changed from bacteriostatic to bactericidal
  - 18 experiments in total

Experiment Schedule Using New Model
- Concentrations tested (in triplicate):
  - Minimum inhibitory concentration (MIC)
  - 3 times MIC
  - 2 times MIC
  - Intermediate concentrations
  - 12 experiments in total

Key Findings
- Saved over $100 for each experiment that was eliminated using the new model
- Eliminated approximately 1/3 of experiments required to achieve complete results

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Total Savings $54.72

Explaination/Impact
- Targeted approach can be used to reduce the number of experiments required to achieve similar experimental outcome
- Over $515 in cost savings

Experiment Results Using Old Model

Experiment Results Using New Model

Summary
- Scientific integrity was maintained
- Unnecessary redundancies were eliminated
- Economic efficiencies were realized

Acknowledgements
Research and Creative Experience for Undergraduates, UAH
The CLEAR Project Team
Joseph G. Leathy, PhD, Department of Biological Sciences
Ellis D. Adams, PhD, CNM, College of Nursing
Marsha Adams, PhD, RN, CNM, ANEF, FAAN, Dean, College of Nursing

Figure 13. Development of a New Model for Laboratory Testing to Improve Efficiency and Reduce Costs, UAH Research Horizons Day, Undergraduate Nursing Award, April 2016
Figure 14. Efficacy of Triclosan and Povidone-Iodine as Disinfectants Against *Escherichia coli* Isolated from Neonatal Oronasopharyngeal Suctioning Devices, Health Sciences Paper winner, Alabama Academy of Science Conference, Florence, Alabama, February 2016
Identification of Effective Disinfectants on Bacterial Growth

Linda A. Hanson, Honors BSN Student, College of Nursing
Pam O’Neal, PhD, RN, Joseph G. Leahy, PhD

Introduction

- Oronasopharyngeal suctioning (ONPS), using Blue Bulb Syringes (nasal aspirators), has been a traditional intervention used on neonates immediately after delivery for decades.
- Studies suggest that ONPS may cause infection as a result of repeated use of the suctioning device.
- There are virtually no studies which have specifically examined disinfection techniques for ONPS devices.

Significance to Nursing

- Microorganisms such as bacteria cause disease.
- Blue Bulb Syringes are used repeatedly and are considered multi-use devices.
- Pathogenic bacteria have been identified in clinical isolates taken from Blue Bulb Syringes (nasal aspirators) used on neonates.
- Disinfection is needed if ONPS is to be used repeatedly.
- Effective disinfection methods to kill the most common bacteria found in Blue Bulb Syringes have not yet been identified.
- An effective, low-cost disinfection method safe for use on ONPS devices used on neonates and young children is essential for nurses and parents in providing better health care to babies in their charge.

Key Findings & Results

- Triclosan does not appear to be effective in inactivating bacterial cells.
- Povidone-iodine appears to be effective in inactivating bacterial cells, especially at high concentration.

Conceptual Framework

- Abdeliah’s Theory of 21 Problems in Nursing
  Nurses have a duty to promote safety by taking action in patient care to prevent disease.
- Germ Theory of Disease
  Pasteur – Heat can kill bacteria.
  Koch’s Postulates – Specific microorganisms cause specific diseases.
- Lister – Antiseptic surgical technique significantly reduces infection and/or death.
- Henle – Investigated how microorganisms are spread.

References


Acknowledgements

Research and Creative Experience for Undergraduates, UAH
The CLEAR Project Team

Figure 15. Identification of Effective Disinfectants on Bacterial Growth, Research and Creative Experience for Undergraduates, Summer 2015 and Alabama State Nurses Association conference: Abuse, Neglect and Human Trafficking, September 2015.