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Review Article

Sterilisation of Infant Feeding Bottles: Focus on the Cold-Water Method Using Chlorine Containing Compounds

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Abstract

Sterilisation of infant feeding equipment is fundamental to reduction in microbial contamination and protecting infants from the risk of infection. The cold-water method of sterilisation using chlorine-containing compounds has an excellent anti-microbial action, but Health care professionals (HCPs), parents and carers must be educated in its appropriate use if it is to work effectively. This review explains the need for sterilisation in the context of the infant's immature immune function and describes the bacteria, viruses and fungi that colonize infant feeding equipment if it is not cleaned and sterilised. It highlights official guidance in this area and compares the main methods for sterilisation with a focus on the cold-water method and use of chlorine-containing compounds (hypochlorite and troclosene). This review explains how chlorine-containing compounds work and presents data showing their efficacy in removing microbes from feeding equipment. In also emphasizes the need for education on sterilisation of feeding equipment if it is to work effectively.

Keywords: Feeding bottles; Hypochlorite; Chlorine; Sterilisation; Infants; Microbial contamination; Infection

Introduction

Public health guidelines globally recommend sterilization of infant feeding equipment for the first 12 months of an infant's life to reduce the risk of infection. Several methods of sterilisation are recommended of which the cold-water method using chlorine containing compounds (e.g. hypochlorite and troclosene) is one method with boiling and steam sterilisation (including microwaving) being two others. This short review paper evaluates the need for sterilisation of baby feeding equipment and the efficacy and safety of chlorine-containing compounds used in the cold-water sterilisation method.

Health care professionals, parents and carers are provided with information on feeding, breast milk, types of infant formula (if the infant is to be bottle fed) and feeding equipment. However they may be given little information on care and sterilisation of feeding and associated equipment. Such equipment can easily become contaminated with microbes risking the health of the infant and it is vital to ensure that infant feeding equipment (bottles, bottle caps and rings) and pacifiers (dummies) are appropriately sterilised.

The Need for Sterilisation

The need for sterilisation of baby feeding equipment (e.g. bottles, teats, rings and caps that go with the bottles) and associated equipment (e.g., pacifiers) arises from the potential for microbial colonisation in the context of an infant's immature immune function and risk of infection. Milk provides an ideal breeding ground for microbes (bacteria, viruses and yeasts) that could make an infant very ill. This is why sterilising of bottles and feeding equipment is recommended by national authorities such as the UK National Health Service (NHS) for babies of 12 months and under.

A newly born infant – and for the first 12 months of life - has an immature immune system which matures and acquires 'memory' as the infant develops and grows. The immune system consists of two parts – the innate immune system which provides the first line of defense provided by a range of white cells (neutrophils, monocytes, macrophages and dendritic cells) and the adaptive immune system which interacts with the innate immune system and consists of various types of T cells and B cells. Some antibody protection is transferred by the mother to the infant in utero. Infants are at high risk of bacterial, fungal and viral infections with immune response increasing through childhood and young adulthood before declining in older age [1].

Risks of infection in infants arise from feeding equipment and, in those fed powdered infant formula, may occur with infection in the formula itself due to *Enterobacter sakazakii*. During manufacture of powdered infant formulae, contamination may occur with *Enterobacter sakazakii* and *Salmonella enterica* [2]. This is because during current manufacturing processes, it is not feasible to produce sterile product.

During preparation of infant feed itself in the home or care setting, inappropriate handling practices by parents, carers and healthcare professionals, including poor sterilisation practice, can exacerbate the problem of microbial contamination. Extrinsic contamination can occur when contaminated utensils (e.g. spoons, blenders, bottles, teats) are used for preparing or feeding infant formula, or contamination may occur from the preparation environment. Risk of infection can be significantly reduced if formula is prepared safely and handled correctly with appropriately sterilised equipment.

Which Microbes Colonize Infant Feeding Equipment?

A range of microbes (bacteria, viruses and fungi) may colonise infant feeding equipment. Microbial growth in feeding bottles is facilitated by the tendency of milk or formula milk to become a culture medium and by inappropriate cleaning and sterilisation of bottles.

Infant feeding bottles may be contaminated with *Escherichia coli* and a range of other coliforms such as *Klebsiella* species, *Enterobacter* and *Citrobacter*. *E coli* was found in 56.3per cent of feeding bottles in this study and all the bottles contained coliforms. *Staphylococcus aureus*, enteropathogenic *E. coli, Bacillus cereus*, shigella species, salmonella [3] and *Serratia marcescens* [4] have also been isolated from some bottles. A highly detailed study from Japan revealed a vast range of other bacteria in bottled milk stored for more than 3 hours with bacteria transferred to artificial teats [5].

Outbreaks of *Enterobacter sakaazakii* have been attributed to feeding equipment. *E. sakazakii* is widespread in the environment and has been shown to attach and grow and form 'biofilms' on surfaces commonly used in infant feeding equipment, such as latex, silicon and stainless steel. It is therefore important that all infant feeding and preparation equipment (e.g. feeding cups, bottles, rings and teats) has been thoroughly cleaned and sterilised before use, since the formation of biofilms on such equipment may result in reservoirs of infection that can continually contaminate feeds [6].

Pacifiers (dummies) are also repositories of microbes. A study of the surface of 40 pacifiers found that microorganisms were isolated from 21 (52.5 per cent) pacifiers. The number of colonies per pacifier varied from between one and 35 (average six). The isolates included eight alpha-haemolytic streptococci, six *Staphylococcus epidermis*, five *Candida albicans*, five alpha-haemolytic streptococci, three Neisseria species and two *Staphylococcus aureus*. A further study of 28 children using pacifiers found contamination by *Streptococci mutans* [7]. *Candida albicans* is also found on pacifiers [8].

Infants at greatest risk from microbial contamination are term infants <1 year and with neonates and infants <2 months of age at greatest risk. Infections may occur in both hospital and out of hospital settings so it is important that educational messages on safe handling of powdered infant formulae and feeding equipment are required for health care workers, parents and other infant carers. They may not be aware of the risks nor familiar with best practice in sterilising feeding equipment.

Guidance on Sterilisation

Official guidelines (where they exist) recommend sterilisation of feeding equipment during the first 12 months of an infant's life [2,9]. It is very important that all equipment for feeding infants and preparing feeds is thoroughly cleaned and sterilised.

Cleaning and sterilising are two different but overlapping processes and both are required. Cleaning is a process that entails physical removal of microorganisms and anything else that is not part of the feeding equipment. It is achieved by rinsing and washing with soap and water. Cleaning achieves removal of milk deposits that can harbour germs. Sterilisation describes a process which eliminates all microorganisms with a reasonable degree of assurance [10] with the exception of bacterial spores from a surface. Cleaning enables sterilisation – that is removal of most pathogens – to be achieved more easily because milk deposits have been removed from the feeling equipment. Compared with sterilisation, cleaning does not have the same effect on killing microbes but must be done to allow for effective sterilisation.

Comparison of Methods for Sterilisation of Infant Feeding Equipment

Sterilisation Method	**Advantages**	**Disadvantages**
Cold water sterilisation	• Cheap, simple low-tech, flexible: can sterilise small or large numbers of pieces of equipment • Does not take energy (heat) • Developed to sterilise feeding equipment within 15 minutes • Used to sterilise all breast feeding and infant bottle-feeding equipment • Items remain sterile for 24 hours if left in the solution • No need to wait for bottles to cool down as no heat used • No need to rinse • Can take items out of the sterilising fluid when needed • Portable: convenient to use at home, travel and overnight stays	• No need to rinse, but a weak smell of chlorine may be evident when the bottles are removed from the fluid.
Boiling	• Cheap	• User may burn themselves with hot water. • Will not kill many bacterial spores, such as tetani, gas gangrene and anthrax bacilli. • Does not penetrate well into organic materials that may harbour microbes. • Teats tend to get damaged faster than with other methods • May cause release of microplastics into feed and the environment
Steam sterilisation	• Large capacity: can accommodate up to 8 bottles • May include an integrated dryer • Takes 8 minutes to sterilise. • Steam (because it's a gas) can easily spread across the entire bottle or other equipment (unlike UV beams) regardless of shape	• Need to rinse after the process • Bulky equipment • Contents remain sterile only if the steriliser door is kept shut • May release microplastics into the feed and the environment
UV sterilisation	• Effectively kills bacteria (like steam)	• Effectiveness determined by where the light shines on items to be sterilised • Parts of bottle teats and dummies likely to be missed by the straight path where the light shines • May require 60 minutes to remove 99.9 per cent of germs • UV rays may degrade plastics used in bottles and other equipment • UV sterilisers tend to have small capacity so need to be used more frequently • Some feeding equipment brands advise against their use

Table 1: WHO (2007) guidance on cleaning and sterilising infant feeding equipment.

1. Hands should always be washed thoroughly with soap and water before cleaning and sterilising feeding equipment.
2. Wash feeding and preparation equipment (cups, bottles, teats, spoons) in hot, soapy water. Where feeding bottles are used clean bottle and teats brushes should be used to scrub the inside and outside of all bottles and teats to make sure all traces of feed are removed.

3. After washing equipment, rinse in microbiologically safe water (does not need to be sterile unless the infant is at particular risk)
4. For sterilisation itself, follow manufacturer instructions.
5. Hands should be washed thoroughly with soap and water before handling sterilised bottles and equipment.
6. To prevent recontamination remove feeding and all equipment just before use. If not used immediately it should be covered and stored in. a clean environment. Bottles can be assembled fully to prevent contamination of inside of the bottle and inside and outside of the teat from becoming contaminated.

Methods of Sterilisation

There are four main methods of sterilising infant feeding equipment:

- Cold water sterilising solution
- Boiling
- Steam sterilising
- Ultraviolet (UV) sterilisers

It is difficult to tell which of these four methods is the most effective. One study comparing a hypochlorite-based solution and three heat-based methods for sterilising bottles found no significant difference in effectiveness between the four methods. The study found that strict adherence to all four methods allowed effective decontamination with all four methods [11].

Cold water sterilisation involves use of chemicals, which are mixed with cold water to produce a sterilising solution. They are available in concentrated liquid form (e.g. sodium hypochlorite solution) or a tablet (e.g. troclosene sodium), can be added to water. They are also available as a ready-to-use sterilising solution. Chlorine-containing compounds are effective in sterilising within 15 minutes. Items for sterilisation should be submerged in the sterilising solution for 30 minutes.

Boiling at 100 degrees Centigrade for 10 minutes kills the majority of bacteria. Boiling can damage equipment due to the heat so items may need replacing more frequently.

Steam sterilisation works on the same principle as boiling. Water is added to the sterilising unit and the water boils creating steam that kills the bacteria. Several different types of steam sterilisers are on the market. Some steam sterilisers are used in a microwave, while others are stand-alone units that plug into the mains. Some microwave steam sterilisers can also be used as cold-water sterilisers. Reusable steriliser bags are also available for use in the microwave, or bottles that can be sterilised alone in the microwave.

It has been reported that heating plastics frequently to the high temperatures needed for sterilisation by boiling or steaming, then shaking when making up formula, releases microplastics from the bottles. The effect of these microplastics on humans is currently unknown.

Ultraviolet (UV) bottle sterilisers use UV light rather than steam to sterilise bottles. UV lights may eventually degrade the plastics used in bottles and pump parts. These plastics include polypropylene and low-density polyethylene. UV rays can break the bonds within the plastic and cause cracking or discolouration. A few infant feeding bottle brands specifically advise against the use of UV sterilisers on their products, as the rays can seriously decrease the durability of the bottles.

Chlorine Compounds Used for Cold Water Sterilisation: Sodium Hypochlorite and Troclosene Sodium

What are these compounds?

Sodium hypochlorite provides a source of chlorine and when made into a solution dissociates to form hypochlorous acid (HOCL) [12]. Sodium hypochlorite is a broad spectrum disinfectant effective for the removal of viruses, fungi, bacteria and mycobacterium. Sodium hypochlorite is not effective for the removal of bacterial spores and prions. It is found in low concentrations (2%) in some commercially available sterilising solutions (e.g. Milton) used to sterilise baby feeding equipment (bottles, bottle tops, teats), dummies and bottle brushes.

The advantages of sodium hypochlorite (see also Table 1) are that hat it fulfils the requirements for sterilisation in the baby feeding environment. Sodium hypochlorite has a broad antimicrobial spectrum with rapid bactericidal action. It is soluble in water, easy to use and it is stable and non-toxic in the dilute solutions used in infant feeding sterilising solutions. Only in high concentrations (which are not relevant to sterilising baby feeding equipment) does sodium hypochlorite display some toxic features such as irritation to mucous membranes. Sodium hypochlorite is colourless, non-flammable and non-staining.

Troclosene (sodium dichloroisocyanurate) is also a source of chlorine and available in dry form (e.g. tablets to dissolve in water for sterilisation of infant feeding equipment). In dry form and in dry storage, troclosene is very stable. When troclosene is dissolved in water it forms a 50:50 mixture of hypochlorous acid (HOCl) and monosodium cyanurate [13]. These ingredients remain in a 50:50 ratio within the solution so as the chlorine is used up (due to reaction with microbes and organic material) part of the chlorine in HOCl is freed to retain the 50:50 ratio to continue the sterilising process.

Antimicrobial Efficacy of Chlorine Containing Cold Water Sterilisation Solutions

Chlorine containing cold water sterilisation solutions for infant feeding equipment have a long history of use with the Milton Method (cold water sterilisation using 2 per cent hypochlorite solution), for example, being used for the first time in 1947. In England during the late 1940s, there was a widespread outbreak of gastroenteritis, which led to the death of 4,500 babies under the age of 1 year. Following a request from the Ministry of Health, on the BBC, to develop a better method of sterilising baby feeding equipment, Milton pioneered a new approach to sterilising baby bottles. The Milton method of cold-water sterilising was born and Milton was credited for saving many babies lives over the years.

Scientific studies since then have provided evidence that hypochlorite cold water sterilisation is effective for removing microbes from infant feeding equipment. A 1970 study which evaluated 758 infants' feeding-bottles and teats collected aseptically by health visitors in four areas of Great Britain and examined in public health laboratories, found fewer than two-thirds of the bottles and just over half of the teats produced results within an arbitrary "satisfactory" level. The 22 per cent of mothers who said they used the hypochlorite method of sterilisation and for storage of bottles and teats produced significantly better results [14].

In a 1998 study [15] 20 infant feeding bottles, contaminated with different levels of enterotoxigenic *Bacillus cereus*, were subjected in triplicate to a variety of commonly used cleaning and sterilisation procedures. Thorough cleaning reduced microbial numbers, but it did not remove all *B. cereus* present. Three commercially available sterilisation procedures (i.e. one chemical and two thermal) successfully eliminated this organism when the level of contamination was <10(5) organisms $ml^{(-1)}$. However, the chemical sterilisation method did not eliminate enterotoxigenic *B. cereus* totally at potentially hazardous contamination levels (i.e. greater than or equal to 10(5) organisms ml^{-1} which the authors stated may be encountered in the home if infant feeding bottles are stored and used incorrectly.

In a 2009 study, researchers artificially contaminated infant feeding bottles with low and high inoculum of bacterial enteric pathogens and evaluated the efficacy of several cleaning and chlorine sterilisation protocols. Rinsing with soapy water followed by tap water was the most effective cleaning method and reduced pathogen load by 3.7 and 3.1 log(10)s at the low and high inoculum levels, respectively. Submersion in 50 ppm hypochlorite solution for 30 minutes produced a 3.7-log(10) reduction in pathogens, resulting in no identifiable pathogens among bottles. When combined with handwashing and appropriate cleaning, immersion of bottles in hypochlorite solution improved the microbiological safety of infant feeds [16].

In another comparative study [11] a hypochlorite-based chemical solution and three heat-based methods were evaluated for infant feeding bottle sterilisation. Bottles were sampled in four sites (on the bottle). Before cleaning and sterilisation, the inner screw cap and inner-teat were the most heavily contaminated sites with 1.6-7.4 x 10^3 cfu/per-area-sampled; the bottle interior was more contaminated overall with 1.2 x 10^4 cfu/per-area-sampled. After disinfection, adherence to recommended procedures (combined with good hygiene) enabled effective decontamination to be achieved using all methods. Small differences in disinfection ability were not significant ($p>0.05$). Cumulatively, 800 sites were sampled and no *B. cereus* or *E. sakazakii* were isolated. *S. aureus* was isolated from 0.1 per cent of sites with one site exceeding 1 cfu/ml. These study findings indicate the potential for bottle contamination and that strict adherence to four currently used methods allows effective decontamination.

Data from Laboratoire Rivadis (makers of Milton products) demonstrate that the sterilising fluid (containing sodium hypochlorite) fulfils European (EN) standards for antimicrobial activity as follows:

- Bactericidal in 5 minutes (including Methicillin- resistant *Staphylococcus aureus* MRSA), and also effective on *Escherichia Coli*, *Enterococcus hirae* and *Pseudomonas aeruginosa*.
- Fungicidal in 15 minutes (including *Candida albicans* and *Aspergillus brasiliensis*)
- Virucidal in 15 minutes (including Bovine Coronavirus).

Laboratoire Rivadis company data also show the sterilising tablets (containing troclosene) are:

- Bactericidal in 5 minutes (including MRSA), and are also effective on listeria, salmonella and campylobacter, *Pseudomonas aeruginosa* and *Escherichia Coli*.
- Fungicidal in 15 minutes (including *Candida albicans* and *Aspergillus brasiliensis*)
- Virucidal in 15 minutes on Adenovirus type 5, in 1 minute on Bovine Coronavirus and in 5 minutes on rotavirus.

Sterilisation and hence removal of 99.9% of microbes is achieved within 15 minutes for a duration of up to 24 hours.

Mechanism of action of hypochlorite and troclosene

Sodium hypochlorite (NaOCl) and troclosene act as a source of chlorine. Both sodium hypochlorite and troclosene in solution form hypochlorous acid (HOCl), which dissociates readily to form the hypochlorite ion(-OCl) and the proton (H+).

Troclosene in solution also produces monosodium cyanurate. Together with NaOCl monosodium cyanurate remains in a 50:50 ratio within the solution. As the chlorine is used up (due to reaction with microbes and organic material) part of the chlorine in HOCl is freed to retain the 50:50 ratio and continue the sterilising process [13].

HOCl is the main active species in sodium hypochlorite's germicidal action, but both HOCl and -OCl are strong oxidising agents which react with a wide variety of biological molecules such as proteins, amino acids, peptides and lipids found in infant milk.

HOCl penetrates the microbial cell across the cell wall and membrane. It is thought that the germicidal activity is due to the inhibition of enzyme activity essential for the growth of microbes, damage to the membrane and DNA and possibly injury to membrane transport capacity [14].

OCl is not able to penetrate the microbial cell membrane because of the existence of the lipid bilayer which is hydrophobic. Some structures in the microbial cell wall also protect the microbial cell from -OCl penetration. Mycobacteria, for example, possess a peculiar cell wall structure that consist of mycolic acids that represent a hydrophobic barrier to -OCl entry. -OCl exerts its oxidising action outside rather than inside the cell whilst HOCl, on the basis that it can penetrate the lipid bilayer, can attack the microbial cell from both inside and outside the microbial cell.

Compared with -OCl, HOCl is 80 times more effective against Escherichia Coli, 40 times more effective against pseudomonas species and 100 times more germicidal to bacillus species. HOCl may also reduce the availability of ATP to the cell, meaning a decrease in ATP availability for the growth and metabolism of microbial cells.

As an oxidising agent, HOCl also reacts rapidly with several cellular components such as porphyrins, purine and pyrimidine bases, amino acids and sulphydryl groups. The oxidation of these compounds results in the loss of microbial cell functions. HOCL may also cause DNA damage. In summary the primary effect of HOCl is either the oxidation of the sulphydryl groups of essential enzymes and antioxidants and/or the damaging effects on DNA synthesis [17].

How to use these compounds

WHO and national guidelines emphasise the need to follow the manufacturer's instructions to make up these cold-water sterilising solutions. HCPs, parents and carers must be extremely rigorous. Sterilisation of feeding equipment is achieved within 15 minutes, but feeding equipment should be left in the sterilising solution for 30 minutes. It is important to make sure no air bubbles are trapped in bottles or teats when putting them into the sterilising solution. All equipment should be kept under the solution and the steriliser provides for this with a floating top or plunger. Fresh sterilising fluid must be prepared every 24 hours using the correct amount of water to sterilising tablet(s) or fluid.

Adherence to correct usage

Care must be taken to make the appropriate dilution as instructed by the manufacturer. Dilution errors and using too concentrated a solution or too dilute a solution must be avoided. If the solution is too dilute there is an increased risk of microbial contamination. If too concentrated there is a possible risk of toxicity particularly in susceptible infants [10].

Importance of education

Education on infant feeding for health professionals and parents and carers tends to focus on methods of feeding and types of infant formula. If infant formula is chosen, evidence shows that parents and carers are not well educated and informed about cleaning and sterilising all feeding equipment – bottles, teats, bottle rings and caps and dummies. Some equipment will also be used by breast feeding mothers. This too must be sterilised properly. Misconceptions on this topic abound with some non-professional advice suggesting that sterilisation is not necessary. Attention needs to be paid to official guidelines which clearly state that feeding equipment must be sterilised for the first 12 months of an infant's life and that equipment must be thoroughly cleaned with soap and water before sterilising. If using the cold-water method for sterilising, manufacturer's instructions must be accurately followed.

Safety

Hypochlorite solution as prepared for the cold-water sterilisation method is safe. In vitro tests have suggested safety issues with hypochlorite sterilisation in feeding equipment for neonates and premature infants. But the in vitro data have been challenged suggesting it is not reflective of exposure in infants with real concentrations being lower in milk feeds [18]. This author also suggested that sterilisation of bottles and teats with hypochlorite and no final rinsing cannot be considered hazardous. Terminal rinsing with tap water is safe in healthy term infants but is not advisable in infants at serious risk of infection (e.g. premature or sickly infants) when sterile water may be advised.

Conclusion

Global and national official guidelines state that sterilisation of infant feeding bottles and associated equipment is essential for the first 12 months of life. This is due to the infant's immature immune system and consequent risk of infection. Chlorine containing compounds (hypochlorite and troclosene) produce effective sterilisation using the cold-water method. These compounds remove bacteria, yeasts and viruses with data showing the eradication of, amongst other microbes, MRSA, *E coli*, *Pseudomonas aeruginosa*, *Candida albicans* and Coronavirus within 5-15 minutes depending on the microbe in question. They work through oxidation which damages components of microbial cell membranes and the arrest of microbial cell DNA synthesis.

Hypochlorite has a long history of use for sterilisation of infant feeding equipment beginning with the 'Milton Method' which had its origins in 1947 due to the deaths of many infants from gastroenteritis in the 1940s. Cold-water sterilisation using chlorine containing compounds remains in widespread use today as in addition to being effective, it is convenient, flexible and affordable, sterilises equipment within 15 minutes without the need for heat and items remain sterile if left in the solution for 24 hours [19-22].

Education of HCPs, parents and carers is essential if sterilisation of feeding equipment is to be done properly, eradicate microbes according to the evidence base and reduce the risk of infant infection. It is important to distinguish between cleaning and sterilisation with both processes being essential for infant health.

Very few studies have compared the efficacy of the different methods of sterilising feeding equipment and from those that have it is difficult to tell which shows the greatest efficacy so more research is needed. What is known, however, is that cold water sterilisation is effective if conducted properly and it is convenient and cost effective without some of the disadvantages associated with the other methods.

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