



Microbial Contamination of Infant Feeding Bottles and Caregiver Compliance to Disinfection and Sterilization Techniques, Pediatric Hospital, Libya

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPR/2023/v12i6259

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://www.sdiarticle5.com/review-history/110294>

Original Research Article

Received: 04/10/2023
Accepted: 09/12/2023
Published: 14/12/2023

ABSTRACT

Introduction: Microbial contamination of infant feeding bottles imposes a big health issue that cause increase in the infant morbidity and mortality rate, and this infection can be resulted from poor hygiene practices and using inadequate cleaning and disinfection methods of feeding bottles.
Aim: to evaluate the rate of bacterial infection of infant feeding bottles of infants who were admitted to pediatric hospital.

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Materials and Methods: The data were collected by filling questionnaires from the mothers and taking swabs from feeding bottles of infants who were admitted to pediatric hospital. The data included 24 questionnaires and 60 swabs; which 3 swabs were taken from three different area of the same bottle for each 24 children separately. then the swabs were tested in a laboratory to detect the types of isolated bacteria.

Results: This study found that 91.7% of the bottles were infected, and the isolated bacteria from these bottles were *staphylococcus*, *Klebsiella*, *E. coli* and *salmonella*. Moreover, it found a slight elevation in the bacterial infection rate in plastic bottles more than glass bottles. Besides, it found a positive relationship between bottle body infection and the educational level of mothers. On the other hand, it did not indicate any statistical association between infection of teats, bottles body and covers and occupational status of mothers.

Conclusion: The microbial bottles contamination is caused due to unsuitable cleaning and sterilization methods of feeding bottles and this leads to increase gastrointestinal infection as well as increase the morbidity and mortality rate among infants.

Keywords: Bacteria; contamination; disinfection; infant feeding bottles; infection; sterilization.

1. INTRODUCTION

According to WHO, one of the main causes of new-born death is infection in the worldwide [1]. The bacterial infection is considered as the major cause of elevated the rates of morbidity and mortality among infants who receiving artificial feeding in the developing countries [2]. These infections involve gastroenteritis and diarrhea, which are an intestinal infection developed among children who feed by milk bottles, which the risk of fecal bacterial transmission during the feeding bottle is very high levels [3].

This infection can be transmitted by caregiver hands which is the main route of transmission, caregivers may not wash their hands properly before preparation of bottle milk and after changing the baby' diapers [4]. Also, it can be transmitted through an unclean bottle, to which the added milk could be contaminated inside the bottles or by using contaminated water [4]. Besides, store spoons in the Powder Infant Formula (PIF) may lead to direct contact between PIF and spoons that have been touched directly by caregiver's hands and this increase the risk of infection [5]. Furthermore, the remaining milk in the bottles can lead to multiplication and growth microorganisms inside the feeding bottles [6].

The previous studies identify several types of bacteria that can be found in the infant feeding bottles involving *Escherichia Coli* [7,8,9,10,11,12], *Enterobacter*, *Klebsiella* [7,8,10], *Staphylococcus Aureus* [7,10,11,12,13], *Bacillus Cereus* [7,12,13], *Vibrio Cholera* [8], *Acinobacter Baumimi* [9], *Salmonella* [11,12,13], *Streptococcus* spp., *Citrobacter* spp. [13],

Aeromonas [8], *Candida* and *Pseudomonas* spp. [10].

This infection can be prevented by several ways; one of the best effective ways is washing hands [14], washing baby caring hands helps to prevent and reduce the spread of germs, The caregivers must wash their hands before and after eating, after touching animals, after changing a diaper of baby or using the toilet and after sneezing or coughing [15]. Besides, using the appropriate methods of cleaning, sterilizing and storing infant feeding items to avoid the transmission of bacteria through the infant feeding materials [16], which sterilization play an important role in removing and destroying the microbes from inside the feeding bottle [17]. Additionally, educating the care givers regarding causes and routes of bacteria transmission pathway to the feeding bottles [12] as well as educating them about the suitable practices of bottle cleaning and sterilization, and they must be aware about the problems that might be happened to the children [18].

Research Aims: To evaluate microbial association to feeding bottles of infants at Pediatric Hospital and making awareness for caregiver on sterilization techniques.

2. MATERIALS AND METHODS

2.1 Study Site

This study was conducted in Pediatric hospital in Benghazi, Libya.

2.2 Study Design

It involves cross sectional design study.

2.3 Method of Data Collection

The data were collected in two ways, the first way was by collecting swabs specimens from the infants' bottles, and the second way was using a multiple-choices questionnaire. This data were collected between November and December 2021.

2.4 Sample Collection

The sample included 24 feeding bottles of the infants who admitted to the hospital in the period of study, the swabs were taken from three different area of each feeding bottle; which involves swabs from teats, bottles and cover. they were collected with sterile cotton swab pre-moistened with sterile 0.9% saline solution. Then, the date and area of the samples were registered on the containers. All the containers were transferred directly to laboratory in less than half of an hour to identify the type of isolated bacteria, and the technicians used the following agar in the laboratory; Blood agar BA, Nutrient Agar NA, and MacConkey Agar MAC.

2.5 Questionnaire Design

The questionnaire was used to evaluate the mother's knowledge and practices regarding hygiene and sterilization of the infant feeding bottles. It involved two sections:

Section one: sociodemographic information about the mothers, which are age, occupation, educational status and number of children they have.

Section two: 10 questions about the practices of cleaning disinfecting and sterilizing the bottles.

2.6 Target Population and Sample Size

The study involved all infants who were admitted to pediatric hospital during the study period, they were 24 infants. Three swabs were taken from each bottle, which involves swabs from teats, bottles and covers. All mothers were informed of the purpose of this study, and their consent was obtained before specimen collection, the swabs were collected and transferred directly to the laboratory, at the same time, questionnaires were filled by 24 caregivers of these children.

2.7 Statistical Analysis

In this study, the software SPSS version 20 is used in coding, entering and analysis the data, which the percentage and frequency are

calculated, and Chi square and ANOVA tests are used to study the statistical relationship between some variables.

2.8 limitation

The limitations of this study were:

- a. the long drawn administrative procedures in this hospital involved a time delay in order to get response from the head of hospital.
- b. the cost of microbial culture is too expensive.

3. RESULTS

Table 1 shows the age of largest number of infants were 0 to 3 months (33.3 %), and 25% of them were 4- 6 months and 10-12 months separately. Moreover, it presents that 66.7% the caregivers were housewives, while only 29.2% were employed. Furthermore, it shows that 33.3% of the caregivers hold a bachelor's degree, and only 16.7% have preparatory degree. Additionally, it reported that half of the mothers have 3 to 4 children in their families. Also, it shows 44.7% of mothers have just 1 bottle for her baby, while 33.3% of the caregivers had 2 bottles.

Furthermore, Fig. 1 shows 14 bottles material were plastic (58.3%), while 8 were glass (33.3%). Besides, Fig. 2 Reported that 22 bottles were infected (91.7%), while only 2 were non infected.

According to Table 2, the gram-positive isolated bacteria from infants' bottle body involved *Staphylococcus Aureus*, however the gram negative were *Klebsiella*, *E. coli* and *Salmonella*.

According to Table, 3, 33% of the teats feeding have *staphylococcus aureus* and 29.2% is *Escherichia coli*, and 25.0% of bacteria rate no growth.

Moreover, Table 4 shows that largest proportion of isolation rate of bacteria from the bottles were *Staphylococcus Aureus* (37.05%) and (29.2%) *Escherichia Coli*, while 20.8% have no growth.

Besides, Table 5 reported that 41.2% of bottles cover were infected by *staphylococcus aureus* and 29.4% *Escherichia Coli*, and 23.5% of covers have no growth.

According to Table 6 , Shows that the high percentage of infection were reported in plastic bottle more than glass bottles. And the largest proportion of infection were belongs to *Staphylococcus Aureus* and *Escherichia Coli* as well as *klebsiella*.

Table 1. Socio-demographic characteristics of respondents

Characteristics		NO. (%)
Infant Age	0-3 months	8 (33.3%)
	4-6 months	6 (25%)
	7-9 months	4 (16.7%)
	10-12 months	6 (25%)
Occupational status of infants 'mother	Housewife	16 (67%)
	Employed	7 (29%)
	Student	1 (4%)
Educational status of infant's mother	Preparatory	4 (16.7%)
	High school degree	5 (20.8%)
	Diploma	7 (29.2%)
	Bachelors	8 (33.3%)
Number of children each mother	Less than 3	10 (41.7%)
	3 to 4 children	12 (50%)
	More than 4 children	2 (8.3%)
Number of babies feeding bottle for each infant	1	10 (41.7%)
	2	8 (33.3%)
	3	2 (8.3%)
	More than 3	2 (8.3%)
	Missed	2 (8.3%)

Table 2. Types of isolated bacteria from infant's bottle body

Gram positive	Gram negative
<i>Staphylococcus Aureus</i>	<i>Klebsiella</i>
	<i>E. coli</i>
	<i>Salmonella</i>

Table 3. Bacteria isolated from teats

Type of bacteria	Frequency	Percent
<i>Staphylococcus Aureus</i>	8	33.03%
<i>Escherichia Coli</i>	7	29.02%
<i>Klebsiella spp.</i>	2	8.03%
No growth	6	25.00%
<i>Salmonella</i>	1	4.02%

Table 4. Bacteria isolated from baby bottles

Type of bacteria	Frequency	Percent
<i>Staphylococcus aureus</i>	9	37.05%
<i>Escherichia coli</i>	7	29.02%
<i>Klebsiella spp.</i>	3	12.05%
No growth	5	20.08%

Table 5. Bacteria isolated from cover

Type of bacteria	Frequency	Percent
<i>Staphylococcus Aureus</i>	7	41.02%
<i>Escherichia Coli</i>	5	29.04%
<i>Klebsiella spp.</i>	1	5.09%
No growth	4	23.05%

Table 6. Types of isolated bacteria according to bottle material

type of bacteria	Plastic	Glass	Both types
<i>Staphylococcus Aureus</i>	5	3	1
<i>Escherichia Coli</i>	5	2	0
<i>Klebsiella spp.</i>	3	0	0
No growth	1	3	1

Table 7. Practices of cleaning and disinfect the feeding bottles

Characteristics		NO. (%)
Washing hands before bottle preparation.	Yes	24 (100%)
	No	0 (0%)
The frequency of clean the baby's' bottle	Once a day	23 (95.8%)
	After each use	1 (4.2%)
Method of cleaning infants' bottle	Water	1 (4.2%)
	Soap and water	17 (70.8%)
	Use sodium hypochlorite	4 (16.7%)
	Chemical cleaner	1 (4.2%)
	Missing	1 (4.2%)
	Yes	19 (79.2%)
	No	5 (20.8%)
Disinfect infant feeding bottles.	Yes	20 (83.3%)
	No	4 (16.7%)
Disinfection method that are used in decontamination of infant bottles	Boling in water	16 (62.5)
	Boling in water and salt	3 (12.5)
	Boling in water with sodium hypochlorite	3 (12.5%)
	Dishwasher	1 (4.2)
	Commercial sterilizer	1 (4.2)
	Don't use any method	1 (4.2)
	hand hygiene after changing infant dipper	Yes
No	0 (0%)	
Sometimes	1 (4.2%)	
Only use wipes	1 (4.2%)	

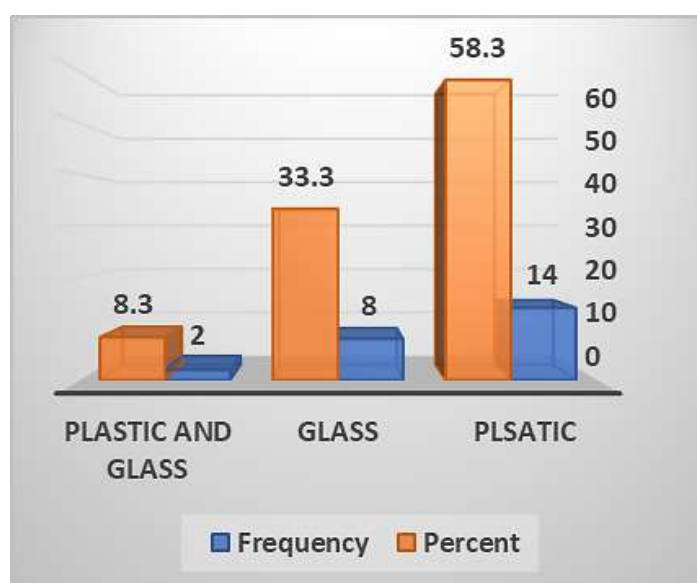


Fig. 1. Type of infant bottle material

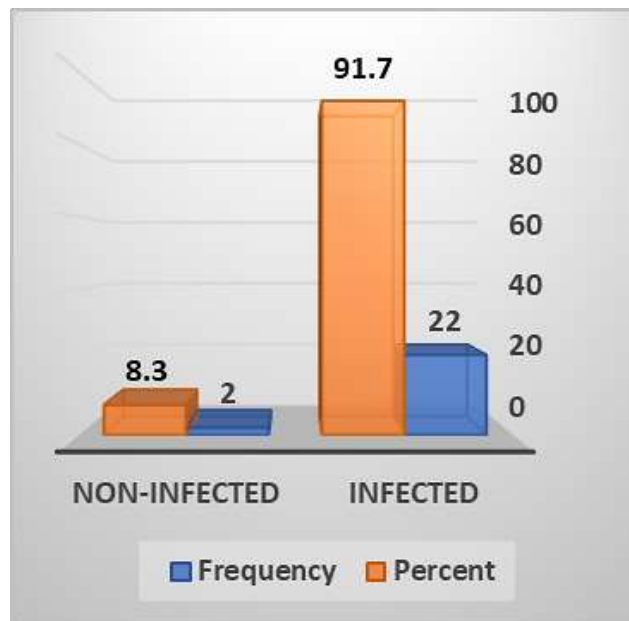


Fig. 2. Infection rate of infant bottles who admitted to pediatric children from November to December (2021)

Additionally, Table 7 reported that all mother said they wash their hands before the preparation of baby's bottle. Also, it reported that 95.8 % of mother clean the baby's' bottle after every feeding and 70.8% of mothers use water and soap, and 16.7% use Clorox and detergents, also, it shows that more than half of caregivers (79%) use the brush during cleaning the bottles, while (21%) do not.

On the hands, 83% of caregivers said they sterilize infant feeding bottles, while only 17% don't, which the most sterilization method that they use is boiling in water (62.5%). Furthermore,

it observed that 92% of caregivers wash their hands after changing the infant's dipper, and 4% of them use wet wipes and 4% said they sometimes wash their hands.

Besides, Table 8 indicated a positive relationship between bottle body infection and educational level of the caregivers which the p value was less than (0.05), while no relationship with a teats and cover infection.

Moreover, Table 9 reported no relationship has been observed between occupational status of participants and teats, bottle body and cover infection which the p value was more than (0.05).

Table 8. Results of ANOVA test for assessing the association between educational level and teats, bottle body and cover infection

Feeding bottle parts	Degree of freedom	Mean Square	P value
Teats	3	3.648	0.085
Bottle body	3	3.467	0.041
Cover	3	0.899	0.654

Table 9. Results of Chi-Square Test for assessing the association between occupational status and teats, bottle body and cover infection

Feeding bottle parts	Chi square χ^2	Degree of freedom	P value
Teats	3.455	4	0.485
Bottle body	2.686	3	0.443
Cover	5.130	3	0.162

4. DISCUSSION

The results of the current study reported that 91.7% of infants' bottles, covers, and teats were infected by bacteria in Pediatric Hospital in Benghazi, which included *Staphylococcus Aureus*, *Escherichia Coli*, *Klebsiella Spp.* and *Salmonella*, and that impose high risk to these infants. Compared to the previous studies, Erku et al. isolated bacteria from 100 samples of infant bottles in pediatric outpatients in Addis Ababa and they found that most common were *Staphylococci*, then comes *Bacillus spp.*, and *micrococci* [19]. Moreover, other studies found *Klebsiella Spp.* was the most isolated bacteria in infant bottles [8,9]. Besides, Suthienkul et al. observed that 500 infant bottles infected with *Enterobacter spp.*, *Aeromonas* (14.4%), and *Escherichia Coli* which is the least common bacteria in Bangkok Thailand [8]. Alongside this, Ayaz et al. detected *Acinobacter Baumini* and *Escherichia Coli* from the samples of infants who were admitted to hospital in Rawalpindi Pakistan [9]. In the similar line, Morais et al. found that *Coliform bacteria* and *Escherichia Coli* were the most causes of contamination in the lacteal contents of feeding bottles of a Low Socioeconomic group (ESG) in metropolitan São Paulo, Brazil, where the infection in this group was higher than the High Socioeconomic group (HSE) in 1998 [20]. Furthermore, Elegbe et al. indicated the most isolated bacteria in teats was enter-pathogenic *Escherichia coli*, followed by *Staphylococcus Aureus* [21]. And this comes in agreement with Cherian et al. who found *Escherichia Coli* was the most common isolated bacteria in feeding bottle contents and teats in Zaria, Nigeria. Also, it was observed that bottles were more contaminated than teats [22]. On the other hand, Zhang indicated that *Salmonella spp.* was the main cause of infection in infant feeding bottles in less developed communities, and it was followed by *Escherichia Coli* [23]. Additionally, Marege et al. found the common foodborne pathogens of bottle feeding babies who are attending health facilities were *Salmonella spp.*, *E. coli*, and *Staphylococcus aureus* in Arba Minch, southern Ethiopia in 2022[24]. Besides, Musa et al. collected samples from feeding bottles and children faeces, they found the most common isolated bacteria from both of stool and bottles is *E. coli*, additionally, Enteropathogenic *E. coli* and *Shigella* species are isolated from the stool of these children [25], On the other hands, another study assessed the amicrobial contamination in a neonatal intensive care unit in Madrid (Spain), it indicated 9 out of

48 feeding bottles was infected with *P aeruginosa*, and this bacteria was isolated from 3 water samples that were taken from dishwasher [26]. Furthermore, Gibson et al. observed that 88% of the formula feeds were contaminated with 8 total coliforms at a level more than 10 MPN/ml, and 45% were contaminated with *E. coli.*, and only two mothers were compliance with washing hands with soap and water before prepare feeding bottles, while the other mothers were not complained with WHO guidelines about milk preparation methods and cleaning and sterilization methods in East Java in Indonesia in 2017 [27].

Therefore, the finding of this study suggested that these infected bottles could have resulted from contamination in the hospital or poor hygiene of caregivers. Also, the lack of caregiver knowledge and education towards proper methods of cleaning and disinfection. this is in line with pervious study which suggested that the extrinsic contamination of baby feeding bottle would be explained by the inadequate caregiver practices during or after food preparation [28]. Moreover, using one bottle could increase the chance of getting an infection, specifically in hospital environment where there is no proper place to clean and sterilize the bottles .It is important for the facility to provide water resource to be available alongside with proper hygiene among caregivers [29].

Moreover, the current study observed that plastic bottles are more infected with bacteria than glass bottles. Besides, only two types of bacteria were isolated from glass bottles, which were *Staphylococcus Aureas* and *Escherichia Coli*, while *Staphylococcus Aureas*, *Escherichia Coli* and *Klebsiella spp.* were isolated from plastic bottles. In addition, another study observed that most mothers use plastic bottles more than glass, and it found higher amounts of milk formula residue in plastic bottles after complete feeding when compared to glass bottles, and this increase the risk of bottle bacterial contamination, also, the same study reported that the glass bottle is better than plastic bottle for both organic and inorganic hygiene [30].

On the other hand, the present study revealed that mother's qualification has significant correlation with only bottle body infection, however, it does not have any correlation with infection in the other feeding bottles (teats and covers). This comes in agreement with Morais et al. study that indicated higher coliform counts

were associated with lower education levels of mothers, which observed that large number of coliforms in the LSE group more than HSE. Also, coliform was higher in bottles that were prepared by any person other than the mother person who had lower education level [18]. In contrast, Morais et al. reported that mother's educational level has no impact on the contamination degree of feeding bottle in the LSE group [18], this comes in partially agreement with the present study which reported no statistical association between mother's qualification status and bacterial infection present in teats and cover. On the other hand, there was no scientific research studied the relation between occupational status of mothers and contamination of feeding bottles until now.

5. CONCLUSION

This study indicated that the most infant feeding bottle who admitted to this hospital were contaminated with *Staphylococcus Aureus*, *Escherichia Coli*, *Klebsiella spp.* and *Salmonella*. This could be result of improper cleaning and sterilization methods that mothers used while they were in hospital. And this infection may be transmitted to the baby bottles through mothers' hands during hospitalization. Therefore, the mothers should have appropriate education and experience with sterilization methods of the infants' bottle before preparing meals for their babies. They also have to sterilize the infants' feeding bottles with boiled water immediately after every use or meal. Additionally, establishing standard precautions to prevent the spread of hospital-acquired infections to the infants' bottles, including hand washing with soap and water or rubbing hands with alcohol before and after the daily examination of the admitted children as well as before and after preparing food. In addition, a proper education should be given to mothers about the risks of diseases that are caused by contaminated feeding bottles of children and the route of infection transmission. Moreover, hospital administration should provide a policy and procedures for bottle feeding to mothers and caregivers.

CONSENT AND ETHICAL APPROVAL

This study was conducted in this hospital after permission for the same was granted by the manger and the consent of the mothers were obtained to collect samples from their baby feeding bottles.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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