

Original Research Article
**~~Bacterial contamination of infantfeeding bottles at
pediatric hospital and caregivers' compliance to
disinfection and sterilization methods~~**

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

Introduction: Microbial contamination of infant feeding bottles could lead to an increase in the morbidity and mortality rate among infants, and it can be resulted from improper 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.

Material 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 20 questionnaires and 60 swabs; which 3 swabs were taken from three different area of the same bottle for each 20 children separately. then the swabs were tested in a laboratory to detect the types of isolated bacteria.

Molecular identification was done??

Results: This study found that 91.7% of the bottles were infected, and the isolated bacteria from infants feeding bottle involve ~~staphylococcus~~, *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 infection and the educational level of mothers. On the other hand, it did not indicate any statistical association between teats, bottles and covers infection and occupational status of mothers.

Conclusion: The improper cleaning and sterilization methods of infant feeding bottle is the main cause of microbial bottles contamination, which leads to increase morbidity and mortality rate among infants

Key words:Keywords: Baby bottles, infection, Sterilization, Pediatric hospital.

Keywords always written alphabetically

1. Introduction

One of the main causes of new-born death in the worldwide is infection, which includes sepsis/pneumonia, tetanus, and diarrhea, and preterm, and birth asphyxia [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].Also, gastroenteritis and diarrhea are more developed among children who feed by milk bottles, which the risk of fecal bacterialtransmission during the feeding bottle is very high levels [3].

This infection can be transmitted bycaregiver 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. Also, it can be transmitted throughan unclean bottle, which the added milk could be contaminated inside the bottles or 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 multiply 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], *AcinobacterBaumimi*[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 baby caring hands to prevent and reduce the spread of germs, which the caregivers must wash hands before and after eating, after touching animals, after changing a diaper of baby or using the toilet and after sneezing or coughing [14]. Besides, using the appropriate methods of cleaning, sterilizing and storing infant feeding items to avoid the transmission of bacteria through the infant feeding materials [15]. 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 could be happened to the children [16].

Research Aims: To evaluate bacterial infection rate of feeding bottles of infants who admitted to pediatric hospital in Benghazi, and to assess the knowledge and compliance of caregivers regarding sterilization and disinfection methods of feeding bottles.

2. Material 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 is taking swabs specimens from the infants' bottles, and the second way is using a multiple-choices questionnaire. These 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 involves 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, 24 questionnaires were filled from the 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 Ethical consideration:

This study was conducted in this hospital after the manager give the authors permission to start it and then getting agreement of the mothers to take samples from their baby feeding bottles.

2.9 limitation:

The limitation of this study was the difficult of administrative procedures in this hospital which it took a long time to get response from the head of hospital. Also, the cost of microbial culture is considered too expensive.

3. Results

According to table 4, table 3.1 the largest number of infants (33.3 %) were 0 to 3 months, and 25% were 4- 6 months and 10-12 months separately. Moreover, more than half of the caregivers were housewives (66.7%), while only 29.2% were employed. Furthermore, the largest proportion of the participants (33.3%) hold a bachelor's degree, and the smallest of the sample (16.7%) have preparatory degree, and half of the mothers have 3 to 4 children in their families. Also, 44.7% of mothers have just 1 bottle for her baby, while 33.3% had 2 bottles.

Please rewrite the above paragraph.
Better to like Table: 3.1 and Figure 3.1. Under this section

Table 3.1 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 babiesfeeding 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%)

Furthermore, figure 1 shows 58.3% of bottles material were plastic, while 33.3% were glass. Besides, Figure 2 Reported that 91.7% of the bottles were infected, while only 8.3% were non infected.

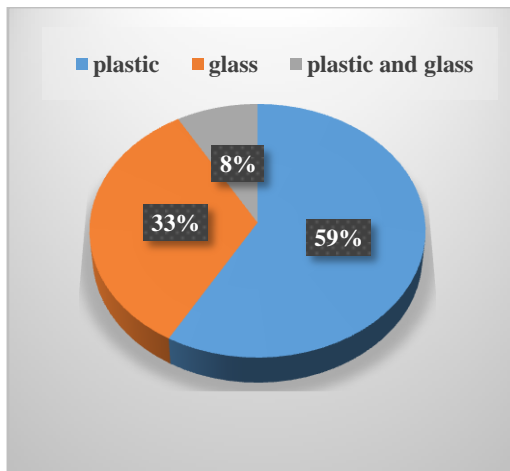


Figure 1: Type of infant bottle material

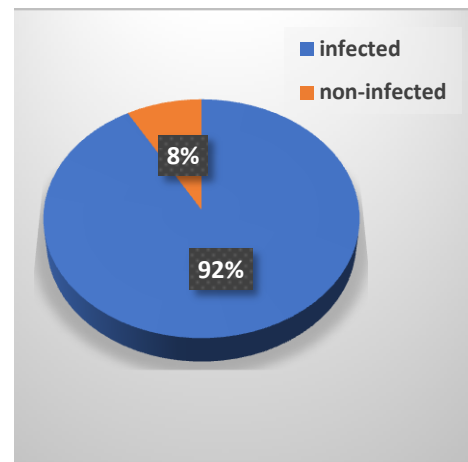


Figure 2: Infection rate of infant bottles who admitted to pediatric children from November to December (2021)

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

Table 2: Types of isolated bacteria from infant's bottles

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

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.

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%

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.

Table4: 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</i> spp.	3	12.05%
No growth	5	20.08%

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.

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</i> spp.	1	5.09%
No growth	4	23.05%

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 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</i> spp.	3	0	0
No growth	1	3	1

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, 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.

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	Once aday	23 (95.8%)
	After each use	1 (4.2%)

baby's' bottle

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%)
disinfect infant feeding bottles.	Yes	19 (79.2%)
	No	5 (20.8%)
of disinfection method that are used in decontamination of infant bottles	Yes	20 (83.3%)
	No	4 (16.7%)
of 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	22 (91.7%)
	No	0 (0%)
	Sometimes	1 (4.2%)
	Only use wipes	1 (4.2%)

Besides, table 8 indicated a positive relationship between bottle 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.

Table 8: Results of ANOVA test for assessing the association between educational level teats, bottles and cover infection

Feeding bottle parts	Degree of freedom	Mean Square	P value
Teats	3	3.648	0.085
Bottle	3	3.467	0.041
Cover	3	.899	0.654

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

Table 9: Results of Chi-Square Test for assessing the association between occupational status and teats' infection

Feeding bottle parts	Chi square χ^2	Degree of freedom	P value
Teats	3.455	4	0.485
Bottle	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, 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* [17]. 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 *AcinobacterBaumini* 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, which the infection in this group was higher than the High Socioeconomic group (HSE) in 1998 [18]. Furthermore, Elegbe et al. indicated the most isolated bacteria in teats was enter-pathogenic *Escherichia coli*, followed by *Staphylococcus Aureus* [19]. 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 [20]. 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* [21].

Therefore, the finding of this study suggested that these infected bottles could have resulted from contamination in the hospital or poor hygiene from caregivers. Also, the lack of caregiver knowledge and education toward proper methods of cleaning and disinfection. This is in line with previous study which suggested that the extrinsic contamination of baby feeding bottle would be explained by the inadequate caregiver practices during or after food preparation [22]. 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 [23].

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 [24].

On the other hands, the present study revealed that mother's qualification has significant correlation with bottles infection, however, it does not have any correlation with teats and covers infection. 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 non-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 agreement with the present study which reported no statistical association between mother's qualification status and bacterial infection present in the feeding bottle, 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 hospital were contaminated with bacteria; which were *Staphylococcus Aureus*, *Escherichia Coli*, *Klebsiella spp.* and *Salmonella*. And this could be resulted from 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 conducted to mothers about the risks of diseases that are caused by contaminated feeding bottles for children and the route of infection transmission. Moreover, hospital administration should provide a policy and procedures for bottle feeding handling to mothers and caregivers.

6. References:

- [1]. WHO. Sever of infection. 2021. Available online at <https://www.healthynewbornnetwork.org/issue/severe-infections-pneumonia/>
- [2]. Weisstaub G, Uauy R. Non-breast milk feeding in developing countries: challenge from microbial and chemical contaminants. *Annals of Nutrition and Metabolism*. 2012 Jun 1;60(3):215-9.
- [3]. Lu R, Costello A. Brief report. Failure to exclusively breastfeed and the risk of early infant mortality due to infectious disease in poor communities in Lima, Peru. *Journal of tropical pediatrics*. 2000 Oct 1;46(5):309-11.
- [4]. WHO, 2001. Infection and infectious diseases. Available at https://www.google.com/url?sa=t&source=web&rct=j&url=https://www.euro.who.int/_data/assets/pdf_file/0013/102316/e79822.pdf&ved=2ahUKewiLr_v8IKLzAhVFhf0HHW-NAIcQFnoECCIQAQ&usq=AOvVaw3ljansb9LXinwIYSDHd8Sn
- [5]. Cho TJ, Hwang JY, Kim HW, Kim YK, Il Kwon J, Kim YJ, Lee KW, Kim SA, Rhee MS. Underestimated risks of infantile infectious disease from the caregiver's typical handling practices of infant formula. *Scientific reports*. 2019 Jul 5;9(1):9799.
- [6]. Kumari M. *Infant Nutrition, Breast Feeding and Motherhood*. Udit Prakashan; 2019 Dec 23.
- [7]. Tesfaye H. Bacteriological quality of infant feeding bottle-contents and teats in Addis Abeba, Ethiopia. *Ethiopian Medical Journal*. 1992 Apr 1;30(2):79-88.
- [8]. Suthienkul O, Siripanichgon K, Promachot P, Echeverria P, Leksomboon U, Rakue Y. Bacterial contamination of bottle milk in infants under 6 months in Children's Hospital, Bangkok, Thailand. *Southeast Asian journal of tropical medicine and public health*. 1999 Dec 1;30(4):770-5.
- [9]. Ayaz F, Ayaz SB, Furrakh M, Matee S. Cleaning practices and contamination status of infant feeding bottle contents and teats in Rawalpindi, Pakistan. *Pakistan Journal of Pathology*. 2017 Apr 28;28(1).
- [10]. Kejriwal M, Agarwal R, Bacteriology of feeding bottles. *IP Int J Med Paediatr Oncol* 2018;4(1):9-10
- [11]. Redmond E, Griffith C. Disinfection methods used in decontamination of bottles used for feeding powdered infant formula; this study by Dr Elizabeth Redmond and Professor Christopher Griffith shows that four commonly used methods of "sterilising" infant feeding bottles are effective, but health professionals need to advise parents and carers on using these methods correctly. *Journal of Family Health Care*. 2009 Feb 1;19(1):26-32.
- [12]. Rothstein JD, Mendoza AL, Cabrera LZ, Pachas J, Calderón M, Pajuelo MJ, Caulfield LE, Winch PJ, Gilman RH. Household contamination of baby bottles and opportunities to improve bottle hygiene in Peri-Urban Lima, Peru. *The American Journal of Tropical Medicine and Hygiene*. 2019 Apr;100(4):988.
- [13]. Uko EA, Adenugba IT. Detection and Enumeration of Gram-Positive Pathogens in Some Powdered Infant Foods in Ikot Ekpene Local Government Area, Akwa Ibom State, Nigeria. 2020.

- [14]. CDC. Water, Sanitation & Environmentally-related Hygiene. 2019. Available online at <https://www.cdc.gov/healthywater/hygiene/index.html>
- [15]. CDC. Water, Sanitation & Environmentally-related Hygiene. 2020. Available online at <https://www.cdc.gov/healthywater/hygiene/index.html>
- [16]. BAGHIANI MM, Nadrian H, Rahaei Z. The effects of education on formula and bottle-feeding behaviors of nursing mothers based on PRECEDE model. 2009.
- [17]. Erku WA, Ashenafi M. Microbial load and microflora of weaning foods obtained from pediatric outpatients in Addis Ababa. *Ethiopian Journal of Health Development*. 1998;12(2).
- [18]. Morais TB, Morais MB, Sigulem DM. Bacterial contamination of the lacteal contents of feeding bottles in metropolitan São Paulo, Brazil. *Bulletin of the World Health Organization*. 1998;76(2):173.
- [19]. Elegbe IA, Ojofeitimi EO, Elegbe I, Akinola MO. Pathogenic bacteria isolated from infant feeding teats: contamination of teats used by illiterate and educated nursing mothers in Ile-Ife, Nigeria. *American journal of diseases of children*. 1982 Aug 1;136(8):672-4.
- [20]. Cherian A, Lawande RV. Recovery of potential pathogens from feeding bottle contents and teats in Zaria, Nigeria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 1985 Jan 1;79(6):840-2.
- [21]. Ma L, Zhang G, Swaminathan B, Doyle M, Bowen A. Efficacy of protocols for cleaning and disinfecting infant feeding bottles in less developed communities. *The American journal of tropical medicine and hygiene*. 2009 Jul 1;81(1):132-9.
- [22]. Marege A, Regassa B, Seid M, Tadesse D, Siraj M, Manilal A. Bacteriological quality and safety of bottle food and associated factors among bottle-fed babies attending pediatric outpatient clinics of Government Health Institutions in Arba Minch, southern Ethiopia. *Journal of Health, Population and Nutrition*. 2023 Dec;42(1):1-8.
- [23]. Caruso BA, Paniagua U, Hoffman I, Manji K, Saidi F, Sudfeld CR, Vernekar SS, Bakari M, Duggan CP, Kibogoyo GC, Kisenge R. Safe infant feeding in healthcare facilities: Assessment of infection prevention and control conditions and behaviors in India, Malawi, and Tanzania. *PLOS Global Public Health*. 2023 Jun 8;3(6):e0001843.
- [24]. Kuan WH, Chen YL. Bottle material and cleansing procedures of infant feeding bottles. *Southeast Asian J Trop Med Public Health*. 2016 Jan 1;47:142-51.