



## FEEDING BOTTLES HYGIENE IN SOUTHERN PUNJAB HOSPITALS: A MICROBIOLOGICAL EVALUATION

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### Abstract

Bottle feeding, a longstanding practice for supplementing infant nutrition, poses a significant risk of food-borne pathogens transmission and subsequent gastroenteritis among infants, whose immature immune systems make them more vulnerable to these infections. It is responsible for 2.5 billion cases of diarrhea and 1.3 million deaths annually with severe cases leading to meningitis, brain abscesses, and necrotizing enterocolitis (mortality rate: 33%-80%). This study investigated the hygiene status of ready to use infant feeding bottles, and extent of facilities provided at Southern Punjab hospitals. Our analysis targeted potential sources of contamination and cross contamination including formula milk/cow milk, water, and handler's hands. The findings revealed poor hygiene practices among caregivers, inadequate hospital facilities, and a high prevalence of bacteria on the 84% of targeted sources (above acceptable limits of >100CFU/ml/cm<sup>2</sup>/g by FDA). However, improved hygiene status was observed following food safety training for caregivers. To ensure safe feeding practices, we recommend hospitals adopt guidelines for proper sterilization, preparation, and handling of infant feeding bottles. These research findings will enable healthcare providers to develop targeted treatments, significantly reducing the spread of food-borne diseases and ultimately protecting infant health.

**Key words:** Infants, feeding bottles, hospitals, diarrhea, caregivers, hygiene

### Introduction:

According to WHO, 79 percent of infants globally are fed using feeding bottles for longer or shorter periods of time (WHO 2018; Nasrul et al. 2020). Mostly children are fed with at least some formula milk or by using feeding bottles for mother milk (Lakshman et al. 2009). Available data suggests feeding bottles as an important source for harboring food-borne pathogens and subsequently leading to the onset of food-borne diseases in infants. Similarly, inadequately managed feeding and poor handler's hygiene habits have been reported to be the reasons for microbial growth and cross contamination (Negassa et al. 2022). Each year 1.3 million deaths and around 2.5 billion diarrheal

samples, worldwide have been reported among children under five years of age. Owing to poor hygiene, improper feeding practices and lack of awareness of food safety principles among mother's have led to high prevalence of food borne pathogens on feeding bottles used by infants and children are the most vulnerable group to food-borne illnesses, due to under developed immune system (Parra-Flores et al.2022). More than twenty viral, bacterial, and parasitic pathogens have been reported to be associated with acute diarrhea (O'Ryan et al. 2005).

Diarrhea has been regarded as one of the most important causes of death among infants and young children. Also, it is the common reason for an infant's hospital visit. It poses a significant threat to hospitalized infants, necessitating meticulous attention to infection control measures. The hygiene of feeding bottles used by these vulnerable patients is a critical aspect of their care, as it directly impacts their risk of nosocomial infections and complications

Despite the critical importance of infection control measures in hospitalized infants with diarrheal diseases, the hygiene of feeding bottles remains a neglected aspect of care. Existing protocols often overlook the specific risks associated with feeding bottle contamination, leading to a knowledge gap in evidence-based guidelines for optimal hygiene practices. This article examines the importance of proper feeding bottle hygiene in the hospital setting, highlighting the need for stringent protocols to prevent the transmission of diarrheal pathogens and ensure the safe treatment of infants suffering from diarrhea. This study aims to address this research gap by investigating the microbial contamination of feeding bottles and evaluating the effectiveness of improved hygiene protocols in reducing nosocomial infections in infants with diarrheal diseases.

One study carried out in the USA demonstrated that Infant formulas prepared at hospital facilities were 24 times more contaminated as compared to those prepared at home. Furthermore, powdered infant formulas were 14 times more contaminated than ready-to-feed liquid formulae (Steele and Short 2008).

Information is available to confirm that hygiene of feeding bottles, milk, water used to prepare infant formula and hygiene of handler's hands are of considerable significance because these determinants play a vital role in incidence and prevention of diarrhea. Since, hands are the direct source of cross contamination, hence good personal hygiene of those preparing formula milk substantially reduces diarrheal cases among the children. (Ejemot-Nwadiaro et al. 2021).The purpose was to identify the source of contamination within ready to feed milk feeding bottles, facilities provided at hospitals, effect of training on caregiver's practices.

## **Materials and Method**

### **Study site and selection criteria**

Hospitals in Southern Punjab were selected based on accessibility and high diarrheal treatment volume.

### **Ethical Considerations**

Prior to data collection, permission was obtained from the heads of the diarrheal wards at each participating hospital. Additionally, all necessary safety measures were implemented to ensure the well-being of participants and researchers. This study was conducted in accordance with the principles outlined in the Declaration of Helsinki.

### **Study Design**

Feeding bottles of infants who were admitted to government hospitals in Southern Punjab for treatment of diarrhea and associated caregivers were selected using a simple random sampling technique. However, efforts were made to minimize selection bias by including all eligible participants. This observational, pre-post intervention study investigated the impact of food safety training on microbial contamination of feeding bottles and caregivers' hands.

### **Inclusion/ exclusion criteria**

- Infants aged  $\leq 6$  months with diarrheal disease,

- Fed on infant formula or cow's milk via milk feeding bottles (with no solid foods),
  - And their caregivers handling/preparing these bottles were eligible.
- Exclusions applied to infants  $\geq 6$  months, those receiving solid foods, non-diarrheal patients, and those using alternative feeding methods.

**Sampling protocol**

A total of 177 feeding bottles and associated caregivers were selected randomly with 177 samples collected before training (at the time of admission) and 177 after training (at the time of discharge) over 27 months (February 2023-July 2024). Samples were taken from various locations, including the outer teat, inner teat, screw cap inner, interior of the bottle, formula milk/cow milk, water used for preparation, and caregivers' hands. Each location yielded 177 paired samples (before and after training), enabling a thorough evaluation of contamination and cross-contamination risks.

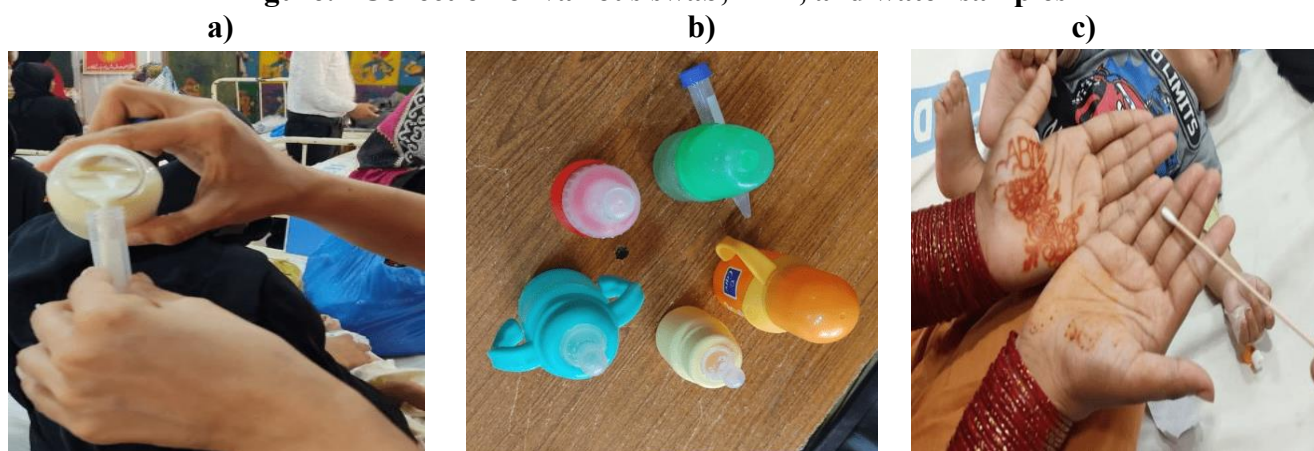
**Table: 1**

Sample type	Before training	After Training
Outer teat	177	177
Inner teat	177	177
Screw cap inner	177	177
Interior of bottle	177	177
Formula milk/cow milk	177	177
Water used to prepare formula milk	177	177
Caregiver's hands involved in preparing bottles	177	177
	=1239	=1239
<b>Sum</b>	1239+1239=2478	

**Sample collection**

Samples were collected using the standard swabbing approach (ISO 18593:2018), employing aseptic procedures. Surfaces were swabbed horizontally and vertically for 20 seconds to detect bacterial presence. Swabs were then placed in transport medium. Additionally, sterile tubes were utilized to collect formula milk, cow milk, and water samples, adhering to FDA Bacteriological Analytical Manual (BAM) protocols outlined by Swanson et al. (1992). Samples were labeled, sealed, and transported to the lab within two hours. They were stored at 4°C and analyzed within 24 hours. Microbial load was assessed aseptically using aerobic plate count to evaluate hygiene status, with test results compared to safety limits set by AOAC and Codex Alimentarius (Brugger et al., 2012).

**Figure:1 Collection of various swab, milk, and water samples**



**Food Safety Training intervention**

Caregivers received a 40-minute food safety training program, consisting

- Lecture on microbial safety and diarrheal disease prevention
- Demonstration of proper hand hygiene and feeding bottle cleaning

## c. Interactive session on milk preparation and storage

**Microbiological Assessment**

Aerobic Plate Count protocol (American Public Health Association) was followed (Swanson et al., 1992; Maturin & Peeler, 2001). Microorganisms were enumerated by counting CFU/mL/g/cm<sup>2</sup>.

**Hospitals Facilities Assessment**

Hygiene status and facilities were noted to analyze correlation between facilities and disease burden.

**Statistical Analysis**

IBM SPSS (version 26) was used. Descriptive statistics, ANOVA, and test statistics (p-value) were calculated.

**Results**

The data presented in the Table 1 illustrates various aspects of hygiene and facilities within a hospital setting.

**Table 2. Facilities provided at hospitals**

Facilities provided at hospitals	N	%
Separate washing area for feeders	Yes	16 9.2%
	No	161 90.8%
Awareness posters about hygiene diarrhea	Yes	20 11.8%
	No	157 88.2%
Water boiling facility	Yes	7 3.9%
	No	177 96.1%
Training in food safety	Yes	3 1.3%
	No	174 98.7%
Sterilization unit steamer	Yes	9 5.2%
	No	168 94.8%
Separate diaper changing area	Yes	18 10.5%
	No	159 89.5%
Hand wash availability in hospital	Yes	20 11.8%
	No	157 88.2%
Feeder washing liquid/ brush	Yes	2 1.0%
	No	175 99.0%

All results were evaluated based on FDA standards for aerobic plate count which considered values less than less than 100cfu/ml/gram/cm<sup>2</sup> as safe (Imada et al; 2016).

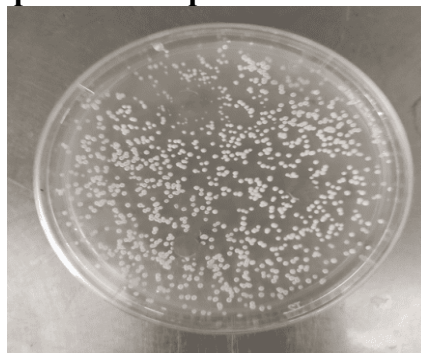
**Table 3. Comparative quality of Various parts of feeding bottles in relation to microbial counts**

Aerobic Plate Count	Training	Mean ±S. D	Range	(Minimum, Maximum)	Test-statistic	p-value
Outer teat Concentration (CFU/cm <sup>2</sup> )	Before	3.44±2.12	7.54	(0.00, 7.45)	7.44	0.000***
	After	1.85±1.58	4.12	(0.00,4.12)		
Inner teat (CFU/cm <sup>2</sup> )	Before	4.60±3.01	9	(0, 9)	9.35	0.000***
	After	1.91±1.88	7	(0,7)		
Interior of bottle (CFU/cm <sup>2</sup> )	Before	3.78±2.64	9.08	(0, 9.08)	5.29	0.000***
	After	2.39±1.84	6.26	(0, 6.26)		
Milk (CFU/mg/ml)	Before	1.02±1.51	5.24	(0, 5.24)	1.61	0.113
	After	0.76±1.30	4.57	(0, 4.57)		
Water added(ml)	Before	1.41±1.72	5	(0,5)	0.90	0.370
	After	1.24±1.58	4	(0,4)		

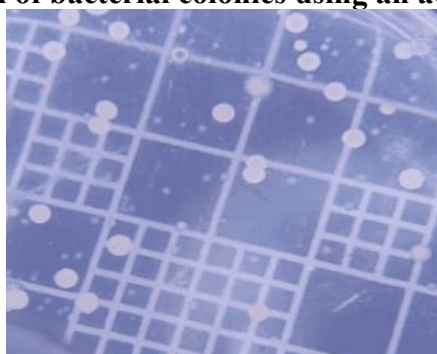
<b>Handler's hands Concentration (CFU/cm<sup>2</sup>)</b>	Before	3.09±2.23	8.43	(0, 8.43)	7.73	0.000***
	After	1.42±1.45	3.57	(0,3.57)		
<b>Screw cap inner (CFU/cm<sup>2</sup>)</b>	Before	5.27±2.59	9.45	(0, 9.45)	-0.72	0.470
	After	3.30±137.27	5.96	(0, 5.96)		

\*\*\* significant

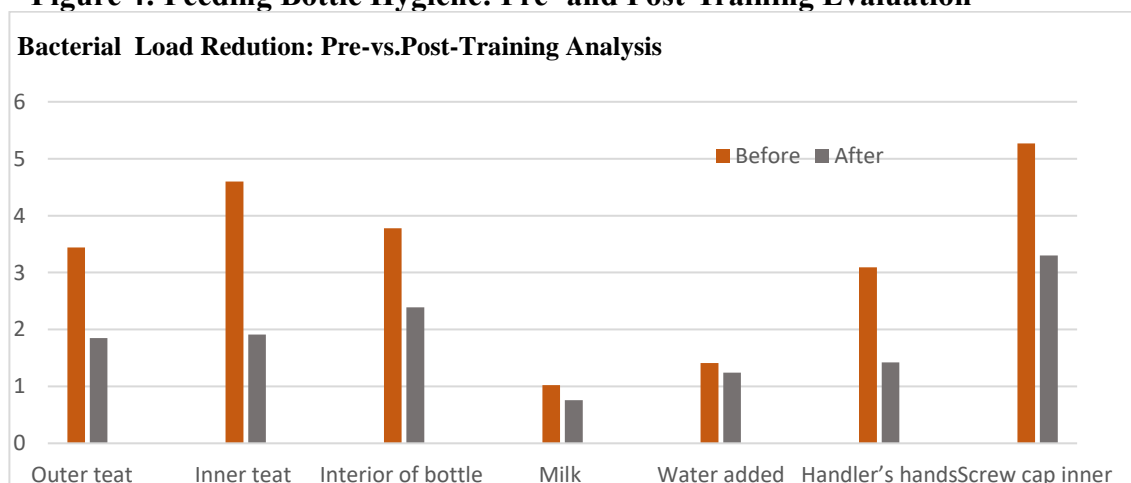
**Figure 2. Representative plate for Aerobic Plate Count**



**Figure 3. Quantification of bacterial colonies using an automated colony counter**



**Figure 4: Feeding Bottle Hygiene: Pre- and Post-Training Evaluation**



**Discussion**

Feeding bottles can become contaminated with harmful bacteria during preparation, particularly when coming into contact with unhygienic surfaces. This contamination can transfer to infant formula, increasing the risk of diarrhea in vulnerable infants. To mitigate this risk, strict adherence to guidelines for sterilization, preparation, and handling of feeding bottles is crucial. In hospital settings, safe infant formula preparation is critical, directly impacting infant health. This study examined the hygiene status of feeding bottles and their potential link to diarrhea. A microbiological assessment using

Aerobic Plate Count (APC), a key indicator of hygiene levels, was conducted to inform evidence-based practices.

The present study investigated the relationship between microbial contamination of feeding bottles and diarrhea among infants. Our results show a significant association between the two variables. Another aim was to identify the primary sources of microbial contamination within ready-to-feed milk feeding bottles. The study revealed disturbingly high levels of microbial contamination on feeding bottle parts, with 87% of samples exceeding the safe threshold of  $10^1$  CFU/g (Table 1). This far surpasses the FDA's recommended aerobic plate count standard of  $<100$  CFU/ml/gram/cm<sup>2</sup> indicating a significant risk to infant health (Imada et al., 2016). Out of the seven targeted potential source of contaminants screw cap inner was highly contaminated following inner teat, interior of bottle, outer teat, handler's hands, water used to prepare milk/formula milk and handlers hands. This is consistent with previous studies identifying similar sources of contamination (Marege et al., 2023; Ayaz et al., 2017).

A study conducted by Remond et al. 2009 are in line with results of our research representing considerable microbial load on screw cap and teat interior of bottle as compared to other parts of ready to use feeding bottles. Consistent with previous studies, feeding bottles exhibited alarming levels of microbial contamination, with aerobic plate counts (APCs) reaching up to  $5.8 \times 10^4$  colony-forming units (CFU) per area (Redmond, 2009; Alqurashi, 2019; Garg et al. 2019; Workie et al. 2019; Alemayehu 2021; Sheth and Obrah 2004;). Lack of feeder washing liquid and brush might be a reason of high level of contamination of feeding bottles interior screw cap or teats.

Several studies have demonstrated the results we have found in our studies and similarity in results validate the feeding bottles with poor hygiene status leave a drastic negative effect on infant health (Musa et al. 2009; Alqurashi, 2019; Garg et al. 2019; Workie et al. 2019; Alemayehu 2021; Bani et al. 2002; Mosisa and Tarekegn 2022; Al-Mamari et al. 2017).

Jalali et. al 2009 indicated that 87% of these contaminated samples had counts greater than 101 CFU/g. In summary, most of the samples had bacterial load beyond the recommended limits hence representing poor hygiene. Many other scientists reported similar findings (Sheth and Obrah 2004 ; Sudershan et al. 2008; Rao et al. 2007 Chaudhary et al. 2004; Khaliq et al. 2022; Emea et al. 2023; Feleke et al. 2022). Load beyond recommended limits demands discarding such milk that is ready to feed infant.

The study's findings highlight significant gaps in hygiene facilities and practices within government hospitals in Southern Punjab, Pakistan (Table 2). Only 9.2% of hospitals had separate washing areas for feeders, and 88.2% lacked awareness posters about hygiene and diarrhea. Moreover, 96.1% of hospitals did not have water boiling facilities, and 98.7% did not provide training in food safety. The scarcity of essential amenities is concerning (Table 2) as proper hygiene practices are crucial in reducing microbial contamination and preventing diarrhea among infants (Ejemot-Nwadiaro et al., 2021; Steele and Short, 2008). The World Health Organization emphasizes the importance of infection control measures and hygiene practices in healthcare facilities (WHO, 2018).

This echoes previous research emphasizing the importance of proper hand hygiene and sterilization practices in reducing microbial contamination (Marege et al., 2023; Ayaz et al., 2017; Musa et al., 2009; Bani et al., 2002; Choube et al., 2014; Mohana et al., 2018; Mukhtar et al., 2011). A significant association between facility quality and microbial load on feeding bottles was observed. This is consistent with previous studies highlighting the prevalence of microbial contamination in healthcare settings (Jalali et al., 2009; Redmond et al., 2009; Alqurashi, 2019; Garg et al., 2019; Workie et al., 2019; Alemayehu, 2021). Researchers reported similar findings where association between disease burden, hospital facilities are directly associated (Bashir et al, 2022; Irfan et al, 2011; Ahmed et al, 2014; Nolan et al., 2001). For instance suboptimal hand hygiene practices or inadequate access to clean water after diaper changes significantly increase the risk of milk contamination when comes in contact with feeding bottles, potentially prolonging treatment of diarrhea and its recurrence in infants. Handlers as a primary source of contamination were provided training through lectures encouraging adherence to guidelines, and enhancing caregivers understanding of infection control principles. The study evaluated the effectiveness of training to reduce microbial contamination on handler's hands.

These findings were in line with research by Zaidi (Szewczak et al. 2018; Zaidi and Smith-Morris 2015). Moreover, studies by Were et al. 2020 demonstrating the importance of proper handling and hygiene practices in reducing microbial contamination.

Besides, study demonstrates significant reductions on several parts of feeding bottles through training caregivers. Key findings were training significantly reduced microbial counts on outer teat ( $p < 0.001$ ), Inner teat ( $p < 0.001$ ), Interior of bottles and handler's hands ( $p < 0.001$ ), but no significant reduction in microbial count after for milk, water and screw cap inner was observed. It highlights more attention is needed for milk, water, and screw cap inner. Despite training, persistent microbial contamination in within formula milk or water used to prepare formula may be attributed to inherently spoiled milk or contaminated water. This underscores that good practices alone are insufficient to minimize risk. Effective mitigation demands quality checks at the point of purchase, ensuring the safety of these critical resources. Moreover, high level of contamination within screwcap even after training represents unavailability of feeding brush or liquids at hospital facility to properly wash the bottles.

Significantly reduction of microbial counts on outer teat, Inner teat, Interior of bottles and handler's hands highlights effectiveness of training interventions. This aligns with previous research highlighting the effectiveness of training programs in improving hygiene practices (Qurayshah et al., 2022; Rao et al., 2007; Sudershan et al., 2008; Sheth and Obrah, 2004).

Similar reductions in microbial counts have been reported in studies evaluating the effectiveness training programs for health care professionals (Qurayshah et al. 2022). Several other studies demonstrated a positive impact of training regarding the onset and quantum of disease (Garg et al. 2019; Marege et al. 2023; Mosisa and Tarekegn 2022; Al-Mamari et al. 2017; Rao et al. 2007).

The study's results have significant implications for healthcare policy and practice. Recommendations include:

- ✓ Implement frequent monitoring of hospital environments along with evaluation of microbial counts and enforce good hygiene principles.
- ✓ Educate parents on proper hygiene, handling, preparation, storage, and sterilization practices related to ready to feed milk feeding bottles
- ✓ Provide handwashing facilities, water boiling facilities, sterilization units, and feeder washing liquids in hospitals.
- ✓ Conduct regular training programs for caregivers handling diarrheal infants.
- ✓ Launch awareness campaigns emphasizing personal and feeder hygiene.

Future studies should

- Elucidate the etiology of diarrhea associated with bottle feeding, focusing on the quality and characteristics of microbial contaminants.
- Assess the long-term efficacy of training programs on sustaining improved hygiene practices among healthcare staff and caregivers.
- Develop, implement, and evaluate evidence-based guidelines for feeding bottle hygiene, incorporating best practices for sterilization, handling, and storage.

### **Limitations**

The study's limitations include:

- Small sample size
- Limited generalizability

### **Conclusion**

The hygiene of feeding bottles used for infants with diarrhea in hospitals across Southern Punjab, Pakistan, falls short of satisfactory standards. This subpar hygiene, coupled with inadequate hospital facilities, contributes to compromised health and well-being of infants., prolonged treatment durations for diarrhea and its recurrence. However, there is a silver lining: training programs for healthcare staff have been shown to significantly reduce bacterial loads in feeding bottles. By addressing these critical issues, hospitals can significantly improve the hygiene standards of feeding

bottles, reduce the incidence of diarrhea, and provide a healthier environment for vulnerable infants. Furthermore, to enhance training impact, educators should identify stage-specific barriers to food safety and hygiene in formula milk preparation and tailor interventions according to international or national standards. Moreover, hospitals require more than just adherence to requirements; they need a comprehensive approach that includes dedicated resources, strong leadership, and a culture that prioritizes food safety and hygiene. By addressing these gaps, hospitals can reduce microbial contamination, prevent diarrhea, and promote a safer environment for infant care.

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The authors declare no conflict of interest

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**Author Contributions:**

Rabab Akhtar & Saeed Akhtar (concept/design), Rabab Akhtar & Sobia Zafar (data acquisition), Rabab Akhtar & Zubair Farooq (data analysis/interpretation).

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