Iranian Journal of Microbiology
Volume 17 Number 4 (August 2025) 606-612
DOI: http://doi.org/10.18502/ijm.v17i4.19251

ORIGINAL ARTICLE

Implementation of hazard analysis and critical control points (HACCP) for microbial safety of enteral feeding solutions at Imam Khomeini Hospital, Urmia, Iran

Mohammad Yousefi[1], Kave Koorehpaz[2], Morteza Bagheri[3], Yaghob Sharifi[4], Amir Rahimirad[5], Yousef Roosta[6*]

[1]Food and Beverages Safety Research Center, Urmia University of Medical Sciences, Urmia, Iran
[2]Department of Theriogenology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
[3]Cellular and Molecular Research Center, Cellular and Molecular Medicine Research Institute, Urmia University of Medical Sciences, Urmia, Iran
[4]Department of Microbiology, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran
[5]Food and Drug Administration, Urmia University of Medical Sciences, Urmia, Iran
[6]Department of Internal Medicine, School of Medicine, Urmia University of Medical Sciences, Urmia, Iran

Received: March 2024**, Accepted:** July 2025

ABSTRACT

Background and Objectives: Enteral feeding solutions (gavage) play a vital role in supporting ICU patients who cannot eat by mouth. However, their preparation is vulnerable to microbial contamination, posing serious health risks. This study aimed to assess and improve the microbial safety of enteral feeding solutions prepared at Imam Khomeini Hospital in Urmia, Iran.

Materials and Methods: A three-phase intervention was conducted involving microbial and PCR analyses, source identification, and corrective measures. Initial testing revealed high contamination levels: coliform bacteria (>5×10³ CFU/mL), fungi (>3×10³ CFU/mL), and total mesophilic bacteria (>10⁴ CFU/mL). PCR analysis confirmed the absence of *Escherichia coli* and *Klebsiella* spp. Corrective actions—such as installing UV lighting, implementing enhanced cleaning protocols, and replacing the mixing device—were introduced.

Results: Post-intervention analyses showed complete elimination of detectable microbial contamination in the gavage solutions.

Conclusion: This study demonstrates that implementing a HACCP-based approach can effectively eliminate microbial contamination in enteral feeding solutions. The findings support the development of national guidelines and highlight the importance of standardized safety practices to improve patient care in hospital settings.

Keywords: Enteral nutrition; Hospitals; Food contamination; Hazard analysis and critical control points; Polymerase chain reaction

*Corresponding author: Yousef Roosta, MD, Department of Internal Medicine, School of Medicine, Urmia University of Medical Sciences, Urmia, Iran. Tel: +98-9143819315 Fax: +4445223471 Email: yroosta@gmail.com

INTRODUCTION

Enteral feeding solutions, often called "gavage", play a crucial role in providing nutritional support to patients in intensive care units (ICUs) who cannot eat by mouth. These solutions are delivered directly into the gastrointestinal tract, bypassing oral intake, and are essential for sustaining the nutritional health of critically ill patients (1). However, the preparation and handling of these solutions can lead to microbial contamination, posing serious risks of infections and complications, especially for those with weakened immune systems (2).

Several studies in Iranian hospitals have highlighted significant microbial contamination in hospital-prepared enteral feeding solutions. For instance, the study "microbiological quality of hospital-prepared blenderised tube feeding in Mashhad, Iran" examined 24 samples of blenderized enteral feeds from teaching hospitals. It found high levels of microbial contamination, including harmful bacteria like *E. coli*, *Salmonella*, and *Listeria monocytogenes* (3). In another study, the authors emphasized the critical importance of maintaining sterile conditions during enteral feed preparation. They explained that contamination risks increase without proper aseptic techniques, leading to potential infections in vulnerable patients. The authors called for strict hygiene protocols and staff training. Ensuring an aseptic environment is essential to protect patient health and ensure feeding safety (4).

In late 2023, initial studies at Imam Khomeini Hospital in Urmia, Iran, uncovered concerning levels of microbial contamination in the prepared gavage solutions. The contaminants identified included coliform bacteria, fungi, and aerobic mesophilic bacteria, all of which can lead to various health problems such as acute or bloody diarrhea, gastroenteritis, food poisoning, sepsis, and systemic fungal infections (1). Given that these solutions are given to ICU patients, who are already vulnerable due to their compromised immune systems, the urgency for immediate action seemed to be clear.

The preparation method at the hospital involved mixing commercial gavage powders with water in PET bottles, which were then sent to ICU units. Although this process appeared simple, it was identified as a major source of contamination. The issue was further complicated by the absence of national microbiological standards for enteral feeding solutions. There were no official guidelines to define acceptable levels of microbial contamination.

To address this issue, a collaborative effort was formed. It included the Food and Beverages Safety Research Center, the Research and Technology Deputy of Urmia University of Medical Sciences, and the Research Deputy of Imam Khomeini Hospital. The project focused on implementing a Hazard Analysis and Critical Control Points (HACCP) plan to guarantee the microbial safety of enteral feeding solutions. The HACCP system, which is well-established in food safety management, was tailored for the hospital environment to identify and control critical points in the preparation process where contamination might occur (5). This study outlines the results of this intervention, presenting the outcomes of that intervention, highlighting the identification of contamination sources, the execution of corrective actions, and the resulting decrease in microbial contamination. The findings emphasize the necessity of establishing national standards for enteral feeding solutions and the importance of ongoing monitoring to ensure patient safety.

MATERIALS AND METHODS

Study design. This study was conducted in three phases: (1) assessment of microbial contamination levels, (2) identification of contamination sources, and (3) implementation of corrective measures. The study protocol was approved by the Ethics Committee of Urmia University of Medical Sciences (Approval No. IR.UMSU.REC.1402.222).

Microbiological analysis. Samples of hospital-prepared gavage solutions were collected and analyzed for microbial contamination. In collaboration with Urmia Food and Drug Administration, the samples were screened to detect the presence of various microorganisms, including: mesophilic aerobic bacteria, *Yersinia*, *Salmonella*, coliforms, *Listeria monocytogenes*, fungi, and *Pseudomonas* species. Subsequent analyses focused on coliforms, fungi, and mesophilic aerobic bacteria, which were identified as the main contaminants. Microbial counts were determined using standard plate count methods, and the results were compared with the U.S. Food and Drug Administration (FDA) manual (6). The 1:10 dilution and the spread plate method were used for bacterial

cultivation, except for collecting samples from surfaces and air. Additionally, YGC medium (Canada, Quelab) was used for fungal cultivation, VRBA (Canada, Quelab) for coliforms, and Nutrient Agar (Canada, Quelab) for total mesophilic bacteria. The spread plate method involved spreading a dilution of 10^{-1} of all microbial sample onto the surface of an agar plate using a sterile L-shaped glass spreader.

PCR methodology. PCR analysis was performed to detect the presence of *E. coli* and *Klebsiella* spp. In this regard, ten samples were examined. All samples were transported to the Department of Medical Microbiology at Urmia Medical School. The samples were cultured and analyzed.

Bacterial DNA was isolated using Nodex Pro Catalog No. NAT0015 (BIO-IDEA, Iran). The Polymerase Chain Reaction (PCR) was performed using specific primers (Table 1). PCR mixture (25 µl) consisted of 2X PCR RED Master Mix (Redimix) Cat. Number: NAT001 (BIO-IDEA, Iran) (12.5 µl), molecular grade water (7.5 µl), 1 µl of forward primer, 1 µl of reverse primer, and 3 µl of DNA template. Thermocycler (Bio-Rad, USA) was used for PCR for *E. coli* and multiplex-PCR for *Klebsiella* spp. A set of primers targeting the uspA gene was used for *E. coli* detection. The primers *SHV-F, LEN-F, OKP-F* and *DEOR-R,* were used in a multiplex PCR system for identification of *K. pneumoniae, K. variicola* and *K. quasipneumoniae,* respectively. The PCR products were subjected to electrophoresis on a 2.5% agarose gel accompanied by DNA safe Stain (Cinna Gen, Tehran, Iran) and visualized using a UV-trans illuminator.

Sampling protocols. Potential contamination sources, including water, ventilation system air, PET bottles, commercial gavage powder, room air, and mixing devices, were investigated. Samples were collected from each potential source and analyzed for microbial contamination using standard sampling methods appropriate for each source. Sampling was conducted over a two-month period (November to December 2024), three times per week. All samples were immediately transferred to the laboratory under controlled conditions with cold chain maintained, and microbiological tests were performed.

Water samples were collected in sterile plastic containers following aseptic techniques. Samples were transported to the laboratory under the cold chain maintained (ISIRI 4208). Samples were preserved at 4°C and analyzed within 24 hours for microbial contamination, including aerobic plate count, coliforms, and *E. coli*. Room air and ventilation system air were sampled using air sampling devices (settle plates) to collect airborne microorganisms at various locations within the room (ISIRI 10847-16). PET bottles were sampled after rinsing the inner surfaces of PET bottles with a sterile peptone water, then the rinse fluid was collected (ISIRI 2305).

Sampling of the mixing device was performed using the swab method. The device was swabbed from three parts, including (blades, floor, inner wall, and outlet valve) using sterile swabs moistened with a neutralizing buffer (INSO 4806). All samples were collected on separate days, with at least six replicates. All microbiological analyses were performed in triplicate.

Corrective measures. Following the identification of contamination sources, corrective actions were implemented, which are described in the results section.

Table 1. Bacteria, primer name, primer sequence, target gene, product size and thermal cycling conditions in this study

Bacteria	Primer Name	Primer Sequence	Target Gene	Size product	PCR program	Reference
E. coli	*uspA*	5’-ccgatacgctgccaatcagt-3’ 5’-acgcagaccgtaggccagat-3’	*uspA*	884 bp	94°C for 5 min; 94°C for 2 min, 70°C for 1 min, 72°C for 1 min (30 cycles); 10 min at 72°C	(7)
K. species	*SHV-F*	5’-gctggcggtacacgccagcccg-3’	bla_{SHV}	995 bp	95°C for 5 min; 94°C for 30 sec; 55°C for 30 sec; 72°C for 1 min (40 cycles); 10 min at 72°C	(8)
	LEN-F	5’-cacgctgcgyaaactactgacygcgcagca-3’	bla_{LEN}	485 bp		
	OKP-F	5’-ggccggygagcggggctca-3’	bla_{OKP}	348 bp		
	DeoR-R	5’- agaagcatcctgctgtgcg-3’	3′ end of the *deo*R			

RESULTS

Microbial contamination levels. Initial microbial testing of the prepared gavage solutions revealed microbial counts significantly exceeding international safety thresholds. Specifically, coliform bacteria reached 3,000 CFU/mL, fungi exceeded 5,000 CFU/mL, and aerobic mesophilic bacteria exceeded 10^4 CFU/mL. According to U.S. Food and Drug Administration (FDA) (6), coliforms and fungi should be absent or below 10 CFU/mL, and aerobic mesophilic bacteria should not exceed 5000 CFU/mL. These elevated levels indicated major hygienic deficiencies in the gavage preparation process, posing a health risk to ICU patients with compromised immune systems.

PCR analysis. PCR testing of ten samples showed no detectable presence of *E. coli* or *Klebsiella* spp. This suggests that while overall contamination levels were high, specific high-risk pathogens were not detected in the tested samples. However, due to the small sample size, this finding should be interpreted cautiously.

Identification of contamination sources. Gavage preparation at Imam Khomeini Hospital was carried out in a designated room (Fig. 1). Following all samplings and analyses, the mixing device was identified as the main source of contamination, accounting for over 95% of the microbial load. Minor contamination was also attributed to airborne fungi in the preparation area. Figures illustrate the microbial contamination (coliforms, fungi, and total mesophilic bacteria) in water, PET bottles, gavage powder (Fig. 2), the ventilation system, and room air (Fig. 3).

To confirm the contamination of the mixing device, sampling was initially conducted in two stages. In the first step, gavage samples were prepared in two ways: using the mixing device and manually with a thoroughly cleaned hand mixer. These samples were then subjected to microbial analysis. The results, as shown in Fig. 4, revealed a significantly higher level of contamination in the gavage samples prepared using the mixer device. In the next stage, surface swabbing was performed on three parts of the mixing device, including the blades, floor, the inner wall, and the outlet valve. The results of this sampling are illustrated in Fig. 5. High contamination levels were found on the blades and floor as well as the outlet valve, while the inner wall showed above-threshold levels of fungi and coliform bacteria.

Fig. 1. Preparation room of gavage solution in Imam Khomeini Hospital, Urmia, Iran.

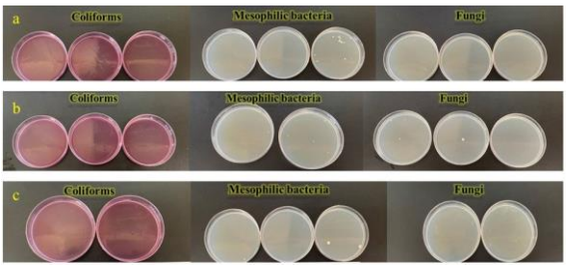

Fig. 2. The results depict the number of coliform bacteria, fungi, and total mesophilic bacteria in the analyzed (a) water sample, (b) PET bottles, (c) gavage powder.

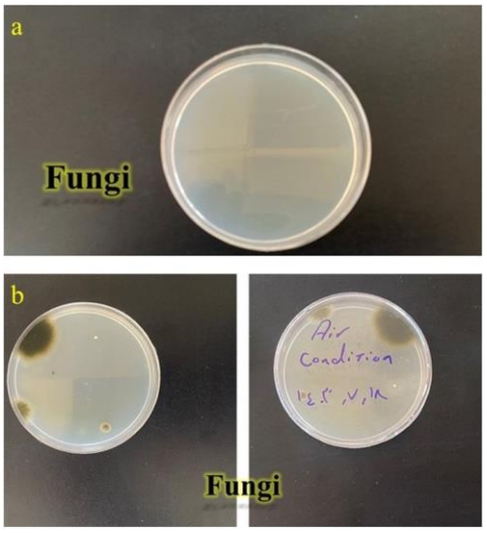

Fig. 3. The results depict the number of fungi in the analyzed (a) ventilation system air, (b) room air.

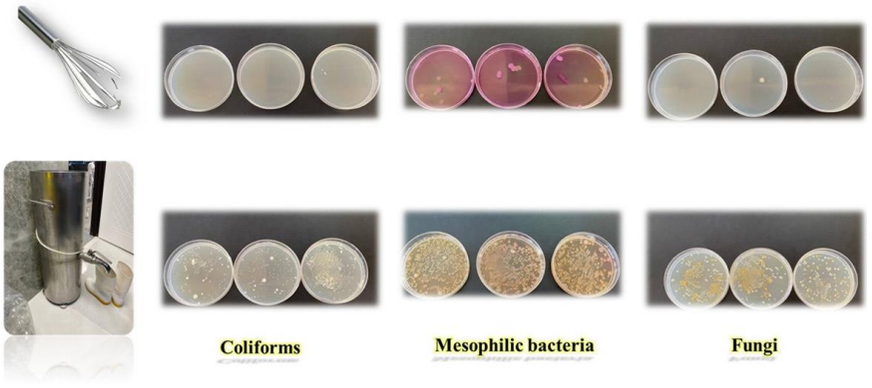

Fig. 4. The results depict the number of coliform bacteria, fungi, and total mesophilic bacteria in the analyzed gavage solutions prepared by a hand mixer and a mixing device.

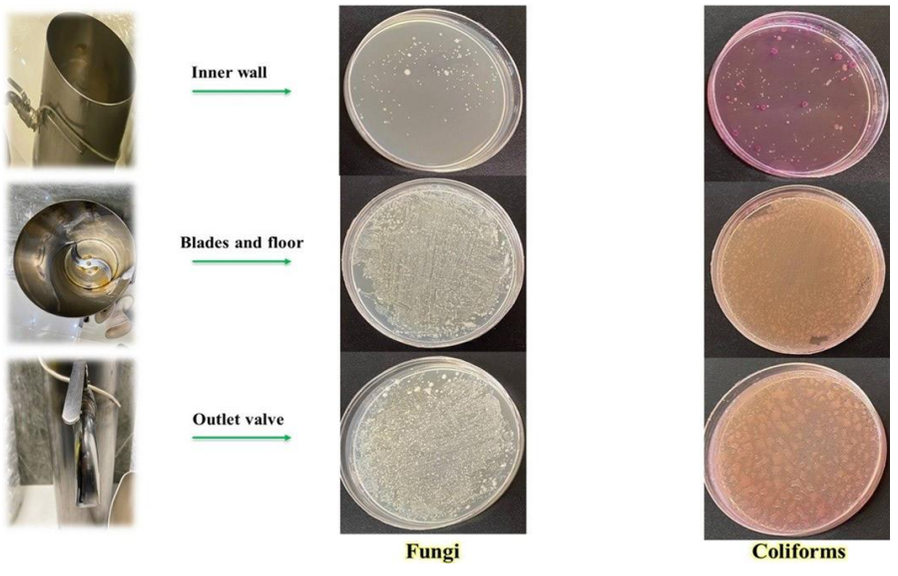

Fig. 5. The results depict the number of coliform bacteria and fungi, in the analyzed three areas of the mixing device, including the blades and floor, the inner wall, and the outlet valve.

Implementation of corrective measures. Based on the findings, inadequate washing and disinfection, poor room air quality, and the mixing device were identified as three critical control points. Based on this, three main interventions were implemented to address the contamination:

a) UV Lighting Installation: UV lamps were installed in the preparation area and were operated for 15 minutes both before and after each preparation session to minimize airborne contamination (9).

b) Enhanced Cleaning Protocols: The newly installed mixing device underwent a thorough cleaning with hot water and disinfectants, with special attention paid to hard-to-reach areas like the floor and outlet tube (10).

c) Mixing Device Replacement: A new device with an easily accessible outlet valve was acquired to facilitate cleaning and disinfection (11).

Post-intervention testing revealed complete elimination of microbial contamination, with no detectable levels of coliform bacteria, fungi, or aerobic mesophilic bacteria in the gavage solutions ($p<0.05$). It is important to note that post-intervention tests were conducted using the same method as the pre-intervention tests. The results exceeded expectations, confirming the effectiveness of the interventions. The results are summarized in Table 2.

Table 2. Microbial counts before and after intervention

Microorganism	Pre-Intervention (CFU/mL)	Post-Intervention (CFU/mL)
Coliform bacteria	3000 ± 400, n=10	0, n=10
Fungi	>5000, n=10	0, n=10
Aerobic mesophilic bacteria	>104, n=10	0, n=10

n: Sample size

DISCUSSION

This study highlights the urgent need for standardized safety protocols and ongoing microbiological monitoring of enteral feeding solutions in hospitals across Iran. The significant levels of microbial contamination found in the initial analysis align with results from other research conducted in similar environments. Hubbard, Van Wyk (12) reported that inadequate handling and storage of enteral feeding solutions were the major factors contributing to microbial contamination in hospital settings.

Despite the promising outcomes, this study had certain limitations. The PCR sample size was relatively small (only ten samples), which may limit the generalizability of the findings regarding the absence of *E. coli* and *Klebsiella* spp. Additionally, the study was limited to a single hospital setting, and the findings may not fully represent conditions in other Iranian healthcare facilities. The effectiveness of the interventions, particularly the use of UV lighting, enhanced cleaning protocols, and mixing device replacement, highlights the importance of addressing critical contamination points. These interventions are not only practical but also cost-effective in the long run, as they can reduce infection-related complications, which can lower treatment costs and hospital stay durations. Ultimately, these improvements contribute to improved patient safety and overall hospital hygiene.

Comparison with other hospitals in Iran. Numerous studies have reported disturbingly high rates of contamination in hospital-prepared enteral feeding solutions in various regions of Iran:

Isfahan: An investigation of hospital-prepared tube feedings from three ICUs in Isfahan showed that 70% of samples were contaminated with coliforms and 90% with *Staphylococcus aureus* at the time of preparation. After 18 hours of storage, these numbers rose to 90% and 95%, respectively, indicating rapid microbial proliferation during storage (13).

Kerman: A study conducted in Kerman found various pathogens, including *E. coli* and *Klebsiella pneumoniae*, in blenderized feedings, with average bacterial contamination levels reaching 5.5×10^6 CFU/ml. In contrast, commercial feeding solutions showed contamination levels below 1 CFU/mL, indicating better microbial safety (14).

Tabriz: Research in Tabriz assessed the nutritional adequacy and bacterial contamination of enteral feedings used in ICUs. The findings revealed that while blenderized tube feedings had a higher energy density compared to commercial powder feedings, 33% of commercial samples were found to contain coliforms, and *E. coli* was only found in commercial preparations. No *Staphylococcus aureus, Salmonella,* or *Listeria monocytogenes* were detected in either type of the feeding (15). The high contamination levels reported in these studies can be linked to several factors commonly found in Iranian hospitals, including poor preparation practices, inadequate equipment sanitation, and environmental contamination.

Recommendations for mitigation. Based on findings from this and other studies conducted in Iranian hospitals, to tackle the significant problem of microbial contamination in enteral feeding solutions, the following measures are suggested:

a) Adoption of Commercial Enteral Feeding Products: Studies have shown that commercial enteral feeding products have much lower contamination rates than those prepared in hospitals using blenders. Switching to these sterile, commercially available options can significantly reduce the risk of microbial contamination (16).

b) Regular Microbiological Monitoring: There is a need for routine testing of the enteral feeding solutions for microbial contamination. Setting up regular monitoring schedules allows for the early detection of any contamination and timely corrective actions (17).

c) Staff Training and Education: Regular training programs that emphasize proper hygiene practices, equipment sanitation, and safe preparation techniques are essential. Equipping healthcare workers with the necessary knowledge and skills can significantly improve the microbiological safety of the enteral nutrition.

d) Environmental Controls: Keeping a controlled environment in preparation areas, which includes

managing air quality and adhering to regular cleaning protocols, can help minimize the risk of airborne contaminants entering feeding solutions.

e) Development of National Standards: The absence of national standards and protocols for enteral feeding solutions in Iran poses a serious challenge to patient safety. There is an urgent need to establish these standards in line with international guidelines. This study supports the creation of a national policy for preparation and monitoring of enteral feeding solutions, along with regular audits to ensure adherence.

CONCLUSION

This study demonstrated that a HACCP-based approach can effectively reduce microbial contamination in enteral feeding solutions. The findings underscore the need to establish national standards and conducting regular monitoring to guarantee the safety of these solutions in hospitals across Iran. Future research should evaluate the long-term sustainability of these interventions and their potential use in other healthcare environments.

ACKNOWLEDGEMENTS

This study was funded by Urmia University of Medical Sciences, Urmia, Iran, with ethical number: IR.UMSU.REC.1402.222.

REFERENCES

1. Sinha S, Lath G, Rao S. Safety of enteral nutrition practices: overcoming the contamination challenges. *Indian J Crit Care Med* 2020; 24: 709-712.
2. Mohamed Elfadil O, Velapati SR, Patel J, Hurt RT, Mundi MS. Enteral nutrition therapy: historical perspective, utilization, and complications. *Curr Gastroenterol Rep* 2024; 26: 200-210.
3. Adibi S, Afshari A, Norouzy A, Nematy M, Ehsani A, Hashemi M. Microbiological quality of hospital-prepared blenderised tube feeding. *J Hum Nutr Diet* 2023; 36: 181-190.
4. Rismanbaf A. The necessity of an aseptic environment for the preparation of enteral feeding solutions in Iranian Hospitals. *J Res Pharm Pract* 2020; 9: 60.
5. Al-Akash H, Abu Arrah A, Bhatti F, Maabreh R, Abu Arrah R. The effect of food safety training program on food safety knowledge and practices in hotels' and hospitals' food services. *Ital J Food Saf* 2022; 11: 9914.
6. Pharmaceutical Microbiology Manual. Office of Regulatory Affairs. Silver Spring, Maryland, USA: FDA; 2020. Available from: https://www.fda.gov/media/88801/download
7. Kayis FB. Antibiotic resistance profiles of *Escherichia coli* isolated from the floating islands and water of Çat Dam Lake, Adiyaman, Turkey: Antibiotic resistance of *E. coli* from Floating Islands and water. *Indian J Exp Biol* 2024; 62: 49-55.
8. Fonseca EL, Ramos ND, Andrade BG, Morais LL, Marin MF, Vicente AC. A one-step multiplex PCR to identify *Klebsiella pneumoniae*, *Klebsiella variicola*, and *Klebsiella quasipneumoniae* in the clinical routine. *Diagn Microbiol Infect Dis* 2017; 87: 315-317.
9. Memarzadeh F, Olmsted RN, Bartley JM. Applications of ultraviolet germicidal irradiation disinfection in health care facilities: effective adjunct, but not stand-alone technology. *Am J Infect Control* 2010; 38(5 Suppl 1): S13-S24.
10. Rutala WA, Weber DJ. Disinfection and sterilization in health care facilities: an overview and current issues. *Infect Dis Clin North Am* 2021; 35: 575-607.
11. Best C. Enteral tube feeding and infection control: how safe is our practice? *Br J Nurs* 2008; 17: 1036, 1038-1041.
12. Hubbard GP, Van Wyk J, Grinyer L, Onley R, White S, Fleming CA, et al. Appropriate handling and storage reduce the risk of bacterial growth in enteral feeding systems reused within 24 hours. *Nutr Clin Pract* 2024; 39: 437-449.
13. Jalali M, Sabzghabaee AM, Badri SS, Soltani HA, Maracy MR. Bacterial contamination of hospital-prepared enteral tube feeding formulas in Isfahan, Iran. *J Res Med Sci* 2009; 14: 149-156.
14. Ahmadinejad M, Moshafi MH, Izadi F, Mousavipour F, Sohrevardi SM. Bacterial contamination of hospital-prepared enteral tube feeding formulas in Kerman, Iran. *Adv Pharmacol Ther J* 2024; 4: 27-35.
15. Mahinkazemi M, Tarighat-Esfanjani A, Safaiyan A. Bacterial contamination and nutritional adequacy of enteral tube feedings in Iran. *Prog Nutr* 2017; 19: 283-290.
16. Oliveira MR, Batista CR, Aidoo KE. Application of Hazard Analysis Critical control points system to enteral tube feeding in hospital. *J Hum Nutr Diet* 2001; 14: 397-403.
17. Kozow JFC, Rabito EI, Kruger JF, Medeiros CO, da Costa RL, Beux MR, et al. Microbiological contamination present in enteral tube feeding prepared in hospitals and/or at home: a systematic review. *Nutr Rev* 2024; 82: 1226-1238.