

Original Article**Bacterial contamination of hospital-prepared enteral tube feeding formulas  
in Isfahan, Iran**

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**Abstract**

**BACKGROUND:** Hospital-prepared tube feedings from three intensive care units of two hospitals in Isfahan, Iran were analyzed for microbial contamination.

**METHODS:** A total number of 152 samples (76 samples each at the time of preparation and 18 hours following preparation) were collected. Standard plate count, coliform count and *Staphylococcus aureus* count for all samples were conducted. Samples were analyzed also for the presence of *Salmonella* spp. and *Listeria* spp.

**RESULTS:** At the time of food preparation, out of 76 samples, 53 samples (70%) had coliform contamination and 87% of these contaminated samples had counts greater than  $10^1$  cfu/g. Also, 68 samples (90%) had *S. aureus* contamination greater than  $10^1$  cfu/g. In standard plate count, 74 samples (97%) had counts greater than  $10^3$  cfu/g, while 54 samples (71%) had counts greater than  $10^4$  cfu/g. In second sampling occasion, out of 76 samples, 68 samples (90%) had coliform contamination and 84% of these contaminated samples had counts greater than  $10^1$  cfu/g. Also, 72 samples (95%) had *S. aureus* contamination, 98.6% of these contaminated samples had counts greater than  $10^2$  cfu/g. In standard plate count, 74 samples (97%) had counts greater than  $10^4$  cfu/g. No *Salmonella* or *Listeria* was detected from samples.

**CONCLUSIONS:** The results indicated that a majority of the blenderized enteral tube feedings in those hospitals are not safe. In comparison to the standard limits, these enteral tube feedings are highly contaminated and posed substantial risk for developing a foodborne disease or nosocomial infection.

**KEYWORDS:** Enteral Feeding, Microbial Contamination, Nosocomial Infection, Standard Plate Count, Coliform.

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Enteral nutrition is essential in the care of patients who are unable to eat. Potential complication of enteral feeding is microbial contamination of the solution.<sup>1</sup> Enteral feeding fluid is a good medium for exponential growth of most of the foodborne microorganisms.<sup>2,3</sup> Contaminated feeding increases the risk of nosocomial infections such as diarrhea, pneumonia and septicaemia.<sup>4,6</sup> Occurrence of various microorganisms in tube feeding formula, have been investigated in several coun-

tries.<sup>2,6-11</sup> In the Philippines, 75% to 96% of blenderized tube feeding samples were reported to have standard plate counts greater than  $10^1$  cfu/g.<sup>12</sup> Higher bacterial contamination of hospital-prepared tube feeding formulas have been reported in Saudi Arabia. Nearly all samples had aerobic plate counts greater than  $10^4$  cfu/g in this country.<sup>13</sup> Although sterile ready-to-eat feeding is available in developed nations, blenderized feedings continue to be used in most parts of Iran, mostly due to

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economic and cultural reasons. Since there is no information on the level of contamination of hospital-prepared tube feeding systems in Iran, the present study aimed to evaluate the microbial quality of blenderized enteral tube feedings in three intensive care units of two university hospitals in Isfahan, Iran.

## Methods

Three intensive care units selected from two university hospitals (A and B), were participated in this study. Feedings used in this study, were prepared in the main kitchen of each hospital by chef under nutritionist supervision. All tube feeding ingredients (egg, milk, meat, etc.) were cooked and mixed (blenderized) to provide appropriate calories. The feeds were prepared daily in quantities that would allow feeding patients for about 24 hours. The feedings were shipped to the wards every day (between 11-12 am) in closed containers and stored in refrigerator for 24 hours. In the period between October 2005 and September 2006, a total number of 152 samples were collected (46, 46 and 60 samples from hospital A-Central ICU, hospital A-Trauma ICU and hospital B-Neurosurgery ICU, respectively). Feeding samples were marked and 50mL of feeds were collected in two occasions, immediately and 18 hours following preparation, for microbial analysis. All samples were transported to the Food Microbiology Laboratory in School of Public Health of Isfahan University of Medical Sciences in an icebox for microbiological analysis. Standard plate count, coliform count and *Staphylococcus aureus* count for all samples were conducted. Samples were analyzed also for the presence of *Salmonella* spp. and *Listeria* spp.

The number of aerobic bacteria, coliforms and *S. aureus* were determined using pure plate technique.<sup>14</sup> For total count, coliform count and *S. aureus* quantification, ten-fold serial dilutions were prepared in 0.1% sterile Buffered Peptone Water (Oxoid). From each dilution a 1mL aliquot was added to Nutrient Agar (NA, Merck, Germany), Violet Red Bile Agar (VRBA, Merck) and Baird-Parker Agar

(BPA, Merck). Colony counts per mL of feed were done after incubation at 37°C for 24-48 hours. Typical colonies on VRBA and BPA were also examined using suitable biochemical tests.<sup>12</sup> Results are expressed as colony forming units (cfu)/ mL of food.

Samples were analyzed for the presence of *Listeria* spp. and in particular for *Listeria monocytogenes* using selective enrichment and isolation protocol, recommended by United States Department of Agriculture (USDA).<sup>15</sup> Twenty-five grams of a sample was aseptically taken, homogenized for 2 minutes in 225mL of UVM *Listeria* enrichment broth (UVM I) (Difco, America) and incubated at 30°C for 24 hours. One mL of primary enrichments were transferred to 9mL of UVM II (Fraser broth) (Amyl Media, Australia) and incubated at 35°C for 48 hours. Secondary enrichments were streaked on Oxford Agar (Merck) and Palcam Agar (Merck) and incubated at 37°C for 48 hours. The plates then examined for typical *Listeria* colonies (black colonies with black sunken) and at least 3 suspected colonies were sub cultured on Tryptone Soy Agar supplemented with 0.6% of yeast extract (TSAYE) and incubated at 37°C for 24 hours. All isolates were subjected to standard biochemical tests such as Gram's stain, catalase test, motility at 25°C and 37°C, acid production from glucose, manitol, rhamnose, xylose,  $\alpha$ -methyl- D- manoside, and nitrate reduction, hydrolysis of esculin and MR/VP test. For further confirmations of *Listeria* spp., other biochemical reactions,  $\beta$ -haemolytic activity, and CAMP test were performed according to the Bergey's Manual of Systematic Bacteriology.<sup>16</sup>

Samples were also examined for the presence of *Salmonella* spp. by the reference method (No 1810) recommended by the Iranian Standard Organization for the isolation of *Salmonella*.<sup>17</sup> Briefly a 25g portion of each sample was weighed aseptically in a sterile stomacher bag containing 225mL sterile Buffered Peptone Water (BPW) and shaken for 2 minutes. BPW was used for pre-enrichment at 37°C for 18-24 hours. One mL of the pre-enriched sample was then inoculated into 10mL of Modified Rappa-

port-Vassiliadis (RV, Merck) broth and 9mL of Selenite Cystine (SC, Merck) broth and was incubated at 42°C and 37°C respectively for 24 hours. Xylose Lysine Deoxycholate (XLD, Merck) medium was used as selective isolation media and incubated at 37°C for 24 hours. At least three characteristic colonies were picked from each plate and purified by streaking on Tryptone Soy Agar (TSA, Merck). Cultures were further subjected to analysis for Gram's stain, motility, ONPG, urease, lysine decarboxylase and reaction on Triple Sugar Iron Agar. Results were expressed as presence or absence of *Salmonella* or *Listeria*.

### Statistical Analysis

Statistical analyses were conducted after

computing log cfu/g and by using SPSS package, version 13.0.<sup>18</sup> Wilcoxon Signed Ranks Test was used to determine a statistically significant difference between onset and 18 hours after food preparation. Differences were considered significant when  $p < 0.05$ .

### Results

At the time of food preparation, there were the range of total viable counts, coliform count and *S. aureus* count among 23 samples of food in hospital A-Trauma ICU, 23 samples in hospital A-Central ICU and 30 samples in hospital B-Neurosurgery ICU. There were significant increases in counts 18 hours after food preparation ( $p$  value  $< 0.001$ ). The results have been shown in table 1.

**Table 1.** Bacterial contamination of hospital-prepared tube feeding samples in three intensive care units, at the time of food preparation and 18 hrs after preparation.

Microbial count	No.	Range of contamination (cfu/g)		Z-test*	P-value
		At the time of food preparation	18 hours after food preparation		
Total viable microorganisms					
Hospital A/Trauma ICU	23	$1.9 \times 10^2$ to $3.5 \times 10^6$	$6.2 \times 10^3$ to $3.7 \times 10^7$	-4.20	$< 0.001$
Hospital A/Central ICU	23	$2.0 \times 10^2$ to $2.9 \times 10^5$	$3.0 \times 10^3$ to $4.5 \times 10^7$	-4.20	$< 0.001$
Hospital B/Neurosurgery ICU	30	$7.0 \times 10^3$ to $6.4 \times 10^7$	$1.2 \times 10^6$ to $1.1 \times 10^9$	-4.78	$< 0.001$
Coliform					
Hospital A/Trauma ICU	23	$< 10^1$ to $2.8 \times 10^3$	$< 10^1$ to $8.6 \times 10^4$	-3.82	$< 0.001$
Hospital A/Central ICU	23	$< 10^1$ to $4.1 \times 10^3$	$< 10^1$ to $4.4 \times 10^4$	-3.82	$< 0.001$
Hospital B/Neurosurgery ICU	30	$1.9 \times 10^1$ to $4.1 \times 10^6$	$4.8 \times 10^1$ to $1.5 \times 10^8$	-4.60	$< 0.001$
<i>S. aureus</i>					
Hospital A/Trauma ICU	23	$< 10^1$ to $4.0 \times 10^4$	$< 10^1$ to $4.2 \times 10^5$	-4.02	$< 0.001$
Hospital A/Central ICU	23	$< 10^1$ to $4.8 \times 10^4$	$< 10^1$ to $2.1 \times 10^5$	-3.63	$< 0.001$
Hospital B/Neurosurgery ICU	30	$2.0 \times 10^1$ to $3.8 \times 10^5$	$1.6 \times 10^3$ to $4.2 \times 10^6$	-4.78	$< 0.001$

\* Wilcoxon Signed Ranks Test  
cfu = colony forming units

**Table 2.** Number of contaminated hospital-prepared tube feeding samples in three intensive care units, at the time of food preparation and 18 hrs after preparation

	Hospital A/Trauma ICU ( n = 23 )						Hospital A/Central ICU ( n = 23 )						Hospital B/Neurosurgery ICU ( n = 30 )					
	Total count		Coliform count		S. aureus count		Total count		Coliform count		S. aureus count		Total count		Coliform count		S. aureus count	
	I*	II†	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II
0	0	0	11	4	4	2	0	0	12	4	4	2	0	0	0	0	0	0
< 10 <sup>1</sup>	0	0	4	1	0	0	0	0	3	3	0	0	0	0	0	0	0	0
10 <sup>1</sup>	0	0	4	6	2	0	0	0	7	6	3	1	0	0	2	1	1	0
10 <sup>2</sup>	1	0	1	4	7	2	1	0	0	2	4	3	0	0	2	1	1	0
10 <sup>3</sup>	10	1	3	6	8	8	9	1	1	6	9	4	1	0	4	1	3	2
10 <sup>4</sup>	8	1	0	2	2	9	11	4	0	2	3	10	1	0	10	9	19	1
10 <sup>5</sup>	4	5	0	0	0	2	2	6	0	0	0	3	7	0	7	0	6	19
10 <sup>6</sup>	0	12	0	0	0	0	0	5	0	0	0	0	9	4	5	6	0	8
10 <sup>7</sup>	0	4	0	0	0	0	0	7	0	0	0	0	12	6	0	11	0	0
10 <sup>8</sup>	0	0	0	0	0	0	0	0	0	0	0	0	0	19	0	1	0	0
10 <sup>9</sup>	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Total number of positive samples (%)	23 (100)	23 (100)	12 (52)	19 (83)	19 (83)	21 (91)	23 (100)	23 (100)	11 (48)	19 (83)	19 (83)	21 (91)	30 (100)	30 (100)	30 (100)	30 (100)	30 (100)	30 (100)

\* I = at the time of food preparation

† II = 18 hours after food preparation

cfu = colony forming units

*Salmonella* spp. and *Listeria* spp. were not isolated from any of the feeds.

As indicated in table 2, in first sampling, out of total 76 samples taken from three wards of two hospitals at the time of food preparation, 53 samples (70%) had coliform contamination, 87% of the contaminated samples had counts greater than 10<sup>1</sup> cfu/g. Out of samples collected immediately after preparation, 68 samples (90%) had *S. aureus* contamination greater than 10<sup>1</sup> cfu/g. In standard plate count, 74 samples (97%) had counts greater than 10<sup>3</sup> cfu/g, while 54 samples (71%) had counts greater than 10<sup>4</sup> cfu/g. In a second sampling occasion, 18 hours after preparation, out of total 76 samples, 68 samples (90%) had coliform contamination, 84% of these contaminated samples had counts greater than 10<sup>1</sup> cfu/g. Also, 72 samples (95%) had *S. aureus* contamination, 98.6% of these contaminated samples had counts greater than 10<sup>2</sup> cfu/g. In standard plate count, 74 samples (97%) had counts greater than 10<sup>4</sup> cfu/g. No *Salmonella* spp. and *L. monocytogenes* were detected from any samples at the time of preparation and 18 hours after storage in the wards.

## Discussion

In recent years, there has been a significant shift in the use of enteral nutrition over parenteral nutrition.<sup>19-22</sup> Using enteral nutrition can greatly impact the functional and structural integrity of the gastrointestinal tract.<sup>20,23</sup> One potential complication of enteral feeding, is microbial contamination of the solution. Enteral feeding solutions support the growth of a wide variety of microorganisms, creating a risk factor for many patients. Many studies have associated nosocomial infections, namely diarrhea, bacteremia and pneumonia to contamination of enteral feeding. Due to the increasing amounts of nosocomial infections occurring as a result of contaminated tube feeding formulas, regulatory agencies are requiring improved control procedures during preparation and administration of enteral feeding. Recent guidelines of Food and Drug Administration (2006) regarding microbial quality of medical foods, including tube feeding formulas, stated that action must be taken if any such products contain more than 10<sup>4</sup> cfu/g or if three or more

samples exceeded  $10^3$  cfu/g. Also they limit the acceptable level of coliforms to 3 organisms/gram.<sup>24</sup> According to the Parenteral and Enteral Nutrition Group of British Dietetic Association, the critical limit for total microbial counts of tube feeding samples is  $10^1$  cfu/g at the start of administration and less than  $10^3$  cfu/g at the end.<sup>25</sup> The Spanish legislation limits the *S. aureus* count to  $10^1$  organisms/gram.<sup>6</sup> Using a definition of "unacceptable" contamination as a standard plate count greater than  $10^3$  cfu/g, the results of present study show that 98.6% of the foods were unacceptably contaminated at the time of preparation and 18 hours after preparation. Also, 72% and 92% of all samples had unacceptable counts for coliforms and *S. aureus*, respectively (Table 2). Similar study on bacterial contamination of hospital-prepared tube feeding formulas in Saudi Arabia indicated that nearly all samples had aerobic plate counts greater than  $10^4$  cfu/g. It also reported maximum coliform counts of about 50 cfu/g.<sup>13</sup> The results of the present study show much higher maximum level of coliform contamination of about  $4.1 \times 10^6$  cfu/g at the time of administration (Table 1). This figure demonstrates poor hygienic condition in preparation of tube feeding in the kitchen. Poor hand hygiene and handling procedures are identified as the main source of microbial contamination.<sup>26</sup> The occurrence of coliforms in tube feeding samples receiving heat treatment indicates poor hygienic condition.<sup>14,27</sup> The presence of high *S. aureus* counts (maximum count of  $4.2 \times 10^6$  cfu/g) in sample may also indicates poor hygienic condition of the personnel. In the Philippines, 75% to 96% of blenderized tube feeding samples were reported to have standard plate counts greater than  $10^1$  cfu/g.<sup>12,28</sup> These results are also similar to our finding. The results of this study show significant increase in bacterial counts in the period of storing at wards (Table 1). The maximum level of standard plate counts, coliform and *S. aureus* counts significantly increased 18 hours after preparation and reached to  $1.1 \times 10^9$  cfu/g,  $1.5 \times 10^8$  cfu/g and  $4.2 \times 10^6$  cfu/g, respectively, which indicates the poor hygienic conditions

or more contamination of feeds during storage in the wards. Temperature abuse of feeds during storage in the wards is the other possibility which may allow exponential growth of bacteria in feeds. Regular measurement of refrigerator temperature (results are not shown) in three wards indicates that average temperature was above  $9.9^\circ\text{C}$ . Note that 0 to  $7^\circ\text{C}$  has been suggested as a proper temperature of domestic refrigerator.<sup>14</sup> Moreover, in some occasions, the feeds kept outside refrigerator for a long time. Similarly, other studies have also demonstrated substantial increase in bacterial counts over time (baseline of  $10^1$ - $10^2$  cfu/ml, increasing to  $10^5$ - $10^8$  cfu/ml over 8 hrs).<sup>3,29</sup>

In the present study, an attempt also was made to isolate two important foodborne pathogens. *Listeria monocytogenes* which is considered as a foodborne pathogen is able to cause meningitis, encephalitis, septicemia, endocarditis, abortion, premature birth, abscesses and local petulant lesions.<sup>30</sup> Although listeriosis occurs infrequently, the mortality rate is high, up to 75% in high risk persons such as immunocompromised people suffering from cancer, AIDS, etc.<sup>31</sup> Although, the presence of *L. monocytogenes* has been reported in a wide variety of foods in Iran,<sup>32</sup> we were not able to isolate any *Listeria* from food samples. As far as our knowledge, similar to our findings, *Listeria* has not been yet detected from tube feeding formulas.

*Salmonella* spp. is also established as one of the leading causes of foodborne disease. *Salmonella* is a causative agent of foodborne diarrheal disease worldwide.<sup>33</sup> Salmonellosis remains a major public health problem in many parts of the world<sup>34</sup> including Iran.<sup>35</sup> *Salmonella enteritidis* contamination of enteral tube feeding has been already reported.<sup>36</sup> In contrast, we were not able to detect *Salmonella* from 152 tube feeding samples.

Tube feeding formulas become contaminated at several points. Anderton et al (1993) highlighted both the main sources of contamination and possible detrimental effects of administering contaminated feeds to patients. Main sources of food contamination include

the feed ingredients, administration systems and their design, mishandling during assembly of systems, inadequate cleaned equipments, kitchen and ward environment, personnel and patients themselves.<sup>37</sup> Recently, Mathus-Viegen et al (2006) also studied sites of bacterial contamination of enteral feeding system.<sup>38</sup> In order to reduce microbial contamination of enteral tube feeding, various multidisciplinary approaches have been used.<sup>39,40</sup> For example, Hazard Analysis Critical Control Points (HACCP) system is internationally recognized as the best method of assuring product safety by controlling foodborne safety hazards. In a study, HACCP system was implemented for improvement of microbial quality of enteral tube feeding in a hospital, and when the control measures applied and monitored, the bacterial counts in feeds reduced from  $10^5$  cfu/mL to  $< 10^1$  cfu/mL.<sup>41</sup>

### Conclusions

The results of present study indicate that the microbial quality of the majority of blenderized enteral tube feedings in both hospitals is not within published guidelines for safety. It is

### Conflict of Interests

Authors have no conflict of interests.

### Authors' Contributions

MJ carried out the design and coordinated the study, participated in most of the experiments and prepared the manuscript. AMS provided assistance in the design of the study and participated in manuscript preparation. ShSB carried out all the experiments and participated in manuscript preparation. HAS participated in the design of the study and manuscript preparation. MRM carried out statistical analysis of the data. All authors have read and approved the content of the manuscript.

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also important to note that there are no microbiological criteria or recommendation for tube feeding formula in Iran. The data we presented, demonstrate that it is urgent to assure strict hygienic methods including the development of protocols for clean techniques in the preparation, handling and storage of feeds and cleaning and sanitization of preparation equipments. In addition, recommendations for microbial quality of enteral tube feeding need to be made by authorities. In conclusion, these solutions must be handled in an aseptic manner during preparation and administration. The implementation of HACCP system will be required in a near future for better quality control of enteral nutrition formulas. The use of commercial products may also provide an additional margin of safety for hospitalized patients.

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