

## Bacterial Safety of Commercial and Handmade Enteral Feeds in an Iranian Teaching Hospital

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### ABSTRACT

**Background:** This study aimed to investigate and compare the bacterial safety of handmade and commercial ready-to-use enteral feeding formulas used in an Iranian teaching hospital.

**Methods:** In this experimental study, a total number of 70 samples (21 handmade formulas sampled at two sampling times, i.e. the time of preparation and 18 h after preparation, and 28 commercial ready-to-use formulas) were studied. Total count of viable microorganisms, coliform count and *Staphylococcus aureus* count for all samples were conducted.

**Results:** Out of 42 handmade samples, 16 samples (76%) had total viable counts greater than  $10^3$  CFU/g in the first sampling time and 17 samples (81%) had total viable counts greater than  $10^3$  CFU/g in the second sampling time. Also, 11 (52%) had coliform contamination in the first sampling time which reached 76% (16 samples) in the second sampling time. Regarding contamination with *S. aureus*, 5 samples (24%) were contaminated in the first- and 13 samples (62%) were contaminated in the second-sampling time. Out of 28 commercial formulas, 27 samples (96%) had total viable counts greater than  $10^3$  CFU/g. Also, 24 samples (86%) were contaminated with *S. aureus* and 27 samples (96%) were contaminated with coliforms. In order to compare these two formulas, the results of Mann-Whitney test showed that contamination of ready-to-use formulas in all three microbiological samples was significantly more than that for handmade samples.

**Conclusions:** The results of the present study indicate that the microbial safety of enteral feeding solutions in this hospital is much lower than standard values, demonstrating that the development of protocols for clean techniques in the preparation, handling and storage of both commercial and handmade enteral feeds is necessary.

**Keywords:** Bacterial contamination, enteral feeding, handmade formulas, microbiological safety, ready-to-use formulas

### INTRODUCTION

Enteral nutrition has been used to maintain or return nutritional health in ill patients or chronic illnesses for many years.

Malnutrition is a common problem which many specialists may encounter in hospitalized patients especially in those with high metabolic needs. Nowadays, timely provision of enteral nutrition is a therapeutic method to attenuate disease severity, adjust immune response, reduce complications and would have a favorable effect on therapeutic results of ill patients.<sup>[1]</sup> Tube feeding is used for patients who have an efficient digestive system, but are not able to receive food orally, or receive less food than they need for maintaining vital body functions. In patients who use this feeding method, two factors of “nutrient contents” and “microbiological safety” are very important and have been investigated in many studies.<sup>[2-4]</sup>

All types of enteral feeding formulations, whether handmade at hospital kitchen, or made by dilution of ready-to-use formulas, contain various amounts of proteins, carbohydrates and lipids in different combinations. The nature of these foods (in terms of pH, nutrient contents, water activity, etc.) is so that if they become contaminated, they would immediately grow microorganisms inside and put the patient at the risk of infection.<sup>[5,6]</sup> In addition, microbial contamination of these foods slows the trend of patient's recovery and can even cause dangerous conditions like pneumonia, nosocomial infections and sepsis.<sup>[3,7,8]</sup> Moreover, microbial infection can impact nutritional value of food by microbial spoilage.<sup>[9]</sup> Regarding the amount of acceptable bacterial contamination in different food products, there are different registered standards. American Food and Drug Administration (FDA) established standards for different aspects of these foods in 2006, especially for health and nutritional quality. Health quality means observing microbiological indexes in food.<sup>[10]</sup> On the basis of this guideline, in addition to the elimination of pathogen microorganisms, these foods must meet certain standards, which are determined by evaluating indicator microorganisms. Since the presence of certain pathogen microorganisms in food may cause severe illnesses, foods should be studied regarding the presence of such microorganisms. FDA guideline has mandated to assess the foods in terms of total colony count of aerobic microorganisms, count of coliforms, *Escherichia coli*, *Bacillus cereus*, detecting the *Salmonella* spp. and *Listeria monocytogenes* and determining

Staphylococcal enterotoxins. According to this guideline, food products that have one of the following conditions are considered below the standards of microbial safety and inappropriate for consumption: Contamination with aerobic microorganisms  $>10^4$  CFU/g in one sample, contamination of over  $10^3$  CFU/g in 3 or more samples, coliform count over 3 organisms/g, or positive for *L. monocytogenes* or *Salmonella* spp. Similar standards have been devised in several other countries. According to regulations in Spain, maximum acceptable bacterial contamination for *Staphylococcus aureus* is  $10^1$  organisms/g.<sup>[11]</sup> Unfortunately, no microbial standards have been devised for such foods in Islamic Republic of Iran.

Currently, enteral feeding solutions are provided in two ways in hospitals. One way is to mix the kitchen food at the hospital and the other way is to prepare the ready-to-use formula (usually in powder form) by diluting it with water. Ready-to-use formulas have been used for over 20 years, but most hospitals prefer to make feeding solutions by mixing nutrients in the kitchen, due to economic reasons or cultural considerations. Several studies have examined and compared ready-to-use formulas with handmade foods in terms of bacterial contamination. The results of all such studies confirm the fact that commercial ready-to-use formulas are considerably less contaminated than handmade formulas. In many studies, commercial formulas were completely sterile.<sup>[11-14]</sup>

In 2006, a study was conducted on handmade tube feeding formulas in two educational hospitals in Isfahan to assess them in terms of bacterial contamination. The results showed that bacterial contamination of these foods was much higher than FDA standards. They only examined handmade formulas and finally recommended using commercial ready-to-use formulas in order to reduce the probability of contamination.<sup>[15]</sup>

In recent years, there have been a lot of studies on microbial contamination of tube feeding formulas and its contributive factors in different countries including Saudi Arabia,<sup>[16]</sup> and the Philippines.<sup>[13]</sup> However, despite the grave need, such studies are rare in Iran. With regard to the increasing trend of using commercial ready-to-use formulas for patients and that handmade solutions are still used in some hospitals, this study aimed to investigate the bacterial contamination of

handmade and commercial ready-to-use formulas used for patients in intensive care units (ICU). The amount and kind of bacterial contamination was measured and then handmade and ready-to-use formulas were compared with this regard. In this study, qualitative studies were conducted to determine *L. monocytogenes* and *Salmonella* spp. which were emphasized by FDA standards.

## METHODS

In this experimental study, which was done from September 2010 to January 2011, seventy random samples of enteral feeding solutions made in one the University hospitals in Isfahan, Iran were studied in terms of the amount and kind of defined microorganisms.

### Preparation, storage and administration of feeding solutions

In this hospital, feeding formulas were made in two ways: Handmade (made from available nutrients in the kitchen of the hospital), or ready-to-use (made by diluting commercial powders). Commercial formulas were prepared several times, each 30 min before patient's feeding time in the kitchen of the hospital under the supervision of the nutrition department head. The necessary amount of powder needed for 200 mL of feeding formula was added to the cool boiled water, poured in disposable glasses, capped and taken to the wards. The ingredients of handmade foods were prepared once a day (in the morning) by mixing different food items like dry milk, green beans, carrot, orange juice, chicken, etc., and keeping in refrigerator. So, all the needed food volume for 1 day was made in the morning and used until the next day. For each feeding course, a part of the prepared solution was warmed to about 45°C, poured in disposable dishes, capped and taken to the wards. It is noteworthy that all feeding solutions were taken to the wards at certain times and sometimes patients were not ready to take their food. This delay sometimes took 30 min or more. The containers of the feeding solutions were kept out of the refrigerator and at room temperature at all this time elapse.

### Data collection

Samples were collected from two ICUs in this hospital from two types of feeds; handmade and

ready-to-use formulas. Sampling of handmade feeding solutions was performed twice. The first time was in the morning immediately after food preparation in the kitchen. The second sampling was done 18 h after the first time for handmade solutions. At this time, sample was taken from the same solution that was prepared in the kitchen in the morning and kept in the refrigerator. For ready-to-use formulas, sampling was done immediately after the powder was mixed with water and ready for consumption.

In each sampling time, 60 mL of the feeding solution was taken to sterile disposable dishes under aseptic conditions. Then samples were placed in ice containers and taken to the microbiology lab in 30 min. Of the 70 samples, 42 (21 samples in two sampling times) were made manually in the kitchen and 28 were made from diluting commercial formulas (mostly Ensure®). All stages of this study were conducted to assess the actual condition of the hospital without any changes in the usual settings.

### Laboratory examinations of the samples

Immediately after samples were taken to the microbiology laboratory, defined tests were conducted on them. These tests were divided into two general categories for each sample:

- Quantitative tests, which include determining the counts of indicator microorganisms. To do so, total colony count of viable aerobic microorganisms, count of coagulase positive *S. aureus* and coliforms were performed.

In order to perform quantitative tests, different dilutions were prepared using serial dilution method in sterile normal saline 0.9%. Then appropriate dilutions were cultured and the microorganisms' colonies were counted using pour plate method. The mean of colony counts was reported as colony forming units per gram.

Total colony count of viable aerobic microorganisms was measured based on standard number 356 of Iranian Standard and Industrial Research Organization (ISIRO) using nutrient agar culture medium.<sup>[17]</sup> CFU of coliforms was counted based on standard number 437 of ISIRO using culture medium Violet Red Bile agar.<sup>[18]</sup> Colony count of *S. aureus* was performed based on standard number 1194 of ISIRO using Baird-Parker agar.<sup>[19]</sup>

- Qualitative tests include detection of specific microorganisms in samples such as *L. monocytogenes* and *Salmonella* spp.

Detection of *Salmonella* spp. was performed based on standard number 1810 of ISIRO using buffered peptone water enriching media and selenite cystine broth culture medium to boost its growth.<sup>[20]</sup> Then selective culture medium of xylose lysine deoxycholate agar was used.

*L. monocytogenes* was recognized according to The U.S. Department of Agriculture/Food Safety and Inspection Service protocol.<sup>[21]</sup> In this method, two levels of enrichment in culture media of Listeria Enrichment Broth (UVM-I) and Fraser broth (UVM-II) were used, which yields suitable growth of *Listeria*. Then, Oxford agar culture medium, which is selective for *L. monocytogenes*, was used.

All stages of the tests were performed immediately after samples reached the laboratory, under aseptic conditions and under laminar airflow hood. Standard microbial species were also used to verify the accuracy of microbiological tests.

### Data analysis

For quantitative tests, colony counting was done manually and the result was reported as CFU/g. Any microbial growth was considered contamination. Because of limitations of serial dilution technique, it is not possible to show CFU less than 1 log CFU/g. therefore, if there was fewer than 10 colonies on each plate, the result was reported as  $<10^1$ .

Finally, statistical studies were conducted using SPSS for windows (SPSS, Chicago, IL, USA) version 16.0, to compare the amount of contamination of the first and second handmade samples after calculating the log (CFU/g) of each sample using Wilcoxon signed ranks test. This comparison was done to find the difference of contamination between the two samples regarding passage of time and quality of storing feeding solutions. Furthermore, Mann Whitney test was applied to compare the contamination results between first-sampling handmade samples and commercial ready-to-use samples. The results were meaningful if  $P < 0.05$ .

## RESULTS

The results of microbiological tests showing the amount and kind of bacterial contamination

of feeding solutions are demonstrated in Table 1. The comparison of bacterial contamination of first-time handmade samples and ready-to-use samples are shown in Table 2. Considering the extensive and non-normal distribution of data, parametric statistical tests could not be used to compare the contamination of samples. Therefore, non-parametric tests of Mann-Whitney and Wilcoxon signed ranks were used.

### Handmade samples

Regarding that any positive bacterial growth was considered contamination, in the first-time sampling, of 21 samples, 11 (52%) had coliform contamination, 5 (24%) had *S. aureus* contamination and 16 (76%) had total viable counts greater than  $10^3$  CFU/g; whereas in the second-time sampling, of 21 samples, 16 (76%) had coliform contamination, 13 (62%) had *S. aureus* contamination and 17 (81%) had total viable counts greater than  $10^3$  CFU/g.

Wilcoxon test was used to compare the contamination of the first- and the second-time sampling after calculating log (CFU/g). As can be seen in Table 1, the difference between total viable contamination of these two groups of samples was significant ( $P=0.004$ ). For these two sampling times, maximum coliform contamination was  $1.7 \times 10^3$  and  $5.0 \times 10^4$  CFU/g, respectively. The results for *S. aureus* were  $2.3 \times 10^1$  and  $4.0 \times 10^2$  CFU/g, respectively. Therefore, 18 h of keeping the feeds had increased coliform contamination about 1.5 logs and *S. aureus* contamination about 2 logs. The increased contamination after this time was not significant for coliforms ( $P = 0.085$ ), but was significant for *S. aureus* ( $P = 0.008$ ).

None of the samples was contaminated with *Salmonella* spp., or *L. monocytogenes*.

### Commercial ready-to-use samples

The range of contamination of ready-to-use samples for total viable count, coliforms and *S. aureus* are shown in Table 2. It can be seen that their contamination in all three tests is considerable. Out of 28 commercial formulas, 27 samples (96%) had total viable counts greater than  $10^3$  CFU/g. Also, 24 samples (86%) and 27 samples (96%) were contaminated with *S. aureus* and coliforms, respectively. In order to compare ready-to-use and handmade samples, Mann-Whitney test was used. As can be seen in Table 2, contamination of



**Table 1:** Contamination of handmade samples in two sampling times

Test	Range of contamination (CFU/g) (n=21)				Z-test	P value*
	First sampling		Second sampling			
	Min	Max	Min	Max		
Total viable count	<10 <sup>1</sup>	8.2×10 <sup>5</sup>	<10 <sup>1</sup>	1.9×10 <sup>7</sup>	-2.85	0.004
Coliforms	<10 <sup>1</sup>	1.7×10 <sup>3</sup>	<10 <sup>1</sup>	5.0×10 <sup>4</sup>	-1.72	0.085
<i>Staphylococcus aureus</i>	<10 <sup>1</sup>	2.3×10 <sup>1</sup>	<10 <sup>1</sup>	4.0×10 <sup>2</sup>	-2.67	0.008

\*Wilcoxon signed ranks test. First sampling=At the time of food preparation, second sampling=18 h after food preparation. Min=Minimum contamination, Max=Maximum contamination

**Table 2:** Comparison of the contamination of the first handmade samples with ready-to-use samples

Test	Range of contamination (CFU/g)				Z-test	P value*
	The first handmade samples (n=21)		The ready-to-use samples (n=28)			
	Min	Max	Min	Max		
Total viable count	<10 <sup>1</sup>	8.2×10 <sup>5</sup>	2.0×10 <sup>2</sup>	5.0×10 <sup>6</sup>	-3.173	0.002
Coliforms	<10 <sup>1</sup>	1.7×10 <sup>3</sup>	<10 <sup>1</sup>	2.6×10 <sup>4</sup>	-4.982	<0.001
<i>Staphylococcus aureus</i>	<10 <sup>1</sup>	2.3×10 <sup>1</sup>	<10 <sup>1</sup>	1.2×10 <sup>2</sup>	-3.947	<0.001

\*Mann-Whitney test. First handmade sample=Sampling of handmade formulations at the time of food preparation. Min=Minimum contamination, Max=Maximum contamination

ready-to-use formulas in all three microbiological samples is significantly more than that for handmade samples.

None of the samples was contaminated with *Salmonella* spp., or *L. monocytogenes*.

## DISCUSSION

In the present study, 96.4% of the ready-to-use formulas and 78.6% of handmade formulas had total aerobic microorganism count of over 10<sup>3</sup> CFU/g. Furthermore, coliforms were over 3 organisms/g in 57.1% and 17.8% of handmade and ready-to-use samples, respectively, which means they cross the standard limit (the details are not mentioned in the result section). This high rate of contamination shows lack of observing health standards in different stages of preparation and transportation of both types of feeding solutions.

Bastow *et al.* compared handmade and ready-to-use foods in terms of bacterial contamination. The contamination of all handmade samples immediately after preparation was on average 10<sup>2</sup>-10<sup>3</sup> organisms/mL while none of the commercial samples were contaminated.<sup>[22]</sup> In two other studies by Muytjens *et al.* and Simmons *et al.*, the results were different. They reported bacterial contamination in ready-to-use products.<sup>[23,24]</sup>

Relevant researches have shown that contamination of ready-to-use formulas are directly related to the stages of preparation.<sup>[25]</sup>

Bacterial contamination of handmade formulations could be attributed to several sources including original food items, food-making devices, blenders, environmental contamination of the kitchen regarding hygiene of the floor and air conditioner, process of food preparation, not observing the hygienic principles by kitchen staff and nurses and process of food carriage to the wards.<sup>[7,9,26-28]</sup> Comparison of the results of the handmade samples of both sampling times shows the significant increase of contamination in the second time. This increase indicates the sub-standard conditions of keeping feeding solutions during this period. The conditions have provided suitable medium for growth and proliferation of bacteria due to the temperature, long time of storage and environmental contamination.

With regard to powder samples, there was not any data indicating their sterility neither on the packaging nor on the package inserts of these products; contacting the manufacturing companies of these ready-to-use formulas, we realized that these products are not produced under the sterile condition, so their microorganism count could not be zero; however, high contamination shows lack of

hygiene in manufacturing companies. International organizations have established standards to control the contamination of nutritional solutions, however, inadequate observation of personal hygiene and the process of food preparation is one of the main reasons for contamination, which indicates the break between theoretical and practical standards.<sup>[26]</sup> Although ready-to-use formulas are prepared 30 min before each feeding time, total viable count of microorganisms, coliforms and *S. aureus* count were all higher than those in handmade formulas. It can be attributed to lack of hygiene during preparation time as well as presence the same source of contamination for both types of formulas. Moreover, contamination of ready-to-use formulas can be caused by lack of hygiene in the production line of the manufacturer to some extent.

In a similar study by Jalali *et al.*, only handmade formulas were studied. They found contamination of most samples to be over the standard limits for total viable count, coliforms and *S. aureus*. They recommended using ready-to-use formulas instead of handmade formulas to reduce contamination.<sup>[15]</sup> However, the present study found contamination in both handmade and ready-to-use formulas. These results call for designing comprehensive and documented guidelines for preparation, storage and transportation of these products in Iran. Such regulations will clarify the conditions for the involved personnel (cooking staff, nurses and workers carrying food) and also the basic rules of periodical monitoring of places that such foods are prepared or stored. Moreover, considering the fact that ready-to-use samples used in the present study were contaminated at the time of preparation and transportation, it seems that closed system ready-to-use formulas that do not need further process for preparation and can be used at patient's bed can reduce bacterial contamination.<sup>[13]</sup>

Several studies including the present one have shown that even the commercial formulations can be contaminated at the time of opening the can, diluting the powder formulation or pouring the formula into the patient's dish.<sup>[29]</sup> Furthermore, any steps of the processing of handmade formulas could be considered as the cause of contamination. This introduces the subject of future studies in order to determine the sources of these formulas' microbial overload. Moreover, the nature of these

foods is so that they grow other somewhat pathogen microorganisms inside including anaerobes, molds, or yeasts. This also warrants more comprehensive studies to evaluate the probability of these kinds of contamination.

## CONCLUSIONS

The results of present study indicate that the microbial safety of the majority of enteral feeding solutions in this hospital is lower than standard values published in relevant guidelines. The presented data demonstrate that the development of protocols for clean techniques in the preparation, handling and storage of both commercial and handmade enteral feeds is necessary.

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