Microbiological Quality of Commercial Enteral Feedings used in Two Public Hospitals in Shiraz, Iran

Mahsa Moazen[1], MSc; Samane Rahmdel[2], PhD student; Seyedeh Maryam Abdollahzadeh[1], PhD student; Maryam Ranjbar Zahedani[1], MSc; Seyed Mohammad Mazloomi[3]; PhD

[1]Department of Nutrition, School of Nutrition and Food Sciences, Shiraz University of Medical Sciences, Shiraz, Iran; [2]Department of Food Hygiene and Public Health, School of Veterinary Medicine, Shiraz University, Shiraz, Iran; [3]Department of Food Hygiene and Quality Control, School of Nutrition and Food Sciences, Nutrition and Food Sciences Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

Correspondence:
Seyed Mohammad Mazloomi, PhD; Nutrition and Food Sciences Research Center, Department of Food Hygiene and Quality Control, School of Nutrition and Food Sciences, Shiraz University of Medical Sciences, Shiraz, Iran
Tel: +98 71 37251001
Fax: +98 71 37260225
Email: mazloomi@sums.ac.ir
Received: 2 January 2014
Revised: 27 January 2014
Accepted: 3 February 2014

Abstract

Background: Although enteral feeding solutions are used to reduce the morbidity and mortality in many malnourished and hospitalized patients, microbial contamination of these products may lead to severe infections, especially in immune suppressed ones. The aim of this study was to evaluate the microbiological quality of commercial enteral feedings in two hospital settings in Shiraz.

Methods: Twenty commercial enteral feedings were collected immediately after preparation and then homogenized and serially diluted on the same day. MPN methods were used to evaluate the total viable count and *Escherichia coli* count. They were also tested for total coliform (pour plate method) and the presence of coagulase positive *staphylococci*. The results were compared with standard limits.

Results: All samples had a total viable count lower than 10^4 colony forming units (CFU) per g (the maximum recommended level of FDA standard).For all enteral feedings, coliform counts were not detectable (<10 CFU/g).E*scherichia coli* counts were also lower than those of Brazilian legislation (<3 CFU/g). Coagulase positive *staphylococci* were recovered just from one sample (5%).

Conclusion: Freshly prepared **c**ommercial enteral feeding samples had acceptable microbial quality. Such products are considered to be more acceptable than hospital prepared ones. However, further steps involved in the preparation and administration of commercial feedings can be sources of microbial contamination. Thus implementation of hygienic practices and monitoring procedures during preparation and administration can be suggested.

Please cite this article as: Moazen M, Rahmdel S, Abdollahzadeh SM, Ranjbar Zahedani M, Mazloomi SM. Microbiological Quality of Commercial Enteral Feedings used in Two Public Hospitals in Shiraz, Iran. J Health Sci Surveillance Sys. 2014;2(2):49-53.

Keywords: Enteral Nutrition; Bacterial counts; *Escherichia coli*; *Staphylococcus*

Introduction

Enteral nutrition (EN) is an efficient artificial feeding method for patients including those under particular medical and/or surgical conditions with adequate functional gut who are unable to eat sufficient food orally.[1-3] There are two main available EN solutions, given in institutional settings, i.e. the blenderized hospital prepared and/or homemade solutions and the commercial sterile formulas available in the form of solution or powder.[4,5]

While hospital-based solutions are still commonly

used, administration of commercial EN formulas has been gradually increased in hospital settings in recent years. However, neither the hospital or homemade feedings, nor the sterile ready to use ones are exempted from the potential microbial contamination.[6] Clinical consequences of contaminated feedings are abdominal pain, vomiting, diarrhea, infection, fever, bacteremia, septicemia, and pneumonia.[2,4,7] Since the population receiving these solutions is mainly those with suppressed immunity function and is often at high risk of developing and/or progressing malnutrition[5], the severity of the mentioned adverse effects can be more deteriorative. Thus, the routine monitoring of the microbiological contaminations occurring in different steps of preparation seems to be crucial. The potential sources of contamination are thought to be during preparation, opening, decanting into delivery systems, and administration to patients and contribute to the number of manipulations.[2,4,8]

Numerous studies have been conducted to evaluate the microbiological quality of these products and the stages of potential contamination as well. However, the microbial contamination of enteral diets, especially sterile ones, has rarely been investigated in Iran.

The aim of the present study was to investigate the microbial contamination of reconstituted powder feedings used in Shiraz hospitals and to compare their microbiological quality with the standard limits as well. Since the feeding is made usually for the proceeding meal in the 2 hospitals participating in the current study, we just evaluated the microbial quality of those immediately after preparation as a preliminary study for further investigations.

Materials and Methods

Sampling

The sample size was calculated according to standard deviation of standard plate count obtained in a similar study,[9] adopting a *z*-score of 1.96 and margin of error (*d*) of 4.9.Twenty samples of commercial enteral feedings were collected from 2 public hospitals (A and B), affiliated with Shiraz University of Medical Sciences, immediately after preparation, on 3 separate days in 2011. Each hospital provided 10 tube feedings made based upon a commercial powdered formula to which tap water was added. These powder formulas were reconstituted in plastic beakers and then instantly served to patients hospitalized in intensive care unit or internal ward through the sterile enteral feeding syringe. Twenty-five mL of each homogenized sample was aseptically put into sterile tube, transported to the laboratory at refrigerated temperature and analyzed the same day.

Microbiological Analysis

Samples were tested for total viable (mesophilic) count, total coliform, *Escherichia coli* (*E. coli*) count and also the presence of coagulase positive *staphylococci*. In this regard, aliquots of 10 g of each sample were mixed with 90 mL of sterile normal saline (9 g/L NaCl; Merck, Darmstadt, Germany) and homogenized completely.10-fold serial dilutions of this mixture were then prepared.

For determination of total viable count (TVC), using a 3 tube most probable number (MPN) procedure, 1 mL aliquots from each of four consecutive dilutions was inoculated into 3 nutrient broth (Merck) tubes. Nutrient broth tubes were incubated at 37 °C for 48 h. The MPN values (MPN/g) were then calculated from the number of positive tubes identified by turbidity at each dilution.

Total coliforms were counted using the pour plate method. In brief, 1 ml of each of ten-fold dilutions of the sample was added onto sterile petri dishes in duplicate and mixed with molten violet red bile lactose agar (VRBLA; Merck) cooled to approximately 45 °C. To prevent surface growth and spread of colonies, the plates were overlaid with about 5 mL VRBLA. The set agars were incubated aerobically at 35°C for 24 h. Colony counts were calculated in CFU/mL.[10]

Enumeration of *E. coli* was carried out as follows: three tubes of lauryl sulfate tryptose broth (LST; Merck) for each decimal dilution (10^{0}-10^{-3}) were inoculated and incubated at 37 °C for 24-48 h. From each gassing LST, a loopful of each suspension was transferred to a tube of EC broth (Merck). The tubes were examined for gas production after 24-48 h incubation at 45.5°C. To perform the completed tests for *E. coli*, streaking on eosin-methylene blue (EMB; Merck) agar and also indole test were carried out. The MPN of bacteria per g was obtained from standard MPN table.[10]

Isolation of coagulase positive *staphylococci* cells was carried out based on Iranian national standard-No 6806-3 with some modifications. To do this, 1 mL of sample homogenate was added to 10 mL of enrichment medium (Giolitti-Cantoni Broth; Merck) containing 2% of potassium tellurite solution (1%; Merck). The surface of each tube was then covered with a layer of sterile agar at least 20 mm thick. After 24-48 h incubation at 37 °C, all tubes (whether blackened or not) were subcultured to Baird-Parker agar (Merck). The plates were incubated at 37°C for up to 48h. The identity of suspected colonies was confirmed using the coagulase test. Positive tubes were those that had yielded the growth of coagulase positive *staphylococci*.[11]

Microbiologic Standard Limits

Both TVC and total coliforms were assessed based on Food and Drug Administrational (FDA) standards.

E. coli count was also evaluated according to Brazilian legislation (table 1).

Statistical Analysis

Data were subjected to descriptive statistical analysis using SPSS 16.0 (SPSS, Inc., Chicago, IL, USA). Regarding TVC, total coliform and *E. coli* count, the results were expressed as the mean ± standard error (SE). However, in the case of no microbial growth, the estimated values were presented. Afterwards, the percentage of samples that were in disagreement with acceptable limits and also the percentage of contaminated samples with coagulase positive *staphylococci* were reported.

Results

TVC of all samples ranged from 3.8 to 1100 MPN/g and were lower than the maximum recommended level of FDA standard.93.75% of freshly prepared feedings had TVC greater than 10^1 CFU/mL. Estimated coliform counts of our samples were lower than 10 CFU/g. However, since no coliform was grown on the plates, these results seem not to deviate from that of FDA values. For all the tested feedings, *E. coli* count was lower than that of Brazilian legislation. Coagulase positive *staphylococci* were recovered just from one sample contributing to hospital B. The results for microbiological quality of the investigated enteral feedings are demonstrated in table 2.

Discussion

Hospitalized patients can profit from EN to prevent malnutrition. On the other hand, microbiological contamination of enteral feedings, even the sterile, ready-to-use ones, may lead to some complications.[4,7] Thus, routine microbiologic monitoring of enteral feedings should be considered in all hospitals.[14] Some countries including Iran have not published any microbiological criteria or recommendations for these solutions. Since reconstituted commercial enteral formulas are frequently used in Shiraz hospitals, the present study was conducted to investigate the microbial quality of these products.

According to FDA guidelines on microbiological quality of medical foods, action must be taken if aerobic plate count of such products exceeds 10^4 organisms/g or if three or more subsamples exceed 10^3 organisms/g. However, Parenteral and Enteral Nutrition Group of British Dietetic Association (BDA) limits the acceptable level of total microbial counts of tube feeding to less than 10^1 and 10^3 CFU/mL at the beginning of and during administration, respectively.[15] On the other hand, the high mesophilic count could be a sign of probable presence of pathogens[13] and the US Center for Disease Control and Prevention considers a count of 10^5 microorganisms/mL as an incriminating level for food-borne disease outbreaks.[9] In the present study, TVC seems to be satisfactory according to the above mentioned standards with the exception of BDA. Low amount of contamination in freshly prepared powder formulas has also been reported by Oie et al.[8] However, 26.25% of reconstituted powder feedings collected from 2 hospitals in Brazil were found to violate Brazilian legislation (> 10^3 CFU/mL).[13] In comparison to commercial feedings, hospital-prepared formulas have been demonstrated to have lower microbial quality.[5,7,9,16] However, they continue to be used in developing nations including Iran for economic or cultural reasons.[1,9] The mean standard plate count for hospital-prepared feedings collected from two hospitals in Isfahan ranged from 1.9×10^2 to 6.4×10^7 CFU/g at the time of preparation which increased significantly after 18 h.[1] Moreover, Moghadam et al[17] reported the aerobic plate count of 22 hospital-prepared solutions to be 2×10^6 CFU/g, up to 4 h after preparation. Lower mesophilic counts were reported by Lopes et al[18]who analyzed freshly prepared blended whole foods taken from a pediatric hospital in Brazil. Actually, the more handling is done, the more contamination occurs. Thus, microbial quality increases with the progression from hospital-formulated feeding through commercial powdered

Table 1: The recommended microbiological limits for enteral feedings

	Standard	Reference
TVC (CFU/g)	$<10^4$	FDA, 2006[12]
Total coliforms (CFU/g)	< 3	FDA, 2006[12]
E. coli (CFU/mL)	< 3	Brazilian legislation, 2000[13]

CFU: colony-forming units

Table 2: Microbiological quality of commercial enteral feedings used in two public hospitals on preparation.

Hospital	Microorganisms			
	TVC (MPN/g)[a]	Total coliforms (Estimated CFU/g)	*E. coli* (MPN/g)	Coagulase positive *staphylococci* (Contaminated samples %)
A	432.38±118.40	<10	<0.3	0
B	132.00±48.30[b]	<10	<0.3	10
Total	319.74±83.42	<10	<0.3	5

[a]Values are given as mean±standard error (SE) of two replicates; [b]The mean value of 6 samples; CFU: colony-forming units; MPN: most probable numbers

formulas, to commercial feeds transferred straight from the can into the feeding containers.[5]

Not only TVC, but also other microbiological indicators should be evaluated to ensure the safety of enteral feedings. Coliforms including *E. coli* are considered to be a sign of poor hygienic practices during food preparation.[4] According to BDA, contamination with any gram-negative organism indicates inadequate sanitary conditions and is undesirable[15]; besides, FDA suggests that coliform counts of medical foods should not exceed 3 CFU/g.[12]Coliforms and *E. coli* were not recovered from any sample and the samples were acceptable based on published guidelines. These results are similar to those reported by Mokhalalati et al[5] for commercially prepared formulas (available in solution form). Such contaminations, however, have previously been reported. In a research conducted in Brazil, out of 160 reconstituted powder feedings, 77 samples had coliform counts ranging from 6.0 to 7.5 × 10^3 CFU/mL and 1.88% of samples were contaminated with *E. coli*.[13] In Philippines, mean coliform counts for samples including blended natural whole foods and reconstituted powdered products were 10.3 MPN/g at the time of preparation which increased significantly over 4 h.[9] High coliform counts (8.9×10^5 CFU/g) were also reported in the study conducted by Moghadam et al[17] on 22 whole food formulas taken from one hospital in Rasht, Iran. 70% of hospital-prepared feedings taken immediately after preparation from two hospitals in Isfahan were positive for coliforms; coliform positivity had increased to 90% after 18 h.[1] Lopes et al[18]reported coliform contamination (4.5->1.4×10^2 MPN/g) in three out of eight freshly prepared hospital-made formulas. The coliform contamination was more common in more manipulated products in the study conducted by Carvalho et al[7]. However, neither hospital-formulated and commercial powdered formulas, nor commercial liquids in cans had *E. coli* contamination. Since raw materials are considered to affect microbial quality of final products, the quality of water, the only substance used to reconstitute our samples, seems to be satisfactory.

The improper manipulation by handlers can result in contamination of enteral feeds with *Staphylococcus aureus* (*S. aureus*).[13] Although enterotoxin production is believed generally to be associated with *S. aureus* strains, the other staphylococcal coagulase-positive species are also known as enterotoxin producers.[19] The Spanish and Brazilian legislation limits the acceptable level of *S. aureus* to 10^1 and 3 organisms/g, respectively.[1,13] In the present study, coagulase positive *staphylococci* were only isolated from 1 sample. Lopes et al.,[18] Oliviera et al.[4]and Mokhalalati et al.[5] found that all enteral feedings analyzed were negative for *S. aureus*. In the study carried out by Borges et al.[13], 1out of 160 reconstituted powder feedings was contaminated with *S. aureus*. High level of contamination was reported by Jalali et al. (2009) in hospital-made feedings.[1]

Our samples taken just after preparation had an approximately acceptable quality; however, dilution water and the beaker used for reconstituting the formula seem to be the potential sources of microbial contamination. On the other hand, since the whole administrated solution for each meal is not covered by the volume of enteral feeding syringe, the syringe is poured with enteral diet several times, leading to contamination. Furthermore, in the period of administration of each meal, the feeding content is kept at room temperature during which the proliferation of the microorganisms can occur. In some cases, the volume of 2 or more meals is made and kept in refrigerator until administration. Keeping this in mind, to ensure the safety of enteral diets, all possible hazards and hazardous points should be considered. As shown by Oliviera et al,[4] the implementation of Hazard Analysis and Critical Control Points (HACCP) can be an option to meet this objective.

Conclusion

In conclusion, although the majority of reconstituted powder formulas analyzed immediately after preparation had microbiological quality within published guidelines, it is not possible to predict the level of contamination occurring at following steps. As a result, commercial enteral feedings are preferred to hospital-prepared ones from the perspective of microbial safety; however, an additional margin of safety can be achieved by the implementation of the HACCP system, involving personnel training, hygienic conditions in the preparation, handling, storage and administration of feeds.

Acknowledgments

The authors acknowledge the technicians in the dietetic unit of the two hospitals participating in the study. This research was entirely financed by Shiraz University of Medical Sciences (Project No. 90-5623).

Conflict of Interest: None declared.

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