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Cross-sectional study

Bacterial contamination of hospital-prepared enteral tube feeding formulas in Kerman, Iran

Running Title: Bacterial contamination of enteral feeding nutrition

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Abstract

Background: Blenderized feedings continue to be used in most parts of Iran, mostly due to economic and cultural reasons. The most important problem of this kind of food is bacterial contamination.

Materials and Methods: 54 samples were taken at the first and the last time of their giving to patients from blenderized food. Furthermore, 10 samples were collected from the commercial brand Fresubin® in the same way. Samples were brought to the laboratory and analyzed for the presence of some microbial pathogens.

Results: *Citrobacter diversus, Enterobacter cloacae, E.coli, Klebsiella pneumonia, Proteus sp, Klebsiella oxytoca, Hafnia alvei, Serratia sp, Shigella sonnei* were identified from blenderized feeding. The mean bacterial contamination of 54 samples that were collected from 3 intensive care units was 5/5×106 CFU/ml. Bacterial contamination of commercial food was obtained less than 1 organism/ml. According to these results, there are significant differences between these foods. Commercial foods are the best choice for feeding patients in the hospital.

Conclusion: 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 pose a substantial risk of developing a foodborne disease or nosocomial infection.

Keywords: Enteral Nutrition, Bacterial Contamination, Malnutrition

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Introduction

Malnutrition is a condition that results from eating a diet that does not supply a healthy amount of one or more nutrients. It is a condition characterized by a lack of one or more essential nutrients from the diet or a surplus of some nutrients that affect the body negatively. Malnutrition consists of two types: undernutrition and overnutrition. Undernutrition involves the deficiency of macronutrients like protein or calories and micronutrients like iron, iodine, and many more. Protein-energy malnutrition is caused by the inadequate intake of protein and calories (1).

Based on reports and observations of physician colleagues, gavage ingredients that are produced in hospitals around the country (Iran), are not nutritious enough to satisfy patients' calorie and protein requirements. According to Investigating the amount of calories, macronutrients, and micronutrients in the cages of hospitals covered by Mashhad University of Medical Sciences compared to the reference, it came out that The mean calories, macronutrients, and micronutrients were significantly lower than the RDI standard. The average levels of vitamins, vitamin B12, vitamin E, and vitamin D were lower than the standard RDI, but vitamin A, folic acid, vitamin C, and vitamin K were optimal. The levels of iron, calcium, zinc, and copper were significant. However, the values of manganese and chromium did not differ significantly (2).

Studies have shown at hospital admission, 30 to 40 % of patients present a certain degree of malnutrition. However, only a small percentage is recognized to be malnourished and receive adequate treatment (3). Another study indicates the prevalence of malnutrition among medical ICU patients in a university hospital in Egypt was 50% (4). Malnutrition is mostly followed by increasing the risk of infections, especially in immunocompromised patients like those with cancer (5) Also, according to previous studies, malnutrition and gastrointestinal/respiratory infections in children are a serious public health problem due to the cyclical relationship between malnutrition, immune system dysfunction, and increased susceptibility to infection (6).

For conditions in which a patient cannot take food and other nutrients normally, physicians suggest enteral feeding. The most critical disadvantage of enteral products is their potential for Microbial contamination; they are an ideal and rich nutrient environment for micro-organisms (7). Hand (home) made enteral products have been used as a nutritional method in hospitals for years, while commercial (industrial) enteral nutrient products have been available for almost 20 years (7, 8), but because of some issues and conditions such as cultural or economic reasons in many countries - especially in developing countries - hand made gavages still being used instead of similar commercial products (7).

FDA guidelines (standards) concerning microbial contamination of hospital foods which also includes gavages, declare if these kinds of products have more than 104 CFU/gr contamination or if the result of sampling them came out with three or more contaminated samples, decontamination procedures

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would be necessary. FDA also declares that the acceptable amount of Coliform in such cases is less than 3 Organisms/g (9). Based on studies, research, and standards admissible limit of microbial contamination in commercial enteral products must be less than 100 CFU/ml (10).

Factors like reduction in the strength of the gastrointestinal immune system as a subsequent of lack of food through the digestive system, stress, and the use of antibiotics, antacids, and H2 blockers, increase the risk of nosocomial (hospital-acquired) infections (7, 11). Infection and septicemia could cause Systemic Inflammatory Response Syndrome (SIRS) which is related to infectious and non-infectious agents. The most important agent causing SIRS syndrome is septicemia (12).

Standard enteral nutrition products are expensive, they are not covered by insurance companies in Iran and they are not available permanently to patients, which is why physicians prescribe gavages produced in the hospital's kitchen or by the patient's family (homemade gavages). This research has been done to investigate microbial contaminations of gavages which are produced in Bahonar Hospital's kitchen in Kerman-Iran, in comparison with similar standard industrial products Fresubin®. It is hoped that this research help us to find new ways of reducing the microbial contamination of such products and identify the most common and effective bacteria which produce infection in such environments. This would be the most proper way to help patients who have to use such products due to their health issues.

Methods and Materials

In this research, different kinds of enteral nutrition formulas produced at Shahid Bahonar Hospital in Kerman, Iran were examined. Differentiating the types and numbers of microorganisms that exist in these kinds of gavages collected from the Intensive Care Unit (ICU) was one of the main goals of this research.

The basic ingredients for preparing soluble gavages usually were: 1-The Soup 2- Fruit juice 3- A mixture of Milk, Banana, Honey and Egg. Different amounts of these ingredients were mixed based on different patient's needs.

Ingredients for preparing the Soup were: rice, vegetables, meat, olive oil, carrot, chicken meat, and different kinds of beans and grains. After cooking all these ingredients, they were ready to be poured into a blending machine to produce a homogenous mixture suitable for use in the nasal-gastric tube.

Sampling was carried out in 2 stages, in the ICU of Bahonar Hospital. In each stage, 27 samples were taken (11 samples from the soup, 11 samples from fruit juice, and 5 samples from a mixture of milk, banana, honey, and egg). The microbial analysis had been made of 54 samples in total. In the first stage, after transferring gavages from the hospital's kitchen into the ICU, at 9:00 a.m. sampling was done. After feeding patients based on their prescription and endurance, the rest of remained gavage was kept in the refrigerator (2 to 8 centigrade degrees), to be used three hours later (based on the ICU routine schedule) at noon for patients. This was the time when sampling was done for the second time.

To provide enough amounts of samples to do all examinations, each time at least 40 milliliters of gavages were taken as a sample. Samples were collected in sterile plastic containers and moved to the microbiology laboratory for further microbial culture and analysis.

To do examinations on industrial soluble gavages, 5 packages of 500 *ml* gavages solution made by the *Fresenius KABI* factory with the brand of *Fresubin*® were used. For sampling, first, 40*ml* of *Fresubin*® was collected with a sterile syringe from each package, and then 250*cc* of gavage was prescribed for patients. The procedure that has been used for hand-made gavages is repeated here also, for *Fresubin*®: the rest of remained gavage was placed in the refrigerator, to be used three hours later (based on the ICU routine schedule) at 12:00 o'clock at noon for patients and also for sampling for microbial analysis (10 samples were collected in total).

Regarding each sample, we could divide all the experiments that had been done into two main

categories

1) Quantitative experimentation category including determination of the number of microorganisms. In this category, *Total Aerobic Viable Count* TVC and counting of Enterobacteriaceae have been done.

2) Qualitative experimentation category including identification of special bacterial species that exist in samples. In this category identification of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and different kinds of *Enterobacteriaceae* had been carried out.

Total Aerobic Viable Count has been carried out based on Standard No. 5272 issued in *Iran Industrial Research and Standard Institute*. The microbial culture environment, used in this examination was *Plate count Agar*; This is a non-specialized microbial culture environment in which a broad range of different kinds of microorganisms could grow and produce colonies.

Counting *Enterobacteriaceae* had been carried out based on Standard No. 2461 issued by *Iran Industrial Research and Standard Institute*. The microbial culture environment used in this examination was *Violet Red Bile Dextrose* VRBD; This is the microbial culture environment that is used to do selective separation of *Enterobacteriaceae* or *Gram-negative bacteria resistant to bile*, non-sterile pharmaceutical products, food products, etc. For doing diagnostic tests in this category these kinds of microbial culture environments have been used: *Triple Sugar*, *Sulfide Indole Motility* (S.I.M), *Plate Count Agar* (P.C.A), *Iron Agar*, *Urease Broth* (U.B.), *Simmons' Citrate Agar* (S.C.I.). Collecting from *Enterobacteriaceae* colonies which have been cultivated and grown in the VRBD environment, cultivation in the PCA environment has been done. Samples were put in an incubator at 37 centigrade degrees for 24 hours. After 24 hours each colony was inoculated in all four environments: TSI, SIM, UB, and SCI.

Identification of the *Pseudomonas aeruginosa* microorganism was carried out based on Standard No.3140 issued in *Iran Industrial Research and Standard Institute*. The microbial culture

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environment used for this examination was *Pseudosel Agar* (*Cetrimide Agar*). Having *cetrimide*, this environment has a quality of inhibitory properties for a variety of *Pseudomonas* bacterial species except for *Pseudomonas aeruginosa*.

Identification of the *Staphylococcus aureus* microorganism was carried out based on Standard No. 6806 issued by *Iran Industrial Research and Standard Institute.* For this examination a microbial culture environment with high performance has been used; Baird-Parker Agar coupled with egg yolk emulsion is used to isolate this specific microorganism from food.

Finally in order to analyze data, *SPSS16* software was used. One-sample and two independent samples T-tests were carried out to compare samples. *Anova* test (analysis of variance) was carried out to make a comparison between several independent samples.

Results

Considering above mentioned standards for hand-made gavages (8), it came out that all collected samples taken from hospital-made gavages for CCU, had considerable microbial contamination.

Results from TVC examination showed that the average contamination (SD±Mean) of soup was $2.1\times10^{6}\pm3.3\times10^{6}$ CFU/ml, the average contamination of fruit juice was $2.3\times10^{8}\pm1.9\times10^{8}$ CFU/ml and the average contamination of milk, banana, honey and egg solution was $4.1\times10^{8}\pm3.6\times10^{8}$ CFU/ml.

The average contamination among 54 samples was $1.7\times10^{8}\pm2.4\times10^{8}$ CFU/ml. Based on statistical analysis it was determined that the average microbial contamination in all samples was higher than the admissible limit dramatically (p=0.0001), and there was a considerable difference between different groups of soups, fruit juice and milk, banana, honey, and egg solutions (0.01>P), it was also determined that the abundance of microbial contamination was the highest in milk, banana, honey and egg solution, and respectively lower in fruit juice and soup it was the lowest.

In terms of contamination with Enterobacteriaceae, all (100%) samples collected from the CCU section in Bahonar Hospital were contaminated - in both times of sampling- and their contamination was higher than the admissible limit.

The average contamination of samples with Enterobacteriaceae in total was $2.5 \times 10^{7} \pm 5.8 \times 10^{7}$ CFU/ml. The results of these experiments showed that the average contamination with Enterobacteriaceae for soup samples was $1.9 \times 10^{3} \pm 4.5 \times 10^{3}$ CFU/ml, for fruit juice samples was $5.2 \times 10^{7} \pm 8.3 \times 10^{7,}$ and for milk, banana, honey, and egg solution samples was $2.4 \times 10^{7} \pm 1.9 \times 10^{7}$.

There was a significant difference in the average rate of contamination with Enterobacteriaceae between different sampling groups of soup, fruit juice, and the solution of milk, banana, honey, and egg (P=0.01). The comparison between averages showed that the contamination with Enterobacteriaceae in fruit juice samples was the highest and the samples of milk, banana, honey egg solution, and soup were in the following stages in terms of Enterobacteriaceae contamination.

In terms of identifying the exact kind of bacteria species, 211 different kinds of bacteria isolated from VRBD environment, were tested. Based on bacterial cultivation in these four different environments: TSI, SIM, SCA, and UB and also based on the indole test, the following results were obtained (table No. 1):

Having done qualitative tests on samples that were collected from CCU sectors in a two times sampling procedure, Staphylococcus aureus and Pseudomonas sp bacterias were isolated from samples, while because of their extreme virulence food products should not contaminated with these types of bacteria.

Table 1: abundance of Enterobacteriaceae in handmade enteral products made in hospital

Bacteria	Percentage
Citrobacterdiversus	25.5%
Enterobacter cloacae	15.1%
Ecoli	11.6%
Klebsiellapneumoniae	11.6%
Proteus sp	9.3%
Klebsiellaoxytoca	8.5%
Hafniaalvei	3.4%
Seratiasp	3.4%
Shigellasonnei	1.1%
Non identified	13.2%

The result of the TVC test for industrial products (Fresubin®) indicated that the rate of microbial contamination was less than 1 unit. Taking into account that the allowable limit of microbial contamination is up to 100 units, we can conclude the rate of microbial contamination in industrial products is considerably lower than the allowed limit ($P<0.0001$).

Conclusion

Malnutrition is a prevalent problem among a huge number of patients who are hospitalized in hospitals and also is a challenging issue in front of specialist physicians. Malnutrition itself could increase the risk of putrefaction and the risk of death also could prolong the period of patient hospitalization and increase the cost of treatments (2, 3, 13-15).

Patients suffering from chronic disease and trauma have higher metabolic needs and they are more at risk of malnutrition. Today, providing enteral feeding at the appropriate time is considered a treatment method and a tool to reduce disease severity, it also could regulate a patient's immune response, reduce the risk of aggressive treatments, and have favorable impacts on the outcomes of treatments on critically ill patients (16).

The results of this research on gastrointestinal nutrition solutions showed that the average microbial contamination of samples was 1.7×10^8 CFU/ml while the maximum allowed limit of contamination for these kinds of products based on FDA standards is 10^3 CFU/m, and that makes all samples which produced in hospital's kitchen, unsuitable for use. This research also revealed that there is no significant difference in the rate of microbial contamination in samples just after producing them or after keeping them for three hours in the refrigerator.

The results of the study which took place in one of the hospitals in Isfahan showed that after 18 hours of keeping these products in the hospital's normal

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situation, the number of all alive aerobic microorganisms was higher than 10^5 CFU/ml (16).

The results of a similar study which took place in Poursina Hospital in Rasht showed that the number of aerobic microorganisms in enteral solutions four hours after production was $2\times10^6 \pm 2.9\times10^5$ CFU/ml, and the total count of all microorganisms in the same samples was $8.9\times10^5 \pm 6.8\times 10^5$ which is significantly higher than allowed limit according to FDA standards.

The results of this research showed that gastrointestinal nutrition solutions produced in Bahonar Hospital had extreme microbial contamination. This fact becomes more important when we know patients in ICU are a vulnerable population and they are at a high risk for infection with food that is contaminated with various kinds of microorganisms. These patients mostly suffered from weak or suppressed immune system functions plus malnutrition and metabolic disorders while 100% of samples taken from the very first gastrointestinal gavages prescriptions were contaminated and non-consumable.

Results from a similar study which took place in Saudi Arabia showed that the rate of contamination in 86% of samples was higher than 10^4 CFU/ml just at the beginning of the prescription period and the average of contamination at the end of the prescription period was higher than 10^5 CFU/ml.

Results from the current study showed a high rate of Enterobacteriaceae contamination in samples, the average was 5.2×10^7 CFU/ml. Studies showed that fruit juice samples had the highest rate of contamination with Enterobacteriaceae while milk banana and soup groups respectively were in second and third place. The existence of Enterobacteriaceae in foods that were under the thermal processes represents a major health defect.

Staphylococcal poisoning is the only type of food poisoning in which food producers play a crucial role. Based on the FDA manual, in order to identify the presence of Staphylococcus aureus in samples, the presence or absence of Staphylococcus enterotoxin was considered.

The presence or absence of this particular kind of microorganism was examined in this research and Staphylococcus aureus was isolated from all gastrointestinal nutrition solutions.

The results of the same study which took place in one of the hospitals of Isfahan showed that from 30 samples collected at the first stage of sampling (just after the production of GI solutions at the hospital's kitchen) 86% of samples had S.aureus contamination higher than 10^2 CFU/ml. The rate of contamination of samples 18 hours after entering the ICU increased considerably to 96% which was unacceptable to use (16).

Because approximately about 30% of the population are carriers of S.aureus, it is obvious that the main source of food contamination with such microorganisms is human skin. Incidence of Staphylococcus food poisoning often occurs as a result of contamination of food through hands and other parts of the human body (such as oral and nasal

secretions, or from a general wound on the catering staff's hand); Hence, personal hygiene has a considerable importance to prevent food from such contaminations (17).

The other microbial contamination that puts patients' health at great risk is the presence of Pseudomonas in gastrointestinal nutrition solutions.

In this research presence or absence of this particular microorganism has been tested in all samples and the result showed that the presence of Pseudomonas was positive in all (100%) samples.

Results of similar studies showed that more than 70% of fruits and vegetables were contaminated with Pseudomonas. In this research *P.fluorescens* and *P.aeroginosa* were the most common species of *Pseudomonas* which were identified in samples. Water used for cleaning and making GI solutions probably is one of the potential contamination sources of this bacteria (10).

The results of this study showed that the contamination of handmade products is considerably higher than the industrial ones.

The results of other studies are similar to the results of this research. Results of a similar study conducted in Saudi Arabia showed that no bacteria were isolated from commercial products (18).

In any case, the use of all kinds of industrial gastrointestinal nutrition solutions that were produced in sterile conditions should always precede the use of handmade gavages produced at hospitals.

It is hoped that the findings of this research may help physicians and staff who work in national health systems and be used to improve the quality of nutrition and reduce the microbial contamination of gastrointestinal nutrition solutions.

Because of the potential risk of contamination in handmade enteral productions, the use of industrial products is recommended.

References

1. Mehraj M, Feroz F, Sri RM, Ravichandran S. Harmful effects of malnutrition and possible sustainable solution.
2. Bigdeli J, Norouzy AR, Jami AT. Investigating the amount of calories, macronutrients, and micronutrients in the cages of hospitals covered by Mashhad University of Medical Sciences compared to the reference in 2016. 2020.
3. Raguso CA, Paoloni-Giacobino A, Gottraux S, Dupertuis YM, Pichard C. Awareness on nutritional problems at hospitals: which solutions? Revue Medicale de la Suisse Romande. 2003;123(6):401-4.
4. Zaki D, Zakaria DM, Abd El-Galil HM. Prevalence of malnutrition among hospitalized medical intensive care unit patients in a university hospital, Cairo, Egypt: Hospital Based Survey. Int J Intern Med. 2018;7(4):54-9.
5. Fan Y, Yao Q, Liu Y, Jia T, Zhang J, Jiang E. Underlying causes and co-existence of malnutrition and infections: an exceedingly common death risk in cancer. Frontiers in Nutrition. 2022;9:814095.
6. Rodríguez L, Cervantes E, Ortiz R. Malnutrition and gastrointestinal and respiratory infections in children: a public health problem. International journal of environmental research and public health. 2011;8(4):1174-205.
7. Sullivan M, Sorreda-Esguerra P, Santos E, Platon B, Castro C, Idrisalman E, et al. Bacterial contamination of blenderized whole food and commercial enteral tube feedings in the Philippines. Journal of Hospital Infection. 2001;49(4):268-73.
8. Moghadam AD, Chabok SY, Ramezani F, Leili EK, Rahimi V. Evaluation of nutritional quality and microbial contamination of enteral feeding solutions in hospitalized patients referred to neurosurgical ICU of Poursina Hospital in Rasht. 2010.
9. Food, Administration D. Compliance program guidance manual CPGM 7321.002; chapter 21. Rockville, MD, USA. 2006.
10. Arias ML, Monge R, Chávez C. Microbiological Contamination of enteral feeding solutions used in Costa Rican Hospitals. Archivos Latinoamericanos de Nutrición. 2003;53:277-81.

  DOI:https://doi.org/10.18502/aptj.v4i1.16711

11. Anderton A. The potential of Escherichia coli in enteral feeds to cause food poisoning: a study under simulated ward conditions. Journal of Hospital Infection. 1984;5(2):155-63.
12. Nathens AB, Marshall JC. Sepsis, SIRS, and MODS: what's in a name? World journal of surgery. 1996;20:386-91.
13. Cook AM, Peppard A, Magnuson B. Nutrition considerations in traumatic brain injury. Nutrition in Clinical Practice. 2008;23(6):608-20.
14. Waitzberg D, Goiburu M, Goiburu MJ, Bianco H, Díaz JR, Alderete F, et al. The impact of malnutrition on morbidity, mortality, and length of hospital stay in trauma patients. Nutricion hospitalaria. 2006;21(5):604-10.
15. Villet S, Chiolero RL, Bollmann MD, Revelly J-P, Cayeux M-C, Delarue J, et al. The negative impact of hypocaloric feeding and energy balance on clinical outcome in ICU patients. Clinical nutrition. 2005;24(4):502-9.
16. Jalali M, Sabzghabaee AM, Badri SS, Soltani HA, Maracy MR. Bacterial contamination of hospital-prepared enteral tube feeding formulas in Isfahan, Iran. Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences. 2009;14(3):149.
17. Varnam A, Evans MG. Foodborne Pathogens: An Illustrated Text: Manson Publishing Limited; 1996.
18. Mokhalalati JK, Druyan ME, Shott SB, Comer GM. Microbial, nutritional, and physical quality of commercial and hospital-prepared tube feedings in Saudi Arabia. Saudi Medical Journal. 2004;25(3):331-41.