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Microbiological contamination of enteral feeding solutions used in Costa Rican Hospitals

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SUMMARY. Enteral feeding is the most common and preferred modality for providing nutritional support to hospital patients with a functional gastrointestinal tract that can not satisfy their nutritional requirements. Nevertheless, enteral feeding may be an important cause of bacterial infection. The aim of this study was to evaluate the microbial contamination level of enteral feedings distributed in Costa Rican hospitals. A total of 124 samples of enteral feeding, coming from five different hospitals from San José, Costa Rica, were evaluated during the second semester of 1997 and first of 1998 for the presence and identification of total and fecal coliforms, *Pseudomonas sp.* and *Listeria sp.* A subpopulation of the *Pseudomonas* isolated was analyzed for their antibiotic susceptibility patterns.
The concentration of Gram negative rods in the samples of enteral feeding solutions ranged from 10^3 to 10^7 CFU/mL, markedly exceeding the permissible level (10^2 CFU/mL or less). The coliforms most frequently isolated included *Enterobacter cloacae, Escherichia coil, Serratia sp.* and *Klebsiella pnuemoniae. Pseudomonas sp.* was isolated in more than 70% of the samples made from commercial based solutions, fruits and vegetables. *P. aeruginosa* and *P. fluorescens* were the species most frequently isolated. *Listeria sp.* was not isolated from enteral solutions samples. The results obtained demonstrate that it is urgent to assure strict hygiene during the preparation and handling of all enteral feed in order to avoid bacterial growth. The implementation of HACCP (Hazard Analysis and Critical Control Points) system will be required in a near future for better quality control of enteral nutrition mixtures.
Key words: Enteral feeding, microbiological contamination, *Pseudomonas, Listeria*, coliforms.

RESUMEN. Contaminación microbiológica de soluciones de alimentación enteral en hospitales costarricenses. El objetivo de este estudio fue evaluar el nivel de contaminación microbiológica presente en fórmulas de alimentación enteral distribuidas en hospitales costarricenses. Durante el segundo semestre de 1997 se evaluó la presencia de coliformes totales y fecales, *Pseudomonas sp.* y *Listeria sp.* en 124 muestras de alimentación enteral provenientes de cinco diferentes hospitales de San José, Costa Rica. También, se determinó el patrón de sensibilidad a los antibióticos a una subpoblación de *Pseudomonas sp.* La concentración de bacilos Gram negativos en las muestras de soluciones enterales osciló entre 10^3 y 10^7 UFC/ml, excediendo de manera importante el límite permisible (10^2 UFC/ml o menos). Los coliformes aislados con mayor frecuencia incluyeron *Enterobacter cloacae, Escherichia coli, Serratia sp.* y *Klebsiella pneumoniae. Pseudomonas sp.* fue aislada en más del 70% de las muestras hechas a partir de soluciones comerciales, frutas y vegetales. *P. aeruginosa* y *P. fluorescens* fueron las especies aisladas con mayor frecuencia. No se aisló *Listeria sp.* a partir de las muestras evaluadas. Los resultados obtenidos demuestran que es urgente el asegurar una higiene estricta durante la preparación y manipulación de la alimentación enteral, de manera que se controle el crecimiento bacteriano. Es necesaria la implementación del sistema ARPCC (Análisis de Riesgos y Puntos Críticos de Control) en un corto tiempo con el fin de asegurar un mejor control de calidad de las mezclas de nutrición enteral.
Palabras clave: Alimentación enteral, contaminación microbiológica, *Pseudomonas, Listeria*, coliformes.

INTRODUCTION

Enteral feeding is the most common and preferred modality for providing nutritional support to hospital patients with a functional gastrointestinal tract that can not satisfy their nutritional requirements, due to an inadequate oral intake of energy and nutrients (1).

Complications occurring during enteral nutrition have been considered traditionally to be rare and essentially non-infectious. As a consequence, infection control procedures during preparation and administration of enteral feeding solutions have been less assiduous than for parenteral nutrition (2). Nevertheless, markedly contaminated enteral feed, containing 10^3 to 10^9 Gram negative bacilli/mL, has been reported to cause, not only diarrhea, but also sepsis, pneumonia and urinary tract infections (3-6). Also, considerable evidence indicates that enteral feeding contaminated with bacteria may be cause of severe nosocomial infection (2).

Several factors contribute to the development of these infections in hospitalary patients, including the fact that the resistance of the digestive tract to bacteria acquired orally is reduced considerably because of different situations, such as stress, severe illness, antibiotic treatment and antiacids or histamine type 2 blockers (2,7).

In Costa Rica, the prevalence of nosocomial infections is very high, around 10% (8), however, little attention seems to be paid to the microbiological quality of enteral feeding solutions. The aim of this study was to evaluate the microbial contamination level of enteral feedings used in Costa Rican hospitals, including the identification of Gram negative rods and the antibiotic susceptibility patterns present in the Pseudomonaceae isolated.

MATERIALS AND METHODS

Between July 1997 and June 1998, 124 samples of enteral feeding, coming from the five major hospitals from San José, Costa Rica, were evaluated for the presence of total and fecal coliforms, *Pseudomonas sp.* and *Listeria sp.* Samples were randomly taken at the nutritional hospitalary services, from the food daily supplied to patients that need this kind of nutritional support.

The methodology used in the sample analysis is the one described by Vanderzant and Splittstoesser for the analysis of microorganisms in food (9). Briefly, for the isolation of Listeria, 25 g of each sample were transferred to 225 mL UVM broth (University of Vermont modified Listeria enrichment broth) and incubated for 22 ± 2 h at 30°C. After this, 0.1 mL was transferred to 10 mL Fraser broth and incubated at 37°C for 24 ± 2 h. After the enrichment, a loop was streaked on the surface of Oxford agar, incubated at 37°C for 48 h and evaluated for the presence of typical Listeria colonies. These were confirmed by Henry illumination, morphology and Gram staining, motility, hemolysis properties, CAMP (Christie, Atkins and Munch-Peterson) behavior with *Staphylococcus aureus*, xylose and rhamnose utilization.

For the total and fecal coliforms quantification, ten fold dilutions were prepared with sterile peptonated water (PW) 0.1%. From each dilution, a 1 mL aliquot was spreaded in bile-red-violet agar (Oxoid), recovered with additional agar and incubated at 37°C for 48h for total coliforms and 44.5°C at 24h for fecal coliforms. Coliform species identification was done using API® system 20E for Enterobacteriaceae.

The isolation and quantification of *Pseudomonas sp.* was done using F agar (Oxoid) and the biochemical confirmation included the oxidase, catalase, triple-sugar-iron (TSI), and fermentation/utilization of carbohydrates assays.

A subpopulation of the *Pseudomonas* isolated was analyzed for their antibiotic susceptibility patterns. A Biomerieux Vitek was used, and the antibiotics tested included amikacin, aztreonam, ceftazidine, ciprofloxacin, gentamicin, imipenem, mezlocillin, piperacillin, ticarcillin and tobramycin.

Data were analyzed using ANOVA, available in the SPSS package, version 6.0.1.

RESULTS

A total of 124 enteral feeding solutions were evaluated for the presence of different bacteria. 50% of the samples were made out from commercial bases (Ensure®), and the other 50% were solutions prepared at the nutritional hospital services with either fresh fruits (18%), cooked vegetables (27%), meat broth (2%) or milk (3%). The average temperature of the samples at the collection time was of 25-30°C and the average pH was of 5.9±1.

The concentration of Gram negative rods found in the samples of enteral feeding solutions ranged from 10^3 to 10^7 CFU/mL (Table 1), markedly exceeding the permissible level (10^2 CFU/mL or less) determined by Anderton et al. in England (10).

TABLE 1
Levels of Gram negative bacteria found in enteral feeding solutions

Enteral feeding solution	Total Coliforms (CFU/ml)	Fecal Coliforms (CFU/ml)	*Pseudomonas sp* (CFU/ml)
Ensure based solutions	$2.5X10^6 \pm 1.1X10^7$	$2.3X10^5 \pm 1.1X10^7$	$1.1X10^5 \pm 1.2X10^5$
Fresh fruits based solutions	$4.4X10^5 \pm 1.5X10^6$	$4.3X10^5 \pm 1.4X10^6$	$3.4X10^5 \pm 1.4X106$
Cooked vegetables based solutions	$4.9X10^6 \pm 1.6X10^7$	$1.4X10^6 \pm 1.0X10^6$	$6.4X10^6 \pm 1.9X10^7$
Meat broth based solutions	$2.2X10^7 \pm 3.7X10^7$	$2.1X10^6 \pm 1.7X10^6$	$3.6X10^5 \pm 2.2X10^4$
Milk based solutions	$1.6X10^6 \pm 3.2X10^6$	$1.0X104 \pm 1.0X10^2$	$1.6X10^6 \pm 1.2X10^6$

Just 20% of the samples analyzed satisfied the mentioned recommendation. There were no significant differences between the levels of Gram negative bacilli found in the different samples analyzed nor among the hospitals evaluated.

The coliforms most frequently isolated are listed in Table 2. From the overall isolates done (n=108), 27% corresponded to *Enterobacter cloacae*, 11% to *Escherichia coli*, 8% to *Serratia sp.*, 5% to *Klebsiella pneumoniae* and 4.6% to *Enterobacter sp.* From the total isolates of E. cloacae (n=29), 38% were done from commercial based enteral feeding samples, 17% from vegetable based samples and 14% from the other solutions. 71% (n=5) of the *E. agglomerans*, and 31% (n=5) of the *Klebsiella pneumoniae* isolates were done from the commercial based solutions. This last Gram negative bacilli was also identified from 25% (n=4) of the isolates done from enteral feeding solution samples elaborated from fruits and 31% (n=5) from the ones containing cooked vegetables.

TABLE 2
Prevalence of coliforms isolated from enteral formulas

Bacteria	Number of isolations	%
Enterobacter sp.	5	4.6
Enterobacter cloacae	29	27.0
Klebsiella pneumoniae	16	15.0
Klebsiella oxytoca	4	4.0
Citrobacter freundii	3	3.0
Enterobacter agglomerans	7	6.6
Serratia sp.	9	8.3
E. coli	12	11.3
E. harmanni	2	2.0
CDC Ent Group 41	2	2.0
Kluyvera	2	2.0
No identification	15	14.2
Total	108	100.0

E. coli was identified in 33% (n=44) of the isolates made from commercial based solutions, in 67% (n=5) of the isolates made from fruit based enteral feeding solution samples and 17% (n=5) of the isolates made from the cooked vegetables based solutions. *Serratia sp.* was identified in 33% (n=3) of the isolates realized from commercial based solutions and in 44% (n=4) of the isolates made out from meat broth or milk prepared solutions.

Pseudomonas sp. was isolated in more than 70% of the samples made from commercial based solutions, fruits and vegetables. *P. aeruginosa* and *P. fluorescens* were the species most frequently isolated. The levels of this bacteria ranged from 10^4 to 10^7 CFU/mL (Table 1). There were no significant differences between the levels of this microorganism in the different formulas ($p>0.05$). From the total number of Pseudomonas strains evaluated for antibiotics sensibility (n=30), 100% were resistant to aztreonam and ticarcillin, and 28% to mezlocillin. All strains were sensible to the other antibiotics tested.

Listeria sp. was not isolated from any of the solutions analyzed.

DISCUSSION

Enteral feeding solutions contaminated with numbers of Gram negative bacilli ranging from 10^3 to 10^9 CFU/ml have been reported as cause of various clinical syntoms (3-6). Although the number of bacteria needed for causing clinical signs and symptoms of diarrhea is unknown, patients that receive this kind of nutritional support present a bigger vulnerability to different pathogens. When a patient is immunocompromised or receiving antibiotic treatment, the number of bacteria needed for colonizing the intestine and developing infection may be considerably lower (11,12). Some researchers have reported that 10^4 organisms/mL of feed are enough to result in colonization (13). The average counts of total and fecal coliforms and Pseudomonas found in this study are very high, indicating that enteral feeding may be an important infectious focus for hospitalary patients. This is more critical due to the fact that some of the Pseudomonas strains isolated present resistance to some of the common antibiotics used in the clinical management of these patients.

The presence of total coliforms in samples receiving heat treatment (including vegetables, meat broth and milk) or commercial based samples (Ensure®) shows up important hygiene deficiencies, since these bacilli are themolabile, so its presence is unjustified.

The presence and association of *E. cloacae*, *E. agglomerans* and *K. pneumoniae* with bloodstream or gastrointestinal infections has been reported in other studies (2,7). Their presence in this study is relevant, since *Pseudomonas* and these Gram negative bacili constitute the principal causative agent of intrahospitalary infections in Costa Rica (8).

The presence of fecal coliforms in enteral feeding solutions represents a risk for the health of these patients due to the possible presence and transmission of enteric pathogens (9). From the five different hospitals evaluated, none presented an adequate number of fecal coliforms in their enteral feeding solutions.

Pseudomonas sp. represents a real problem for patients receiving this kind of nutritional support. The numbers of *Pseudomonas isolated* from each kind of solution analyzed are quite big, and the most common species identified were *P. aeruginosa* and *P. fluorescens*. Although this is a widespread bacteria, it is one of the most important causes of invasive infection in compromised patients. Also, of the pathogenic bacteria, *P. aeruginosa* is the organism most consistently resistant to antimicrobics (14). A possible source of these bacteria may be water used either for the hygiene of equipment or for the reconstitution of commercial bases, since the isolation of *Pseudomonas sp.* from hospitalary water has been reported in Costa Rica. (Mora D. Unpublished data)

Listeria sp. was not isolated from the samples analyzed. This may be interpreted as a slight improvement in the Costa Rican nutrition hospitalary services, since an earlier work reports a 17% isolation of this bacteria from enteral feeding (15). The other indicators evaluated do not show any improvement according to earlier work.

Casewell has pointed out that the bacteriological hazard of contaminated feed may not be only for individual patients who suffer gastroenteritis, colonization or other infection (16). It is also possible that feeds contaminated with Gram negative bacilli bearing plasmids that confer multiple antibiotic resistance may provide new fecal reservoirs for dissemination of these organisms among groups of high-risk patients. Levy et al. have shown that the plasmid profile of Gram negative enteric pathogens recovered from enteral nutrition remained identical for several months, indicating long lasting

contamination with a limited number of strains (6).

It is important to analyze the origin of all this contamination, in order to suggest possible solutions. Muytjens et al. strongly suggest that contamination of feed ingredients themselves may provide the source of pathogenic microorganisms (17). Several studies indicate that fruits and vegetables used in hospital food services carry Gram negative bacteria such as *P. aeruginosa*, *Klebsiella* or *Enterobacter species* (2). Nevertheless, these microorganisms are thermolabil and sensible to disinfecting agents of common use in the hospital kitchen, therefore, its presence in enteral solutions suggests inadequate manipulation procedures. In commercial based preparations, the only possible contamination source is due to bad manufacturing practices. Nevertheless, studies by Simmons et al. and by Muytjens et al. suggest that the contaminating bacteria might be present in the powdered ingredients (17,18).

Previous studies support that the incidence of formula contamination is directly related to the degree of manipulation required for its preparation (10). However, in this study no differences in the levels of contamination of the formulas were observed. Complex preparations such as those prepared with fruits or cooked vegetables showed contamination levels as high as the ones present in the commercial based solutions (Ensure®). This may be reflex of inadequate preparation conditions of the solutions in the hospitalary services, since there is no defined and exclusive area assigned for their elaboration. The equipment is shared for the manufacture of other preparations and the personal does not have adequate training in the field.

In spite of the contamination origin, it is urgent to assure strict hygiene during the preparation and handling of all enteral feed in order to avoid bacterial growth. Anderton has shown that bacteria may survive and multiply even in feeds with low pH and high osmolarity (19). The implementation of HACCP (Hazard Analysis and Critical Control Points) system is required for assuring better quality control of enteral nutrition mixtures.

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