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Antimicrobial susceptibility pattern of Gram negative bacterias isolated from enteral feeding.

Artículo Original

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SUMMARY.

Introduction: Contaminated enteral feeding solutions represent a risk for the development of patients' and nosocomial infections. The aim of this study was to identify 75 Gram negative rod strains isolated from enteral feeding solutions distributed in three Costa Rican hospitalary centers and evaluate their antimicrobial susceptibility patterns.

Material and methods. Two different techniques were used for this purpose, the Kirby-Bauer modified technique and the ATB antibiogram technique (Biomériaux®)

Results. From the samples analyzed, the predominant groups corresponded to *Aeromonas* sp., (22,7%), *Klebsiella* sp. and *Proteus* sp. (18,7% each one) and *Enterobacter* sp. (4%). According to the antimicrobial susceptibility patterns obtained using the modified Kirby-Bauer

technique, 36% of the strains showed resistance to amoxicillin-clavulanic acid, 25,3% to cefaclor and 14,7% to cefuroxime. All strains were sensible to imipenem and ciprofloxacin. Using the ATB antibiogram methodology, bacteria showed resistance to amoxicillin (74,6%), amoxicillin-clavulanic acid (34,6%), ticarcillin-cefalotine (22,6%) and piperacillin (2,6%). All strains were sensible to the other ten antibiotics evaluated.

Conclusions. It is urgent to assure strict hygiene during the preparation and handling of enteral feeding solutions used at hospitals, so that they do not become a potential source of resistant bacteria that can limit the recovery of patients.

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Key words: antimicrobial susceptibility, resistance bacteriological, enteral feeding solutions.

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RESUMEN.

Patrón de sensibilidad a antibióticos de bacterias Gram negativas aisladas a partir de fórmulas enterales.

Introducción. Las fórmulas enterales contaminadas representan un riesgo para el desarrollo de infecciones nosocomiales y de pacientes hospitalarios. El objetivo de este estudio fue identificar 75 cepas de bacilos Gram negativos, aislados a partir de fórmulas enterales, distribuidas en los tres mayores centros hospitalarios costarricenses y evaluar su patrón de sensibilidad a los antibióticos.

Material y métodos. Se utilizaron dos técnicas diferentes para el desarrollo del proyecto: la técnica modificada de Kirby Bauer y la técnica ATB antibiograma (Biomériaux®).

Resultados. A partir de las muestras analizadas, los grupos predominantes fueron *Aeromonas* sp., (22,7%), *Klebsiella* sp. y *Proteus* sp. (18,7% cada una) y *Enterobacter* sp. (4%). De acuerdo a los patrones de sensibilidad a los antibióticos obtenidos usando la técnica de Kirby-Bauer modificada, 36% de las cepas mostraron resistencia a amoxicilina-ácido clavulánico, 25,3% a cefaclor y 14,7% a cefuroxime. Todas las cepas fueron sensibles a imipenem y ciprofloxacina. Utilizando la técnica ATB antibiograma, las bacterias mostraron resistencia a la amoxicilina (74,6%), amoxicilina-ácido clavulánico (34,6%), ticarcilina-cefalotina (22,6%) y piperacilina (2,6%). Todas las cepas fueron sensibles a los otros diez antibióticos evaluados.

Conclusiones. Es urgente asegurar una higiene estricta durante la preparación y manejo de las soluciones enterales utilizadas en los hospitales, de manera que no representen un foco potencial de contaminación con bacterias resistentes que puedan limitar la recuperación de los pacientes.

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Palabras clave: Susceptibilidad antimicrobiana, resistencia bacteriológica, alimentación enteral.

INTRODUCTION.

Enteral feeding is defined as the nutritional support given to hospitalary patients with a functional gastrointestinal tract but, due to different reasons, can not satisfy their nutritional requirements (1). Its primary aim is to provide the patient with essential proteins, calories, electrolytes, vitamins and minerals, so that they can be used in repairing and maintaining the corporal mass, especially the visceral component. These solutions may be artesanal or commercial, should have an energetic density of 1,0-1,5 kcal/mL (2,3) and are administered using nasogastric, nasoyeyunal, gastrostomy or yeyunostomy tubes, depending on the patient's condition.

There is considerable evidence that enteral feeding solutions contaminated with more than 10^2 Gram negative rods/g represent an important risk factor for the development of nosocomial infections (4). The contamination of enteral feeding solutions may be due to inadequate handling during its preparation, deficient quality of the primary materials used, inadequate hygiene of the equipment used or of the preparation area (4). Other contamination sources include the dilution of already prepared formulas, addition of colorants or other substances, rupture of the storage system and long administration periods (5). Patient's administration may also represent a risk, since in some cases, the probes evade the acidic barrier of the stomach and take food directly to an alkaline pH, at duodenum or yeyunum (3).

In Costa Rica, the prevalence of nosocomial infections is high, at about 10% (6) and enteral feeding solutions may have an important role in the dispersion of these, due to the high contamination they present, as has been previously reported (7,8). This situation is increasing important, especially considering that the phenomena of genetic transfer and the appearance of mutant bacteria have allowed the development of strains resistant to one or various antibiotics and can be transmitted through enteral feeding solutions, since the closed environment of hospitals favor their transmission (9).

Antimicrobial susceptibility of Gram negative bacterias from enteral feeding.

The aim of this work was to evaluate the antimicrobial susceptibility pattern of Gram negative bacteria isolated from enteral feeding solutions, coming from three Costa Rican hospitals, in order to evaluate the importance of this kind of nutrition in the dispersion of resistant strains.

MATERIALS AND METHODS.

During the first trimester of 1999, 35 samples of enteral feeding solutions, including Ensure®, Drosobe®, Isocal®, fruit, vegetable and milk preparations, coming from three hospital services were analyzed. The preparations were transported to the Food Microbiology Laboratory, Facultad de Microbiología, in order to isolate Gram negative bacteria and evaluate their antimicrobial susceptibility pattern. Initial isolation was done using McConkey agar; purified strains were identified using Gram staining, motility evaluation and biochemical assays including oxidase, TSI (triple sugar iron), urea, carbohydrate fermentation and aminoacid utilization.

The antimicrobial susceptibility patterns were determined using two different methodologies:

Disk diffusion, according to the methodology described by Bauer *et al.* (10). Briefly, filter paper disks impregnated with a known and standardized quantity of antimicrobial agent were applied over a solid media inoculated with bacteria. The antimicrobial agent diffuses in media forming a concentration gradient, that will inhibit or not bacterial growth. Depending on the inhibition zone diameter produced, after 18-24h incubation, the effect is classified as sensible, intermediate or resistant. The antibiotics used included trimetoprim, gentamicine, cefotaxime, cefaclor, cefpodoxime, imipenem, ciprofloxacin, amoxicillin-clavulanic acid, amikacine and cefuroxime.

ATB antibiogram, according to the methodology described by BioMérieux®. Briefly, a bacteria is inoculated in domes containing different antibiotics for 18-24h, at 35°C. The antimicrobial agent will allow or not the bacterial

multiplication, result that is evaluated by turbidity, and allows classifying the strain as sensible, intermediate or resistant to the agent evaluated. The antibiotics tested included amoxicillin, amoxicillin-clavulanic acid, piperacillin, piper-tazobactam, ticarcillin-cefalotin, cefotaxime, ceftriaxone, ceftazidime, aztreonam-imipenem, ceftazidime, cotrimazol, tobramicine-amikacine, gentamicine, netilmicine, pefloxacin and ciprofloxacin.

RESULTS.

The identification of Gram negative strains isolated from samples of enteral feeding solutions is shown in Table 1. Predominant genera were *Aeromonas* sp. (24%), *Klebsiella* sp. (22,7%), *Proteus* sp. (22,7%) and *Enterobacter* sp. (18,7%).

The antimicrobial susceptibility patterns obtained using the Kirby-Bauer and ATB antibiogram techniques are shown in Table 2. According to the results obtained by the Kirby-Bauer methodology, a high resistance rate to different common use antibiotics is shown by the strains isolated. The highest resistance rates correspond to amoxicillin-clavulanic acid (36%), followed by cefaclor (25,3%) and cefuroxime (14,7%). Using the ATB antibiogram technique, the greatest resistance rate corresponded to

Table 1
Identification of Gram negative bacteria isolated from enteral formulas obtained from national hospitals.

Identification	Number of Isolates	Percentage
<i>Aeromonas</i> sp	18	24
<i>Klebsiella</i> sp.	17	22,7
<i>Proteus</i> sp.	17	22,7
<i>Tatumella</i> sp.	2	2,7
<i>Kluyvera</i> sp.	3	4
<i>Plesiomonas</i> sp.	3	4
<i>Enterobacter</i> sp.	14	18,7
<i>Citrobacter</i> sp.	1	1,3
Total	75	100

Table 2
Antibiotic resistance from Gram negative bacterias
isolated from enteral formulas, using the Kirby-Bauer
and ATB antibiogram methods.

Antibiotic	Kirby-Bauer n(%)	ATB Antibiograma n(%)
Amikacine	5 (6,7)	0
Amoxicillin	ND*	56 (74,6)
Amoxicillin- clavulanic acid	27 (36)	26 (34,6)
Cefaclor	19 (25,3)	ND
Cefotaxime	1 (1,3)	0
Cefpodoxime	12 (16)	ND
Ceftaxidime-		
Cotrimazol	ND	0
Ceftazidime	ND	0
Ceftriaxone	ND	0
Cefuroxime	11 (14,7)	ND
Ciprofloxacin	0	0
Gentamicine	2 (2,6)	0
Imipenem	0	0
Pefloxacin	ND	0
Piperacillin	ND	2 (2,6)
Piper-Tazobactam	ND	0
Ticarcilina-		
Cefalotina	ND	17 (22,6)
Trimetoprim	6 (8)	ND

ND = Non- determined

amoxicillin (74,6%), followed by amoxicillin-clavulanic acid (34,6%), ticarcillin-cefalotin (22,6%) and piperacillin (2,6%).

The most active antimicrobial agents were imipenem and ciprofloxacin, showing 100% sensibility by both techniques.

DISCUSSION.

The contamination of enteral formulas is an actual problem, as has been described by several international and national authors (7,8, 11-16). Bacteriological identification of strains isolated from enteral feeding solutions allows the determining of the possible effect of diverse agents

on the patient, as well as to determine possible nosocomial infection focus. Diverse authors recognize that the closed environment of hospitals favors the transmission of agents through people, fomites or direct contact (9).

Aeromonas sp. was the predominant strain isolated. This bacteria can be found in water, and it has been related to diarrhea and intestinal infections, especially in immunosuppressed patients. In the same way, the isolation of *Klebsiella* sp, *Enterobacter* sp. and *Proteus* sp. represents gastrointestinal and systemic infection risk, especially for granulocytopenic and immunodeficient patients (17,18).

Results obtained show that *Aeromonas* sp., *Klebsiella* sp., *Proteus* sp. and *Enterobacter* sp. have important resistance rates to beta lactamic agents and cephalosporines, an intermediate resistance to sulfa and high susceptibility to quinolone and aminoglycoside agents.

Casewell indicates that the bacteriological risk that contaminated enteral feeding solutions represent is due to the production of gastroenteritis, colonization or development of other infections in patients. Also to the fact that Gram negative rods, carrying plasmids with information for resistance to different antibiotics may be spread through these (19).

Beta lactamic antibiotics are the antimicrobial agents most frequently prescribed worldwide (20), which explains an increasing resistance to these kind of agents (20). The worldwide range of resistance to amoxicillin varies between 46 and 93%, and to amoxicillin-clavulanic acid between 24 and 85% (21).

In Costa Rica the high resistance rates found can be explained based on an indiscriminate and frequent use of common antibiotics such as amoxicillin and first and second generation cephalosporines, the non-efficient control practices of hospitalary infections and a limited medicinal vigilance by health authorities. At the same time, the low resistance rates to agents such as imipenem and ciprofloxacin can be explained by in its low

Antimicrobial susceptibility of Gram negative bacterias from enteral feeding.

consumption and later appearance in the market (22).

The resistance rates obtained by the Kirby-Bauer method and ATB antibiogram show no statistical difference between the techniques, the slight differences between both are due to the fact that ATB antibiogram generally uses antibiotics in pairs.

It is evident that there is an increasing resistance to beta lactamic antibiotics, even the most new and sophisticated ones, not just in Costa Rica but also worldwide. This is due to the ability of bacterial strains to produce beta lactamases, and the development of new enzymes, or alteration in its expression level, associated with an intense environmental pressure (20). It is urgent to define a strict and controlled use of antimicrobial agents, so that they keep their therapeutic value and the existing resistance rates do not increase.

It is urgent to assure strict hygiene during the preparation and handling of enteral feeding solutions used at hospitals, so that they do not become possible resistant strains' carriers, that limit the patient's recovery. Nutritionists shall check for food safety, and its innocuity is part of it.

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ML Arias, R Monge, J Artavia, P González.

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