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Potential Virulence of *Klebsiella* Isolated from Enteral Diets

Simone Cardoso Lisboa Pereira^{1*} and Maria Cristina Danta Vanetti²

¹Federal University of Minas Gerais, School of Nursing, Department of Nutrition, Belo Horizonte - Minas Gerais, Brazil

²Federal University of Viçosa, Department of Microbiology, Viçosa - Minas Gerais, Brazil

Abstract

Objective: To evaluate the potential virulence of *Klebsiella* isolates from enteral diets in hospitals, in order to support nosocomial infection control measures, especially among critical patients.

Methods: Capsular phenotypic expression of the external membrane, production of aerobactin siderophore, quantity of capsular polysaccharide, hemolytic and phospholipase activity and resistance to antibiotics that are used therapeutically were investigated in 15 strains of *K. pneumoniae* and six of *K. oxytoca*. These isolates were obtained from enteral diets in two public hospitals in the state of Minas Gerais, Brazil.

Results: The hypermucoviscous phenotype was observed in one of the *K. pneumoniae* isolates (6.7%). Capsular serotypes of types K1 to K6 were seen to be present, of which four were K5 isolates of *K. pneumoniae* and one was K4. Under the conditions of this study, no aerobactin production, hemolytic activity or lecithinase activity was observed. All the isolates presented resistance to the antibiotics amoxicillin and ampicillin, but they were sensitive to the antibiotics cefetamet, imipenem, cloranfenicol, gentamicin and sulfamethoxazole/trimethoprim. The *K. pneumoniae* isolates that originated from hospital B presented higher frequency of resistance to the antibiotics evaluated and multiple resistances to at least four antibiotics. The variations in the profile of resistance to antibiotics among the *Klebsiella* isolates made it possible to classify them into eight antibiotypes. No production of broad-spectrum β -lactamases was observed among the isolates.

Conclusion: The data obtained through this study favor the hypothesis that *Klebsiella* isolates from enteral diets are potential pathogens for nosocomial infections.

Keywords: *Klebsiella*; Enteral diets; Pathogenicity

Introduction

Bacteria of the genus *Klebsiella* frequently cause nosocomial infections and are associated with high morbidity and mortality [1]. In particular, *K. pneumoniae*, the species that is clinically most important, occurs in nosocomial infections such as those of the urinary tract and in cases of pneumonia and septicemia [1].

Given the severity of the worldwide situation regarding nosocomial infections, control efforts should be directed towards identifying the sources of contamination and the means of transmission, in order to implement preventive measures [2]. The gastrointestinal tract and the hands of hospital professionals are recognized sources of contamination by *Klebsiella* [3,4] but, although this bacterium is frequently found in enteral diets, the involvement of these foods as a source of this opportunistic pathogen has been little described in the literature [5].

Recently, *K. pneumoniae* was identified as the nosocomial pathogen responsible for a large-scale outbreak, via the food chain. This was the first study to address the transmission of multiresistant *Klebsiella* through food in the hospital environment [6]. Moreover, according to Podschun et al. [7], *K. pneumoniae* isolates from other, nonclinical sources are also capable of expressing virulence factors, just like clinical isolates. Among the factors that contribute towards the pathogenicity of *Klebsiella*, the nature of the polysaccharide capsule of the external membrane, the production of siderophores and fimbrial adhesins and the quantity of capsular polysaccharide, as characterized by the mucoid appearance of the colonies, are considered to be the basis for this virulent process [7,8].

Other factors have also been recognized and are considered to be intensifiers of the virulent potential of *Klebsiella*, such as hemolysis and phospholipase production and multiple resistance to antibiotics for therapeutic use [8]. It should be noted that the numbers of outbreaks

of nosocomial infection caused by *Klebsiella* that is multiresistant to antibiotics, particularly extended-spectrum beta-lactamase (ESBL) producers, have been increasing over the last few years [9].

Considering that enteral diets have already been shown to be a potential cause of dissemination of *Klebsiella* in nosocomial environments; that nonclinical isolates may express virulence factors; that a recent study revealed that *K. pneumoniae* was the cause of a significant outbreak of nosocomial infection via the food chain; and that no study similar to the present study on enteral diets was found in the literature consulted; the objective of the present study was to evaluate the pathogenicity of *Klebsiella* strains isolated from modular enteral diets, by means of their phenotypic expression of virulence factors and from their profile of resistance to antibiotics.

Methods

Control bacterial strains and origin of the isolates

Strains of *Escherichia coli* were used as controls in the experiments to determine phenotypes of virulence factors, and these are described

***Corresponding author:** Simone Cardoso Lisboa Pereira, Professor, Federal University of Minas Gerais, School of Nursing, Department of Nutrition, Belo Horizonte-Minas Gerais, Brazil, Tel: 55 31 3409 8028; E-mail: simoneclpereira@gmail.com

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in Table 1. The virulent potential of isolates of *Klebsiella* sp obtained from samples of modular diets used at two public hospitals in Brazil was evaluated and is presented in Table 2.

Phenotypic determination of virulence factors

All reagents used were analytical or HPLC grade, crystalline form of patulin (P1639, analytical reagent grade, Sigma-Aldrich Chemie GmbH Deisenhofen, Germany) and crystalline form ergosterol (E6510, analytical reagent grade, Sigma-Aldrich Chemie GmbH Deisenhofen, Germany) were used to prepare standard solutions. Also ultra-pure distilled water was used for chromatographic analysis.

Mucoid appearance, presence of capsules and serotyping

The mucoid appearance of the colonies was observed after incubation of the *Klebsiella* isolates in Brain Heart Infusion (BHI) agar, at 37°C for 48 hours. Individual colonies were tested regarding their capacity to form viscous chains. Formation of chains greater than 5 mm in length was indicative of a positive phenotype for hypermucoviscosity [10].

The presence of a capsule in the *Klebsiella* isolates was observed by viewing them under an optical microscope (Leica, model DMLS, Leica Microsystems, Germany) using an oil immersion lens, after preparing slides that were treated with Congo red. The unstained region surrounding the red central nucleus of the bacterial cells, on a blue background, indicated the presence of a capsule [10]. In addition, the isolates of *K. pneumoniae* were sent to the Enterobacteria Laboratory, Department of Bacteriology, Oswaldo Cruz Foundation, Rio de Janeiro, for serotyping evaluation regarding the capsular antigens K1 to K6, of the external membrane.

Analysis of aerobactin production

The aerobactin production was demonstrated using the LG 1522 strain of *Escherichia coli* as an indicator (Table 1) [11]. This strain was cultivated in M9 agar contained in Petri dishes, with the addition of 200 µM of 2,2' bipyridine (Sigma Chemical Co, St Louis, MO, USA). One colony of each *Klebsiella* isolate to be tested was inoculated into this agar, already containing the indicator strain. Aerobactin-producing strains were viewed as the halo of LG 1522 *E. coli* cell growth around the inoculated isolates. The positive control consisted of the *E. coli* strain LG 1315, and the negative control was the *E. coli* strain HB101 (Table 1).

Phosphatidylcholine (PC-PLC) and hemolysin activity

The PC-PLC activity was determined according to the methodology described by Coffey et al. [12]. The *Klebsiella* cultures were grown in 4 mL of BHI broth and were incubated at 37°C for 18 to 24 hours. Aliquots of 3 µL of each culture were inoculated into the surface of BHI agar containing 2% NaCl and 5% egg yolk emulsion, previously diluted in 0.85% NaCl (1:1, v/v).

The plates were incubated at 37°C for 96 hours, in a Gas-Pak jar with an anaerobiosis generator (AnaeroGen™, Oxoid, Basingstoke, England), and were observed to see whether a lecithin hydrolysis halo was present surrounding the colonies.

The hemolytic activity of the *Klebsiella* cultures was investigated using blood agar, as described by Kokosharov and Phetisova [13]. BHI plates (Oxoid, Basingstoke, England) containing 5% defibrinated rabbit blood were prepared and small openings of 3 mm were drilled. These openings were used to inoculate 5 µL of bacterial suspension, and the plates were then incubated at 37°C for 12 hours.

Susceptibility to antibiotics

The susceptibility of the *Klebsiella* isolates to the antimicrobial agents was determined through the disk diffusion method in Mueller-Hinton agar, in accordance with the guide from the National Committee for Clinical Laboratory Standards (NCCLS) [14]. The antibiotics used (Sensibiodisc-CECON, São Paulo, Brazil) were: nalidixic acid (NAL) (30 µg); amikacin (AMI) (30 µg); amoxicillin (AMO) (10 µg); amoxicillin + clavulanic acid (AMC) (30 µg/10 µg); ampicillin (AMP) (10 µg); chloramphenicol (CLO) (30 µg); gentamicin (GEN) (10 µg); imipenem (IMP) (30 µg); kanamycin (KN) (30 µg); neomycin (NO) (30 µg); tetracycline (TET) (30 µg); ticarcillin + clavulanic acid (TIC) (75 µg/10 µg); and trimethoprim + sulfamethoxazole (SUT) (1.25/23.75 µg). To diagnose strains producing ESBL, screening was performed using the following antibiotics: cefotaxime (CTX) (30 µg); ceftazidime (CAZ) (30 µg); cefetamet (CEF) (10 µg); cefalotin (CFL) (30 µg); and aztreonam (ATM) (30 µg), in accordance with the recommendations of the NCCLS [14]. After incubation at 35°C for 16 to 18 hours, isolates that, in accordance with the antibiotics tested, presented the following growth inhibition halos were considered to be suspected ESBL producers: cefetamet ≤ 22 mm; ceftazidime ≤ 22 mm; aztreonam ≤ 27 mm; cefotaxime ≤ 27 mm; and cefalotin ≤ 25 mm. As a confirmatory test, the E test was performed (AB BIODISK, Solna, Sweden), using the antibiotics ceftazidime and cefotaxime as the substrate. Reduction of the minimum inhibitory concentration (MIC) of the antimicrobial agent in association with beta-lactamase inhibitor, in comparison with the MIC of the antimicrobial agent beta-lactamase alone (ceftazidime or cefotaxime), was indicative of ESBL-producing strains.

Results

The majority of the *Klebsiella* isolates obtained from the modular enteral diets formed colonies with low intensity of mucoid appearance in BHI agar. Four isolates (26.7%) of *K. pneumoniae* presented moderate mucoid appearance and only the isolate U4 (6.7%) presented a phenotype of hypermucoviscosity. The presence of a capsule in the isolate U4, and also in the isolates with colonies of moderate mucoid appearance was confirmed through observing the preparations under the microscope. In the isolates that presented colonies with low mucoid appearance, it was not possible to observe any capsule through the staining used. In addition, five isolates with the presence of capsule antigens between K1 and K6 were identified (Table 2).

Under the experimental conditions used, the *Klebsiella* isolates from enteral diets did not present any aerobactin production, hemolytic activity or lecithinase activity.

All the 21 *Klebsiella* isolates from enteral diets presented resistance to the antibiotics amoxicillin and ampicillin (Table 3). However, when amoxicillin was used in association with clavulanic acid, which is a beta-lactamase inhibitor, six isolates (28.6%) were resistant (Table 3). This behavior was also observed in relation to the association between ticarcillin and clavulanic acid, but less frequently (Table 3).

The *Klebsiella* isolates were sensitive to antibiotics of the beta-lactamic group: cefetamet, which is a third-generation cephalosporin; and imipenem, which is a carbapenem (Table 3). These isolates were also inhibited by antibiotics of different groups, such as: chloramphenicol (a quinolone); gentamicin (an aminoglycoside); and sulfatrim (an association between trimethoprim and sulfamethoxazole).

It was observed that the isolates of *K. pneumoniae* from enteral diets that originated from hospital B presented greater frequency of isolates resistant to the antibiotics evaluated and multiple resistance to at least

Sample	Strains	Relevant properties or characteristics	Source or reference
1	<i>E. coli</i> LG1315	ara entA lac leu mtL proC rpsL supE thi tonA trpE Xyl, controle negativo	WILLIAMS (1979)
2	<i>E. coli</i> LG1522	Ara azi fepA lac leu mtL proC rpsL supE tonA tsx thi col-V-K30iuc, controle positivo	WILLIAMS (1979)
3	<i>E. coli</i> F205	Produtora de cloacina DF13	WILLIAMS (1979)

Table 1: Strains of *Escherichia coli* used as controls in this study

Isolate/ Origin	Espécie	Virulence phenotypes				
		Aspect mucoid	Capsular serotype	Aerobactin production	Hemolytic	Lecithinase activity
P1/A	<i>K. oxytoca</i>	Absent	- ^a	Negative	Negative	Negative
P2/A	<i>K. oxytoca</i>	Absent	-	Negative	Negative	Negative
P3/A	<i>K. oxytoca</i>	Absent	-	Negative	Negative	Negative
P4/A	<i>K. oxytoca</i>	Absent	-	Negative	Negative	Negative
P5/A	<i>K. oxytoca</i>	Absent	-	Negative	Negative	Negative
P6/A	<i>K. pneumoniae</i>	Weak	ND ^b	Negative	Negative	Negative
P7/A	<i>K. pneumoniae</i>	Weak	ND	Negative	Negative	Negative
P8/A	<i>K. pneumoniae</i>	Weak	ND	Negative	Negative	Negative
P9/A	<i>K. oxytoca</i>	Absent	-	Negative	Negative	Negative
P11/A	<i>K. pneumoniae</i>	Weak	ND	Negative	Negative	Negative
P13/A	<i>K. pneumoniae</i>	Moderate	K5	Negative	Negative	Negative
P14/A	<i>K. pneumoniae</i>	Weak	ND	Negative	Negative	Negative
P15/A	<i>K. pneumoniae</i>	Weak	ND	Negative	Negative	Negative
P17/A	<i>K. pneumoniae</i>	Weak	ND	Negative	Negative	Negative
U1/B	<i>K. pneumoniae</i>	Moderate	K5	Negative	Negative	Negative
U2/B	<i>K. pneumoniae</i>	Weak	ND	Negative	Negative	Negative
U3/B	<i>K. pneumoniae</i>	Moderate	ND	Negative	Negative	Negative
U4/B	<i>K. pneumoniae</i>	Intense	K5	Negative	Negative	Negative
U5/B	<i>K. pneumoniae</i>	Weak	K4	Negative	Negative	Negative
U7/B	<i>K. pneumoniae</i>	Absent	ND	Negative	Negative	Negative
U8/B	<i>K. pneumoniae</i>	Moderate	K5	Negative	Negative	Negative

A= Hospital A

B= Hospital B

ND= not serotyped

b= undetermined serotype, serotype K6 up

Table 2: Virulence phenotypes of *Klebsiella* isolates from modular enteral diets, originating from two public hospitals

Antibiotics	Species/Hospital N (%)			
	<i>K. pneumoniae</i> /A	<i>K. oxytoca</i> /A	<i>K. pneumoniae</i> /B	Total
Amoxicillin + Clavulanic Acid -AMC	2 (25,0)	0 (0,0)	4 (57,1)	6 (28,6)
Nalidixic acid-NAL	2 (25,0)	0 (0,0)	6 (85,7)	8 (38,1)
Amikacin-AMI	1 (12,5)	0 (0,0)	0 (0,0)	1 (4,8)
Amoxicillin-AMO	8 (100,0)	6 (100,0)	7(100,0)	21(100,0)
Ampicillin -AMP	8 (100,0)	6 (100,0)	7(100,0)	21(100,0)
Aztreonam-ATM	2 (25,0)	0 (0,0)	6 (85,7)	8 (38,1)
Cephalothin-CFL	2 (25,0)	0 (0,0)	5 (71,4)	7 (33,3)
Ceftamet-CEF	0 (0,0)	0 (0,0)	0 (0,0)	0 (0,0)
Cefotaxime CTX-	2 (25,0)	0 (0,0)	5 (71,4)	7 (33,3)
Ceftazidime-CAZ	0 (0,0)	0 (0,0)	1 (14,3)	1 (4,8)
Chloramphenicol-CLO	0 (0,0)	0 (0,0)	0 (0,0)	0 (0,0)
Gentamicin-GEN	0 (0,0)	0 (0,0)	0 (0,0)	0 (0,0)
Imipenem-IPM	0 (0,0)	0 (0,0)	0 (0,0)	0 (0,0)
Kanamycin-KN	3 (37,5)	0 (0,0)	0 (0,0)	3 (14,3)
Neomycin-NO	2 (25,0)	0 (0,0)	1 (14,3)	3 (14,3)
Sulfaxotrim-SUT	0 (0,0)	0 (0,0)	0 (0,0)	0 (0,0)
Tetracycline-TET	2 (25,0)	0 (0,0)	3 (42,9)	5 (23,8)
Ticarcillin + clavulanic acid-TIC	1 (12,5)	0 (0,0)	0 (0,0)	1 (4,8)

Table 3: Resistance profile shown by *Klebsiella* isolates to antimicrobial agents for therapeutic use in the hospitals studied

four antibiotics was also more frequent in that hospital (Table 3).

The variation in the profile of resistance to antibiotics between the *Klebsiella* isolates made it possible to classify them into the antibiotypes described in Table 4.

ESBL production was not observed among the isolates of *K. pneumoniae* and *K. oxytoca*. This observation was possible because no reduction in the MIC of the antimicrobial agent in association with beta-lactamase inhibitor, in comparison with the MIC of the beta-lactamic antimicrobial agent alone (ceftazidime or cefotaxime) was seen.

The resistance to the antibiotics used as the substrate for detecting ESBL-producing strains ranged from 0 to 38.1% (Table 3). The highest frequencies of resistance were recorded for the beta-lactamic agents aztreonam and cefotaxime, with rates of 38.1 and 33.3% respectively, while the lowest frequencies of resistance were recorded for ceftazidime and cefetamet: 4.8 and 0% respectively (Table 3).

Discussion

In this study, the phenotype of hypermucoviscosity presented low frequency among the colonies formed by the isolates of *K. pneumoniae* (6.7%). Vernet et al. [15] also observed that this appearance presented low frequency of occurrence, given that out of 241 clinical isolates of *K. pneumoniae*, only 7.0% presented this characteristic. In a study with greater coverage [16], with 455 clinical isolates of *K. pneumoniae* involved in consecutive cases of bacteremia, in seven countries, it was found that in two of these countries, the phenotypic frequency of hypermucoviscosity was 94% in isolates that caused pneumonia and 100% in isolates that caused invasive syndrome. However, in the other countries, this frequency was on average 2%, i.e. lower than the frequency found in the present study.

The mucoid appearance of colonies is related to production of the capsule, which is a virulence factor that is essential for *Klebsiella* to be able to overcome the host's immune system [17]. Furthermore, a recent study implicated a role for the genes *magA* and *rmpA*, as determinants of the phenotype of hypermucoviscosity, in the pathogenesis of the K1 capsular serotype of *K. pneumoniae*, which is involved in bacteremia [18]. The gene *rmpA*, which codes for a positive regulator for extracapsular mucopolysaccharide expression [16], was also identified in two (2/54) clinical isolates out of a total of five isolates with the phenotype of hypermucoviscosity, although they did not present K1-K6 serotyping [8]. The phenotype of hypermucoviscosity is seen predominantly in strains with the serotypes K1 and K2, which are clinical isolates of invasive diseases. This would explain the low rates of hypermucoviscosity among the isolates of the present study [8].

Presence of a capsule is an important virulence factor regarding the severity of the infection. A capsule is generally present in clinical strains, and 78 capsule types have been identified in *K. pneumoniae*. However, determination of capsule types is often difficult, because of the limitations of traditional serotyping [19]. In the present study, it was found that four isolates had the serotype K5 and one had the serotype K4, out of a possible range of six main serotypes for this microbial species (K1-K6). Among the 78 distinct capsule antigens of *K. pneumoniae*, only some of them, such as K1, K2 and K5, are considered to be highly virulent and these are frequently associated with severe infections in humans and animals. One of these types of antigen was found in four of the isolates of this study.

None of the isolates of this study presented aerobactin production. According to several authors, *Klebsiella* isolates with phenotypes positive for aerobactin are rare, independent of the species or source of isolation [7,8,15].

Aerobactin is one of the siderophores (extracellular ferric chelating agents) that are secreted by bacterial cells in order to eliminate Fe³⁺, from iron-binding proteins in the host. These have a critical role in bacterial virulence. Based on the results found here and information in the literature consulted, *Klebsiella* may be included in the group of enterobacteria that present low aerobactin production rates (less than 20%).

Differences in the research relation between industrial diets and modulated diets make them difficult to compare, evidence seems strong enough to take into account the intake Degradation of Food and the Struggle for Healthy Eating which transformation of food from essential nutrients into edible commodities that far too often fail to nourish us. Modulated diets consisted of a control, nutritionally balanced diet, this same diet supplemented with 10% and a diet that was high in fat and carbohydrate and low in protein.

In this regard, considering that the bioavailability of iron in the host is extremely low (10-18 M); that iron is essential for microbial growth; and that the incidence of the phenotype of aerobactin is low among *Klebsiella* isolates, the question of which mechanisms might ensure an iron supply in the host for this bacterium is raised.

It has been shown that enterobactin, another ferric chelating agent, is present in the majority of clinical isolates of *K. pneumoniae* [8,11]. The role of enterobactin in *Klebsiella* virulence is still uncertain, but expression of the enterobactin gene seems to be activated during the infection. A recent study found that, genotypically, enterobactin was present in clinical isolates of noninvasive infections (bacteremia) [8].

The absence of hemolytic activity under the conditions of this

Antibiotype	Isolates *	Antibiotic resistance:
I	P1, P2, P3, P4, P5, P6, P7, P8, P9, P11, P13 e U5	AMO e AMP
II	U3	NAL, AMO, AMP e ATM
III	P15	AMO, AMP, ATM, KN e TET
IV	U2	AMC, NAL, AMO, AMP, ATM, CFL e CTX
V	U4	NAL, AMO, AMP, ATM, CFL, CTX e TET
VI	P17	AMC, NAL, AMO, AMP, CFL, CTX, KN e NO
VII	U1	AMC, NAL, AMO, AMP, ATM, CFL, CTX e CAZ
VIII	U7	AMC, NAL, AMO, AMP, ATM, CFL, CTX, TET
IX	U8	AMC, NAL, AMO, AMP, ATM, CFL, CTX, NO e TET
X	P14	AMC, NAL, AMI, AMO, AMP, ATM, CFL, CTX, KN, NO, TET e TIC

* The isolates are specified in Table 2 and the antibiotics are described in Table 3 Serotyping not determined; serotypes higher than K6

Table 4: Determination of the antibiotypes of the *Klebsiella* isolates from enteral diets

study may be related to a lack of activators for expressing the genes that code for this enzyme, which are often not reproducible under *in vitro* conditions [20]. This virulence factor was first detected by ALBESA in 1980, in a test conducted on blood agar containing rabbit erythrocytes. Since then, this research group has been studying this cytolysin, and its specificity for rabbit erythrocytes has always been confirmed. Hemolysin is a pore-forming toxin that makes nutrients available, such as the iron of hemoglobin. In a recent study, none of the 54 clinical isolates presented hemolysis or presence of the genes *hlyA* and *cnf-1*, which code for this activity. This is consistent with the lack of studies reporting genotypic and phenotypic presence of hemolysin in *Klebsiella*, even though hemolytic activity has been described [20,21].

Lecithinase or PC-PLC activity was absent from all the isolates under the conditions in which this was determined. This result is concordant with that of Singh et al. [22] who reported that lecithinase activity was rare in *Klebsiella* isolates, given that only 2.4% of their 168 clinical isolates of *K. pneumoniae* presented this activity. Furthermore, they found that this activity was absent among the 11 isolates of *K. oxytoca*. Their study was the first report on lecithinase activity in *Klebsiella* isolates, and the role of this virulence factor in the pathogenicity of this genus of bacteria has not yet been fully clarified.

Among the antibiotics used in the present study, it was seen that there was high prevalence of resistance to ampicillin and amoxicillin. This observation regarding the antibiotic ampicillin was also made in a study on clinical isolates of *K. pneumonia* [23] and in a study on isolates of this enterobacterium in expressed human breastmilk [24].

Another point observed was that there was greater prevalence of resistance to antibiotics among the isolates from hospital B. According to Davies [23], the prevalence of resistance to antibiotics may vary between different hospitals and may be related to local epidemiological factors such as control over the use of antibiotics and preventive measures applied by hospital infection committees, in relation to the problem of multiresistant strains.

No correlation between high incidence of resistance to the antibiotic cefotaxime and ESBL production was observed. This is an important clinical result, even though the isolates presented multiple resistance to up to 12 antibiotics.

The condition of multiresistance observed in the present study confirms the assertion that bacterial resistance to antimicrobial agents is a serious public health problem. This is the reality within which control initiatives have been proposed. In Brazil in 2010, a resolution making provisions regarding controls over medications based on substances classified as antimicrobial agents for use under medical prescription singly or in combinations was instituted, such that dispensing of these substance could only be done through a specially controlled prescription [25].

Moreover, the observation of multiple resistance to antibiotics in *Klebsiella* isolates has significant clinical importance. This is because enteral diets are especially prescribed for patients who are highly susceptible to nosocomial infection and who are concentrated in intensive care units. These are patients who are subject to the intrinsic risks relating to complex underlying diseases, nutritional vulnerability, extreme ages (premature infants and elderly people) and effects of immunosuppression or immunodepression drugs, in addition to extended-spectrum antimicrobial agents. In addition, there are extrinsic risks such as prolonged stay in the hospital environment, subjection to routines of invasive procedures and quality that is not guaranteed for all hospital services, given that this is a complex

organization. In this context, the risk to health becomes more severe, given that these situations may be sources of resistance genes for pathogenic microorganisms, particularly those that are toxic-infectious and infectious, since the intestinal tract constitutes an appropriate niche for transferring genes relating to resistance [26].

From results surveyed in the literature, it was seen that some authors have suggested that transfers of plasmids with the capacity to carry more than one associated resistance gene facilitate horizontal gene transmission of different resistance mechanisms between bacteria, thus further worsening the problem of multiple resistance to antibiotics in the hospital environment [23,27].

These transfers are probably more effective when the hosts are simultaneously subjected to selective pressure from an antimicrobial substance to which the microorganisms involved are resistant. From this point of view, hospitals are environments where drug-resistant microorganisms and others that determine such resistance are more likely to be present. Thus, horizontal gene transfer has a greater chance of occurring and the consequences, in terms of therapeutic failure, may be more severe [28,29].

Occurrences of chromosomal mutations alone do not explain the rapid acquisition of gene resistance to antibiotics among bacteria. It has been established that plasmids are particularly important in the evolution of antibiotic-resistant bacteria. It has also been suggested that plasmid transfers carrying resistance genes facilitates horizontal gene transmission between bacteria [23,27]. Foods seem to be an effective source for human acquisition of resistant bacteria and gene resistance to drugs, but the extent and real consequences of this exposure have not yet been sufficiently investigated [30,31].

Final Remarks

The data obtained in this study favor the hypothesis that *Klebsiella* isolates from enteral diets are potential pathogens for nosocomial infections, especially for critical patients. This is because, among the isolates in this study, capsular serotypes that are only identified in severe cases of nosocomial infections were observed. These serotypes include K5 and K4, which are among the serotypes of clinical importance (K1 to K6). In addition, presence of the phenotype of hypermucoviscosity was observed: this intensifies the invasion process in cases of isolates of one of these important capsular serotypes. This profile of virulence, which can be considered to partially form the basis for infection by *Klebsiella*, now represents a risk to microbiological security. This is especially so in situations of propagation in intensive care units, where the intrinsic and extrinsic risks favor conditions of nosocomial infection. Furthermore, the synergy that may exist between these virulence factors can be highlighted: this may especially occur in combination with multiple resistance to antibiotics for therapeutic use, as also seen in the present study. Thus, there is a need to continue this study in order to evaluate the pathogenicity of these isolates under *in vivo* conditions.

In addition, through these results, it can be seen that control measures against nosocomial infections are important in handling enteral diets. Through comprehension of this reality, the need for participation by all sectors of the hospital in the process of controlling this situation, regarding individual and collective practices, is corroborated. It can furthermore be emphasized that rigorous and systematic monitoring of the risks of food origin has to be applied to the hospital environment. Good food handling practices are effective strategies for controlling the dissemination of these and other bacteria in the hospital environment.

In this regard, the present study adds knowledge regarding the nosocomial infections caused by *Klebsiella* and emphasizes the importance of measures relating to outcomes associated with the nutrition sector, with the aim of strengthening the interfaces between infection control and preventive actions and between these controls and promotion of quality within healthcare services overall. The present study also endorses the need to direct healthcare funding policies towards implementation of risk control and prevention measures in strategic sectors of the healthcare services. There is a need to reflect on all the possible strategies that might contribute towards changing the current panorama of nosocomial infections, with investments in research and updates. It can be emphasized that dissemination of data analyses like the present study and other recent studies should be done routinely for all the professionals involved in care, as well as within the institution's administration.

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