

Epidemiological Characteristics, Resistance Patterns and Spread of Gram-Negative Bacteria Related to Colonization of Patients in Intensive Care Units

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Abstract

Our aim was to determine the epidemiological characteristics, the resistance patterns and the spread of Gram negative bacteria related to colonization of patients in adult Intensive Care Units. **Methods:** A prospective cohort of patients colonized and/or infected with Gram negative bacteria was conducted at two adult ICUs from hospitals in Brazil (April 2012 to February 2013). Nasal, groin and perineum swabs were performed. Samples were incubated on MacConkey and ceftrimide agar (48 h at 37°C) and identification tests (Vitek-BioMérieux), antibiogram (Bauer-Kirby method), Carba NP test, Polymerase Chain Reaction (PCR) and sequencing were performed. The patterns of resistant microorganisms were compared by rep-PCR (Diversilab). **Results:** There were 53 cases of colonization. In these cases, we identified imipenem-resistant *Acinetobacter baumannii* (51%), *Pseudomonas aeruginosa* (32%), *Klebsiella pneumoniae* ESBL (38%) or imipenem resistant (5.6%). The use of antimicrobials and medical devices were related to colonization ($p < 0.05$). The resistance patterns expressed by *Klebsiella pneumoniae* were ESBL (CTX-M, SHV e TEM) and KPC₂. A verified profile of *Acinetobacter baumannii* was related to OXA-23 and OXA-253 (OXA-143 variant). The profiles ESBL and KPC₂ expressed by *Klebsiella pneumoniae* were distributed between the both ICUs. The distribution of OXA-23 and OXA-253 was verified only in one ICU. The similarity of strains ranged from 80% to 95%, highlighting the horizontal transference of these microorganisms.

Keywords

Intensive Care Unit, Bacterial, Drug-Resistance, Gram-Negative Aerobic Bacteria, Colonization

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1. Introduction

Among the Gram-negative bacteria the *Enterobacteriaceae* (*Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., *Proteus* spp., *Serratia* spp.) and non-fermenters (*Pseudomonas aeruginosa* and *Acinetobacter baumannii*) are frequently associated with infections in immunocompromised patients, especially in Intensive Care Units (ICU) and, in a several cases show multidrug-resistance profile [1].

The multidrug-resistance among species of *Enterobacteriaceae* has become a global challenge, with high levels of resistance against carbapenems worldwide. The resistance against carbapenems may be facilitated due the widespread use in treatment of infections caused by *Enterobacteriaceae* or non-fermenters (*A. baumannii* and *P. aeruginosa*) with a large profile of resistance to β lactams [2].

Similarly, the emergence and spreading of carbapenemases among Gram-negative bacteria can be verified, especially with *Enterobacteriaceae* species. The first report of carbapenemase was registered with *Klebsiella pneumoniae* in 2001 in the USA. The KPC-2 is the most frequent variant among class A carbapenemases. The genes blaKPC can be located in a wide variety of plasmids, facilitating further resistance [3] [4].

Although the first report of carbapenemases was registered with *Enterobacteriaceae*, the others Gram-negative bacteria, such as *A. baumannii* and *P. aeruginosa*, can also produce this enzyme. The class D carbapenemases or Oxalinases (OXAs) are frequently found in both Gram-negative bacteria. The OXAs have a large spectra of antibiotic hydrolyze, including carbapenems. Currently, more than 150 OXAs variants have already been described [4] [5].

According to the results of the Program of Epidemiological Surveillance and Bacterial Drug Resistance for Latin America and Brazil, the non-fermenter multidrug-resistant Gram-negative Bacilli and *Enterobacteriaceae* with levels of extended-spectrum beta-lactamase (ESBL) constitute the main problem of resistance to antimicrobials in these countries [6] [7].

The identification and characterization of Gram-negative microorganisms with resistance patterns to antimicrobials may improve the management of sources and spreading, enabling plans infection control measures plans in settings with important prevalence of these microorganisms. Therefore, it was aimed to determine the epidemiological characteristics, the resistance patterns and spread of Gram negative bacteria related to colonization of patients in adults Intensive Care Unit.

2. Materials and Methods

A prospective cohort study was approved by the Ethics Committee (0386.0.203.000-11) and performed in two adults Intensive Care Units in two hospitals in Belo Horizonte, Brazil (April 2012 to February 2013). Patients or their next of kin were requested to give consent prior to sampling. All consenting patients colonized or infected with Gram-negative microorganisms with resistance patterns and who were more than 18 years old were followed during their length of stay in the ICU or in wards where the patients was transferred after being discharged from the ICU.

Patient characterisation data were collected via chart review, including age, sex, monitoring by medical devices (mechanical ventilation, venous catheter, drains and, nasal and indwelling catheter), severity index (measured according to the classification of the Average Severity Index Score-ASIS), antimicrobial use (class, previous use, use during the length of stay in the ICU and time of use), and the occurrence of infection by MDROs. The results concerning occurrence of infection were retrieved from patient charts and by checking hospital surveillance data.

2.1. Sampling and Microbiological Testing

The patients were evaluated with bi-weekly microbiological cultures after confirming their colonization status from identifying imipenem-resistant *K. pneumoniae* or ESBL, imipenem-resistant *P. aeruginosa* and *A. baumannii*.

One swab (Haste Plástico-J. P) was rotated two revolutions in the nasal membrane, another was used for the groin and a third was rotated from the anterior to posterior perineum. The samples were plated in the following dilutions: nasal and groins (10^2 and 10^4) and perineum (10^4 and 10^6). Each dilution was distributed with 100 μ l on plates: MacConkey agar (*K. pneumoniae* and *A. baumannii*), cefrimide (*P. aeruginosa*), incubated at 37°C for 48 h. Each selective medium and dilutions were previously standardized using cultivated duplicate plates *K.*

pneumoniae, *A. baumannii* and *P. aeruginosa*. Identification was performed using Gram stain, and biochemistry cards (Vitek® 2 for Gram negative-bioMérieux, Marcy L'Etoile, France).

After each bacterium was identified, the Bauer-Kirby antibiogram method was used (imipenem/meropenem and ceftazidime for *P. aeruginosa*, *K. pneumoniae* and *A. baumannii*), third generation cephalosporin-ceftazidime, cefotaxime and ceftriaxone for *K. pneumoniae*) [8]. The Carba NP test was performed to verify imipenem hydrolysis [9].

Polimerase Chain Reaction (PCR): All samples identified as *K. pneumoniae* were screened with the following genes: NDM, IMP, VIM, KPC and OXA 48 e for those identified as *Acinetobacter baumannii* were screened with the genes OXA-23, OXA-40, OXA-58 and OXA-143, as previously described by Odeh *et al.*, 2002 [10].

2.2. Samples Sequencing

Positive samples for the following genes (NDM, IMP, VIM, KPC, OXA-23, OXA-40, OXA-48, OXA-58 or OXA-143) were sequenced using their respective primers (forward and reverse) as previously described by Odeh *et al.*, 2002 [10].

2.3. Strains Similarity

The Diversilab (Biomérieux) technique was used to analyze strain similarity. The Diversilab is a technique based on the principles of the *repetitive sequence based* PCR (rep-PCR), that amplifies different fragments sizes of non coded DNA (specific for each specie), offering a quick distinction among species and subspecies (*Biomérieux*, 2013).

2.4. Statistical Analysis

Statistical analysis was performed using the T student test, X² test or Fisher exact test. The predictive factors related to colonization or infection with MDROs were analyzed by logistic regression. Bivariate analysis was used to identify the variables with $p < 0.25$ for insertion into a multivariate model. The significance level was 5% to 95% confidence intervals. Analyses were performed using STATA (11.0).

3. Results

Among 53 evaluated patients 51.0% (27/53) showed colonization with *A. baumannii* and 32.0% (17/53) by *P. aeruginosa*, both carbapenems resistant and 38.0% (20/53) had colonization with *K. pneumoniae* ESBL or KPC 5.6% (3/53).

The main characteristics of the evaluated patients ($n = 53$) were: predominance of male gender (52.0%), at time of ICU admission 64.0% of patients were from another sector in the hospital and, for 72.0% of patients the previous use of antimicrobials in hospital sectors prior to ICU transferences was not verified. During the length of stay in the ICU, we verified an important use of meropenem ($p < 0.05$) among others antimicrobials, with a mean of 24 doses per patient.

A total of 114 samples were related to Gram negative bacilli with resistance profile (resistance to third generation cephalosporin and carbapenem). Among these samples 80 we identified as third generation cephalosporin and/or carbapenem resistant *K. pneumoniae* ($n = 41$) and carbapenem resistant *A. baumannii* ($n = 39$).

Five strains of *K. pneumoniae* and six strains of *A. baumannii* were identified in the imipenem hydrolysis. The positive hydrolyses was verified visually by the change of the medium color from red to yellow or orange. The amplification of the DNA of these strains showed presence of genes for antimicrobial resistance such as KPC2 for 3 strains of *K. pneumoniae*. After the sequencing these strains were identified as:

1) Carbapenemase class A-KPC-2 [*K. pneumoniae*—Identification in *genbank*: gb|ACO82075.1|]; 2) Beta-lactamase KPC-2, partial [*K. pneumoniae*—Identification in *genbank*: gb|AFN82205.1| e, 3) Carbapenemase class A-KPC-2 [*K. pneumoniae*—Identification in *genbank*: gb|ACO82075.1|].

Another two strains of the *K. pneumoniae* PCR results tested positive for the following genes: OXA I, CTXM, SHV e TEM (**Table 1**).

Among the multidrug-resistant *A. baumannii* we verified the genes for OXA 23 and OXA 143 (**Table 2**).

After sequencing, the strain of *A. baumannii*-Ab 25 was identified as OXA-253; one variant of OXA-143 [*A. baumannii*] as described by Girlich *et al.*, 2014 [11].

Table 1. Expression of genes of resistance to antimicrobials by *K. pneumoniae* related to colonization of patients in Intensive Care Units—Belo Horizonte, 2014.

Strains	Oxa1	Oxa9	CTXM	SHV	TEM
Kp30	+	-	+	+	+
Kp37	-	-	-	+	+

Table 2. Expression of genes of resistance to antimicrobials by *A. baumannii* related to colonization of patients in Intensive Care Units—Belo Horizonte, 2014.

Strains	Oxa 23	OXA 143
<i>A. baumannii</i> -Ab2	+	-
<i>A. baumannii</i> -Ab25	-	+
<i>A. baumannii</i> -Ab36	+	-
<i>A. baumannii</i> -Ab38	+	-
<i>A. baumannii</i> -Ab39	+	-
<i>A. baumannii</i> -Ab40	+	-

Similarly, analyses for multidrug-resistant *K. pneumoniae* were verified in 3 different patterns. The cross transmission was registered in ICU II with a percentual of similarity higher than 90.0%, between Kp7 and Kp19 (profile KPC₂) isolated from two different patients. Another important similarity higher than 80.0% was verified between Kp4 (isolated from patient colonization in ICU II) and Kp30 (isolated in colonization of another patient in ICU I), suggesting the spread of similar patterns between these ICUs that are located in the same region of Belo Horizonte.

The identified patterns for *A. baumannii* showed a similarity profile of 95.0%. These samples were isolated from colonization cases in different patients in ICU II during the follow up.

4. Characteristics Related to Patient Colonization in the Intensive Care Unit

The main characteristics related to colonization of resistant Gram-negative bacilli were the use of medical devices and antimicrobials during the length of stay in the ICUs ($p < 0.05$). In the colonization with imipenem-resistant *P. aeruginosa*, the presence of pressure ulcers and severity index was highlighted according to the results of bivariate analyses (Table 3).

With imipenem-resistant *P. aeruginosa*, some variables were highlighted in the final logistic regression model, such as the use of mechanical ventilation and fluoroquinolones, which accounted for approximately 32% of colonization with this microorganism. The main colonization sites by carbapenem resistant *P. aeruginosa* were nares ($\rho: 0.8$; $p = 0.001$) and groins ($\rho: 0.7$; $p = 0.001$).

Among colonized patients with imipenem-resistant *K. pneumoniae* or *K. pneumoniae* producing Extended-Spectrum Beta lactamases, the use of fluoroquinolone during the length of stay in the ICU was the most important characteristic. The groin was the main anatomic site colonized by ESBL or carbapenem resistant *K. pneumoniae* ($\rho: 0.7$; $p = 0.0001$).

The presence of pressure ulcers and carbapenems used during the length of stay in the ICUs were also important characteristics of patient colonization with imipenem-resistant *A. baumannii*. In regards to patients colonized with carbapenem resistant *A. baumannii*, the main site for identification of this microorganism were nares ($\rho: 0.7$; $p = 0.001$) and groins ($\rho: 0.6$; $p = 0.001$).

5. Discussion

In colonization with multidrug-resistant Gram negative bacilli, we highlighted *K. pneumoniae*, with a variety of profiles, such as ESBL (CTXM, SHV e TEM) and KPC₂, imipenem resistant *P. aeruginosa* and carbapenem resistant *A. baumannii* with expressions of the genes OXA 23 and OXA 253 (a variant of OXA-143), distributed

Table 3. Variables in colonization with *Pseudomonas aeruginosa* (n = 17), *Klebsiella pneumoniae* (n = 23) and *Acinetobacter baumannii* (n = 27)—Belo Horizonte, 2014.

Variables	<i>P. aeruginosa</i>		<i>K. pneumoniae</i>		<i>A. baumannii</i>	
	N	p	n	P	n	p
Age	51	0.2	48	0.50	61	0.30
Sex						
Male	13		13		16	
Female	4	0.04	10	0.90	11	0.60
Length of stay (ICU)	33	0.10	23	0.40	24	0.40
Pressure ulcer	10	<0.01	9	0.30	12	0.04
Medical devices (% use)						
Venous catheter	13	0.04	15	0.20	17	0.30
Nasal probe	14	0.01	15	0.30	18	0.20
Indwelling catheter	14	0.05	12	0.10	18	0.60
Mechanical ventilation	14	0.01	13	0.80	18	0.09
Drains	7	0.40	60	1.0	11	0.30
Antimicrobials use (days)	9	0.07	9	0.40	10	0.20
Antimicrobials						
Penicillin	9	0.30	12	0.20		0.30
Glycopeptide	-	-	-	-	0	-
Fluoroquinolone	11	<0.00	11	0.01		0.60
Carbapenem	12	0.01	14	0.08		<0.01
Severity						
C	11		19		24	
D	5		3		3	
E	1	0.05	1	0.50		-

in colonization cases in both ICUs. This profile is similar to those registered throughout Latin America and Brazil [6].

In regards to *K. pneumoniae* producing carbapenemases, in the last 10 years this profile has been associated with infections with limited therapeutic options, showing multiple mechanisms of resistance [12] [13]. Infections caused by *A. baumannii*, are associated with mechanical ventilation use, presence of wounds or burns and mainly in critical patients [14] [15].

Among the multidrug-resistant Gram negative bacilli, the ESBL profile was highlighted, such as the CTX-M that have been noted as the most frequent pattern worldwide. The ESBL expression by *Enterobacteriaceae* is frequent in hospital settings. These enzymes hydrolysis cephalosporins of amplified spectra, such as cefotaxime, ceftazidime and Aztreonam [16]. The expression of CTX-M has been verified since the 1990s, mainly in samples of *Escherichia coli* [17] [18].

The large expression of ESBL among Gram-negative bacilli increases the carbapenems use, one such antimicrobial options for treatment of infections caused by these microorganisms. The increased use of carbapenems enables the expression of carbapenemases due to selective pressure [17] [18]. In the present follow up the meropenem, one such example of carbapenem was the most used antimicrobial in ICU I and ICU II.

The strain of *A. baumannii* identified as OXA-253; a variant of OXA-143 [*A. baumannii*]. Others variants of OXA-143 have been registered in Brazil (OXA-231, in Parana state), Honduras and the United States [19] [20]. The OXA-23 expression is registered all over South America and was the main profile identified in ICU I and ICU II in this follow up [21] [22].

A large variation was found with patterns of multidrug-resistant *K. pneumoniae*. For this microorganism we registered 3 different patterns when we evaluated similarity among the strains. The percentual of similarity ranged from 80.0% to 90.0%. The highest similarity was verified with strains of KPC₂ from two patients in ICU II, suggesting cross transmission of the microorganism.

The patterns identified for *A. baumannii* showed high similarity (95.0%), perhaps the most important patterns related to antimicrobial resistance in *A. baumannii* and cross transmission in these hospital settings.

Some factors associated with patient conditions in the ICUs may be related to the acquisition of resistant microorganisms, as verified in this follow up; the antimicrobials and medical devices used were important factors in colonization with BGN.

In one evaluation of clinical samples isolated in patients from 18 hospitals in Germany, 54.3% were identified as ESBL. It was observed that the Gram-negative bacilli carriers had a high length of stay (mean: 37.2 days). The prolonged length of stay in health institutions and the antimicrobials used may increase the risk of multi-drug-resistant microorganisms acquisition [23] [24].

In analyzing patients colonization we evaluated the nares, the groins and perineum regarding the low temporal variability of microorganisms in these skin sites. The main sites for identification of BGN, in this study, were the nares and the groins. Therefore, the identification of resistant bacteria in these sites may imply colonization establishment [25] [26].

For further evaluations of patterns and spread of Gram-negative bacilli, it is important to have a longer period of follow up and to perform multicentre studies. Although, in this study we performed a short follow up of patient colonization with multidrug-resistant Gram-negative bacilli, it was possible to verify different patterns of resistance to antimicrobials and its spread throughout two ICUs in Belo Horizonte, Brazil. The identification of multidrug-resistant microorganisms carriers may facilitate the management of infection control measures and reduce the spread of microorganisms and infections.

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