

Risk Factors for Nosocomial Bloodstream Infection Caused by Multidrug Resistant Gram-Negative Bacilli in Pediatrics

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The aim of this study was to identify the risk factors for nosocomial bloodstream infections by multidrug resistant Gram-negative bacilli. From November 2001 to December 2003, in the Pediatric Department of the Santa Casa de São Paulo, a retrospective case-control study was developed concerning patients who had nosocomial bloodstream infection caused by Gram-negative bacilli. Patients with multidrug resistant infections were designated as case patients, and control patients were those with an infection that did not meet the criteria for multidrug resistance. Previous use of central venous catheter and previous use of vancomycin plus third generation cephalosporins were associated to a higher chance of infections by multidrug resistant Gram-negative bacilli (Odds ratio – 5.8 and 5.2, respectively). Regarding sensitivity of the isolated agents, 47.8% were multidrug resistant, 54.2% were *Klebsiella* spp. ESBL producers and 36.4% were imipenem resistant *Pseudomonas aeruginosa*. The lethality rate was 36.9% in the studied cases and this rate was significantly higher in the group of patients with multidrug resistant infections ($p=0.013$). Risk factor identification as well as the knowledge of the susceptibility of the nosocomial infectious agents gave us the possibility to perform preventive and control strategies to reduce the costs and mortality related to these infections.

Key-Words: Bloodstream infection, Gram-negative bacilli, risk factors.

Bloodstream infections are among the most common hospital-acquired infections in pediatric patients, and are responsible for approximately 10% to 30% of the cases [1-3]. The enhanced complexity of the patient, with a consequent increase in the duration of the hospital stay and greater need for intravascular devices, has increased the risks for acquiring bloodstream infections [4,5].

Among the risk factors associated with the development of bloodstream infections are: prematurity and low birth weight, age inferior to 1 year, admission into intensive care units (ICU), underlying disease, prior surgery, hemodialysis, duration of hospital stay, elevated PRISM score, use of central venous catheters and parenteral nutrition [6-12].

Gram-negative bacteria were the most common causative agents in hospital-acquired bloodstream infections in the 1960s and 1970s. However, from this point on, Gram-positive bacteria became more predominant, probably correlated with the greater use of prophylactic antibiotics in at-risk patients, such as those with neoplasias or using intravascular devices [8,13].

Currently, Gram-negative bacteria are still among the most important agents involved in pediatric bloodstream infections, responsible for approximately 24% to 50% of the cases [5,6,8,9,14]. We have also observed that the frequency of these agents presents variations according to region or medical service studied. When the percentage of infections from these agents are analyzed independently in Latin America, the frequency of Gram-negative bacilli in hospital-acquired bloodstream infections surpass those of Gram-positive [4,8].

Bloodstream infections by Gram-negative bacilli are associated to high morbidity and mortality rates that seem to be correlated with the severity of the underlying diseases and with the use of inadequate empirical antimicrobial drug therapy [4,8,11,15-19].

Despite the impact hospital-acquired infections from multiresistant Gram-negative bacteria have been causing, few studies exist concerning this topic for the pediatric population. The goal of this study was to identify the risk factors involved in the acquisition of nosocomial bloodstream infections by multiresistant Gram-negative bacteria in patients treated in the Pediatric Department of the Santa Casa de São Paulo, also evaluating the sensitivity profile of the isolated agents and lethality rate in the cases studied.

Materials and Methods

The Santa Casa de São Paulo is a third level university teaching hospital located in the central region of the city of São Paulo with 150 beds for the pediatric department.

Patients admitted in the Pediatric Department of the Santa Casa de São Paulo between November 2001 and December 2003 that presented hospital-acquired bloodstream infection by Gram-negative bacteria 72 hours after admission had their medical charts analyzed.

With the goal of identifying the risk factors for hospital-acquired bloodstream infections by multidrug resistant Gram-negative bacteria, the case-control research model was applied. The patients that had positive hemoculture for multidrug resistant pathogens were considered as cases and those that had positive hemoculture for pathogens without multidrug resistant characteristics were considered as the controls.

For analysis, only 1 sample of positive hemoculture for each episode of bloodstream infection was considered. A patient was only included in the study more than once if the

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episodes of bloodstream infection occurred during distinct admissions or were caused by different agents.

Cases of bloodstream infections were excluded if they occurred prior to 72 hours of hospital stay or in situations in which there were more than 1 pathogen per sample.

Conventional methods for bacteria isolation were used and identification was done using manual biochemical tests. Automated methods were not used.

Antibiotic sensitivity tests were done using the disk-diffusion method in Mueller-Hinton Agar, according to the NCCLS standards of the respective year.

For the detection of ESBL producing strains among the *Klebsiella* spp. and *Escherichia coli* isolates, standardized NCCLS screening and confirmatory tests were used [11].

The medical charts were reviewed and the following data was collected: gender, age, duration of hospital stay prior to bloodstream infection, underlying and associated diseases, previous use of antibiotics, unit where the patient was admitted, previous exposure to invasive procedures and final evolution of the bloodstream infection.

Primary hospital-acquired bloodstream infection was defined, according to the criteria proposed by the CDC, as having been acquired 72 hours after initial admission with laboratory confirmation, presence of fever, chills, hypothermia, apnea, bradycardia or hypotension [20].

Previous use of antibiotics was defined as one applied at least 24 hours and up to 30 days prior to the installation of bacteremia.

Previous use of central venous catheters was defined as that which occurred at least 24 hours and up to 30 days prior to the installation of bacteremia.

The isolated strains that presented resistance to 3 or more classes of antimicrobials were considered multidrug resistant.

Death was considered as consequent from the bloodstream infection when it occurred up to 30 days from positive hemoculture and death certificate indicated sepsis as the *causa mortis*.

Statistical analysis was constituted on the descriptive analysis of the possible risk factors, using the Chi-square test, Fisher's exact test, Mann-Whitney test, followed by the logistic regression model. A 0.05 significance level was adopted and the SPSS program for WINDOWS was used.

Results

There were 92 episodes of nosocomial bloodstream infection by Gram-negative bacilli in 85 patients admitted in the Pediatric Department of the Santa Casa de São Paulo in the period ranging from November 2001 to December 2003.

In 55.5% of the cases, the patients were male and the mean age was of 4 months.

In 95.6% of the cases, there was previous use of antimicrobials in the 30 days prior to positive hemoculture.

In 95.2% of the cases, the patients presented underlying disease or comorbidities and 50% had a deficit in immune system function.

In 67.3% of the cases, the patients had used central venous catheters in the 30 days prior to positive hemoculture.

The mean time of hospitalization prior to bloodstream infection was of 13 days, with 29.5% admitted into pediatric nursery, 9.8% in semi-intensive care unit, 27.2% in intensive care unit and 33.7% in neonatal intensive care unit.

Of the 92 episodes of nosocomial bloodstream infections from Gram-negative bacilli, 44 (47.8%) were caused by multidrug resistant agents and 48 (52.2%) by agents without multidrug resistant characteristics. In the analysis of the bacterial isolates, the most prevalent agents were: *Klebsiella pneumoniae*, 37%, *Acinetobacter baumannii*, 21.7%, *Enterobacter* spp., 14%, and *Pseudomonas aeruginosa*, 12%.

The rate of ESBL producing strains among the *Klebsiella* spp. isolates was of 54.2%, and in the *Escherichia coli* isolates was of 33.3%. Resistance to imipenem was detected in 36.4% of the *Pseudomonas aeruginosa* isolates (Figure 1).

A significant difference was not observed between the 2 groups in relation to gender, age, duration of hospitalization prior to bloodstream infection, unit of admission and presence of underlying diseases or comorbidities.

The patients who used central venous catheters presented a higher rate of infections from multidrug resistant strains, with statistical significance.

The previous use of antimicrobials was not associated with a higher rate of infection from multidrug resistant agents. However, the use of vancomycin demonstrated a statistical correlation with infection from multidrug resistant agents. The patients that used vancomycin in association with third generation cephalosporins had higher rates of infection from multidrug resistant agents when compared to patients that used only one of these two antimicrobials.

The total number of antibiotics used was also greater in the group of patients having infection by multidrug resistant agents ($p=0.05$).

Based on this data obtained from descriptive statistical analysis, the risk factors associated to acquiring nosocomial bloodstream infection by multidrug resistant Gram-negative agents were: previous use of central venous catheters, previous use of vancomycin, previous use of third generation cephalosporins in association with vancomycin and number of antibiotics used, all having p-values inferior to 0.05 (Table 1).

In the logistic regression model for infection from multidrug resistant Gram-negative bacilli, the patients that used central venous catheters had 5.8 times more chance of presenting infection from multidrug resistant agents than patients that did not use central venous catheters. The combined use of vancomycin and third generation cephalosporins presented 5.2 times more chance of infection by multidrug resistant agents than the patients who did not use either (Table 1).

Table 1. Risk Factors: bloodstream infection by Gram-negative bacilli vs. Multiresistant Gram-negative bacilli

	Bacteremia GNB	Bacteremia MR GNB	p-value
Age (median)	5 months	4 months	0.636
T. hosp. (median)	12 days	15 days	0.28
Underlying diseases (%)	49.4	50.6	0.999
Invasive proc. (%)	48.1	51.9	0.036
Catheter (%)	40.3	59.7	0.001
Previous antibiotic (%)	51.1	48.9	0.618
Number of antibiotics (median)	2	3	0.05
3rd gen. cephalosporin (%)	41	59	0.066
Vancomycin (%)	36.1	63.9	0.013
3 ^a ceph. + vancomycin (%)	32	68	0.038

Note: T. hosp.=time of hospitalization; Proc.=procedure; 3rd Ceph.=3rd generation cephalosporin; GNB=Gram-negative bacilli; MR=multiresistant.

Table 2. Deaths related to the presence of infections by multiresistant strains

	Multiresistant bacteria		
	Yes	No	Total
Death (N/%)			
Yes	22	12	34
	50%	25%	36.9%
No	22	36	58
	50%	75%	63%
Total	44	48	92
%	100%	100%	100%

P (χ^2)=0.013.

The lethality of the cases studied was of 36.9% and was significantly greater in the group of patients having infection from multidrug resistant agents, with $p=0.013$. (Table 2)

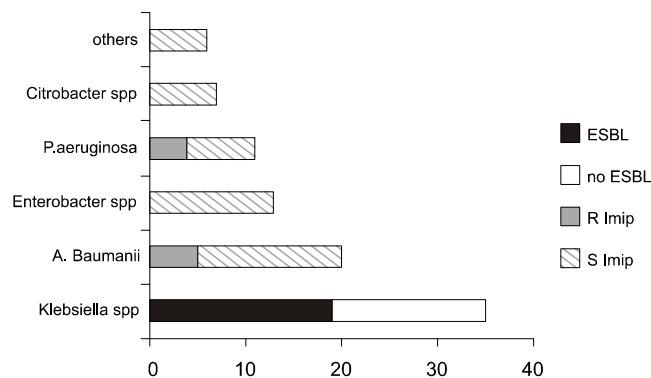
Discussion

When the patient sample of this study is analyzed, it is clear that those who developed nosocomial bloodstream infections by Gram-negative bacilli were exposed to the risk factors that have been well-defined in the literature [1,5,6].

Of all the patients studied, 65.2% were under the age of 12 months and had a mean duration of hospitalization of 13 days prior infection onset. The majority of the patients were exposed to antimicrobials (95.6%) and central venous catheters (67.3%) and had underlying diseases or comorbidities (95.2%).

In the search for risk factors for this type of infection, the patient sample was divided into 2 groups: patients with nosocomial bloodstream infection by Gram-negative bacilli and patients with nosocomial bloodstream infection by multidrug resistant Gram-negative bacilli.

The exposure to invasive treatments, especially those requiring the use of intravascular devices, is considered in the literature as one of the main risk factors for the acquisition of nosocomial infections, as well as bloodstream infection from multidrug resistant agents [4,6,8,9,17,18,21]. It acts as an entry point for the hospital flora that colonizes the patient or

Figure 1. Gram-negative bacilli isolated from the hemoculture of patients of the Pediatric Department of Santa Casa Hospital, between November 2001 and December 2003.

Note: R Imip – resistant to imipenem; S Imip – Susceptible to imipenem.

is brought to the patient by the team of caretakers through contact transmission. In the present study, the use of central venous catheters was associated to the highest rates of infection by multidrug resistant agents, having statistical significance ($p=0.001$).

The selective pressure exerted by the antibiotics, causing bacterial resistance, is another consensus in the medical literature [21-23]. Cases of Gram-negative bacteria infection have a narrow association with the introduction of the new cephalosporins, followed by the emergence and dissemination of ESBL, chromosome inducible β -lactamases and strains of *Pseudomonas aeruginosa* with multiple mechanisms of resistance [21-26].

In this study, the previous use of antibiotics did not demonstrate statistical difference, in general. However, we should consider that there were a small number of patients that had not previously used antibiotics [4].

The total number of antibiotics used was significantly greater in the group of patients that developed infection from multidrug resistant bacteria ($p=0.05$). Nevertheless, we should consider that the use of more antibiotics in this group is

associated to worse clinical conditions, greater time of hospitalization, greater exposure to invasive procedures and use of broad-spectrum antibiotics.

When the antibiotics used were independently analyzed, it was discovered that the use of 3rd generation cephalosporins was greater in the group of patients with multidrug resistant bacteria ($p=0.06$).

The previous use of vancomycin was also greater in patients infected by multidrug resistant bacteria, having statistical significance ($p=0.013$). Thus, the question arises about the association between vancomycin and nosocomial bloodstream infections by Gram-negative bacilli and its role in the emergence of resistance in these agents.

Some studies suggest that vancomycin may act by selecting the Gram-negative flora by eliminating competitive Gram-positive agents. This issue had also been discussed in studies about the action of anaerobic drugs in the selection and emergence of vancomycin resistant *Enterococcus* spp. strains [24].

We should also consider that previous therapy with vancomycin, most of the times, was not done without the association of other antimicrobials. Furthermore, vancomycin is generally used in combination with broad-spectrum cephalosporins, carbapenems or quinolones in patients with severe infections who did not respond to first line drugs and had already been hospitalized for a longer time with subsequent greater manipulation.

In this study, the combined use of vancomycin and 3rd generation cephalosporins was greater in the group of patients that had infection from resistant strains ($p=0.038$ and Odds ratio=5.2). Here, there might be the same factors of interaction that were previously cited.

The duration of hospitalization prior to the occurrence of bloodstream infection is also a factor reported in the literature for cases of nosocomial infections and multidrug resistant nosocomial bloodstream infections [3-6]. We did not find a significant difference in relation to the duration of hospitalization; however, it is worth noting that in both cases the duration was prolonged.

Regarding the ICU, some studies report that the prior PICU and NICU stays are a risk factor for multidrug resistant nosocomial infections [4,17,18,21]. A significant difference was not observed between the different care unit groups. This may be due to the fact that the patients were frequently moved between the care units: infirmary, semi-intensive, ICU. Therefore, it was difficult to determine the origin of the nosocomial infection.

The presence of underlying diseases and comorbidities and its relationship to the severity of the patient's condition (PRISM and APACHE scores) are described in the literature as risk factors for nosocomial infections, as well as nosocomial infections by multidrug resistant agents and higher mortality rates [4-6,16,18,21].

A difference was not observed between the 2 groups in relation to the presence of comorbidities and underlying

diseases even when they were associated to immune deficiency. This fact may be due to the small patient sample that did not have the aforementioned characteristics [11].

The nosocomial bloodstream infection by Gram-negative bacteria is related to elevated lethality, and the main risk factors are: severity of the clinical conditions, presence of underlying diseases and comorbidities and infection from multidrug resistant agents [15,27,28].

In relation to infections from resistant agents, an important association between mortality and implementation of inadequate initial empirical drug treatment is described. In situations in which adequate therapy is instituted, the main risk factor is the severity of the existing clinical conditions [27,28].

In the Pediatric Department of the Santa Casa de São Paulo, the lethality rate of nosocomial bloodstream infections by Gram-negative bacteria was of 36.9% and infection from multidrug resistant bacteria was a significantly greater factor for lethality (64.7%), with $p=0.013$.

The lethality found reflects on sample characteristics, which was mainly composed of very young patients, of high complexity, with underlying diseases or comorbidities.

In conclusion, we should recognize patients exposed to the possible risk factors and recognize our reality in terms of most frequently found agents and their resistance patterns. In this way, we can establish strategies for the control of the infections and adopt adequate initial empirical drug treatment for the cases in question, reducing the impact of bloodstream infections in terms of cost, morbidity and mortality within our service.

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