Nosocomial Bloodstream Infections: Organisms, Risk Factors and Resistant Phenotypes in the Brazilian University Hospital

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Bacteremia is one of the most frequent and challenging hospital-acquired infection and it is associated with high attributable morbidity and mortality and additional use of healthcare resources. The objective of this work was to determine the frequencies of its occurrence, organisms and resistance phenotypes associated to nosocomial acquired bloodstream infections. A total number of 51 nosocomial bacteremia by Gram-negative and 99 by Gram-positive were evaluated and compared during a 15-month period. The risk factors associated with these bacteremias were analyzed and antibiotic use and surgery were associated with bacteremia by Gram-negative and ≥ 2 invasive devices with Gram-positive. The resistance phenotypes ESBL (extended-spectrum beta-lactamases) (23.5%) and AmpC/ others (17.6%) correspond to 41.2 % with predominance of *E. agglomerans* among AmpC (44.4%) and *K. pneumoniae* among ESBLs (38.5%). Among *S. aureus* bacteremia, approximately 40% were associated to MRSA (methicillin-resistant *Staphylococcus aureus*).

Key-Words: Nosocomial bacteremia, resistance phenotypes, risk factors.

Hospital acquired bloodstream infections constitute a serious health problem and are associated to high morbidity and mortality which result in increasing healthcare costs [1]. The two major categories of bloodstream infections are: primary/intravascular, which is originated within the cardiovascular system; secondary/extra vascular, which results from another infection focus. The major risk factor for nosocomial bloodstream infection is intravascular catheterization. It has been estimated that 90% of intravascular device-related bloodstream infections are originated from central venous catheters of many types [2].

Appropriate surveillance techniques constitute the cornerstone of infection control and make possible the implantation of specific preventive approaches. The impact of surveillance programs on the incidence of hospital acquired bloodstream infection needs to be recognized and established as well as its cost-effectiveness for both the patient and the institution [1].

There are multiple mechanisms of antimicrobial resistance that may appear in clinically important bacteria, but ESBL (extended-spectrum beta-lactamases) and AmpC among Gram-negative bacteria and MRSA (methicillin-resistant *Staphylococcus aureus*) and also VRE (vancomycin-resistant enterococci) among Gram-positive bacteria are major resistant phenotypes that have become increasing resistant to most useful antibiotics [3].

In this study we report the frequencies of occurrence, organisms and resistance phenotypes of nosocomial bloodstream infections (BSI) isolated in Brazilian University Hospitals.

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Materials and Methods

Hospital

Universidade Federal de Uberlândia (Uberlândia Federal University Hospital Clinic/ HC-UFU) is a 500-bed tertiary and acute care hospital.

Design of the Study

The study was conducted during a 15-month period, between February, 2000 and April, 2001. The organisms were consecutively collected (only one strain per patient was included) through microbiology laboratory of the hospital by automated cultures (Bacted Alert). Clinical and demographic information about each patient including intrinsic and extrinsic risk factors were obtained from hospital records.

Definition of Hospital Acquired Bacteremia

Bacteremia was considered hospital-acquired if it appeared within 48 hours after its admission and if no evidence of infection was presented on admission [4].

Phenotypes of Organisms

Phenotypes decreasing susceptibility was defined as recommended by NCCLS by using gel diffusion test [5]. The following phenotypes were considered: A: Gram-positive: MRSA and MRCoNS (methicillin-resistant coagulase negative staphylococci) to staphylococci and HLRG (high-Level resistance gentamicin) and HLRS (high-level resistance streptomycin) to enterococci; B: Gram-negative: ESBLs (all Gram-negative bacilli) and AmpC/others (all Gram-negative bacilli, except ESBL positive). The synergy test was performed with double disk to detect the samples producing extended-spectrum beta lactamase. AmpC producers were defined as third generation cephalosporin's (ceftazidime or ceftriaxone) resistant, and this resistance was not reversed by clavulanat (exclusion).

Statistical Methods

All data were analyzed using Epi-info version 5.0. Categorical variables were compared using the likelihood ratio

test or, when appropriate, Fisher's exact test. Odds Ratio (OR) and 95% confidence intervals (CI 95%) were calculated [6].

Study Approval

This study was approved by the Ethics Committee of the Hospital de Clínicas da UFU (Clinical Hospital of Uberlândia Federal University, in the state of Minas Gerais, Brazil).

Results

The main characteristics of nosocomial bacteremia by Gram-positive and Gram-negative organisms' isolation during the study period are shown in Table 1. The use of antibiotics, ≥ 2 antibiotics and surgery were more associated with bacteremia by Gram- negative organisms and central vascular catheter with Gram-positive ones. The relation between male and female prevalence rates were similar with 24:27 in the Gram-negative and 56:43 in the Gram- positive. The average age of patients with Gram-negative bacteremia was 40.4 ± 28.5 years (range 0.01-79 years) and with Gram-positive was 38.1 ± 26.8 years (range 0.005-86 years). The analysis of the population showed that length of hospitalization (33.5 ± 20.2 *vs*. 18.5 ± 17.6) was high and the mortality was similar in two groups**.**

Among the secondary bacteremia prevailed (64.7%) the Gram-negative organisms, being *E. coli* the most common agent, while the Gram-positive organisms were primary bacteremia (67.7%), being CoNS the most common agent (Table 2). The main sources of secondary bacteremia caused by Gram-negative organisms were surgical site (29.4%) and pulmonary (23.5%). In total, in 13/150 (8.7%) the sources of bacteremia were unknown (Table 3).

Our data has shown that 34% (51/150) of bacteremia were caused by Gram-negative bacilli and 66% (99/150) by Gram-positive *cocci*. *K. pneumoniae*, *E. coli* and *P. aeruginosa* were more frequent in the nosocomial bacteremia by Gram-negative and among Gram- positive, *S. aureus* (Table 4).

The major resistance phenotypes are shown in Table 5. The resistance phenotypes of third generation cephalosporins were classified as AmpC (29.4%) and ESBLs (23.5%), respectively, corresponding mainly to samples of *E. agglomerans* and *P. aeruginosa* in the first case (8/15) and *K. pneumoniae* in the second one (5/12).

Among *S. aureus* isolates, 39.2% were resistant to methicillin and only one isolate (0.6%) of *Enterococcus* had high-level resistance to gentamicin.

Discussion

Inadequate antimicrobials treatment is closely associated with the presence of antibiotic resistance in clinically important pathogens. The main cause of this problem is multifactorial, including selective pressure that results from inappropriate use of antimicrobial agents; many hospital medicines are stocked with broad-spectrum antimicrobial agents that are used liberally, in part because of a lack of restrictions and in part because of an already-established problem with resistant hospital flora [7].

The main predisposing factors associated with all nosocomial infections include essentially four key groups: underlying health status (advanced age), acute disease process (surgery, trauma, severity score), invasive procedures and treatment (antibiotic therapy, patients in intensive units) [8], but apart from these overall risk factors more specific ones have showed significance as 87% of primary bloodstream infections were in patients with a central line [9]. In this study only 18.7% of bactaeremic patients were cared in ICUs in spite of most of them were critical with ≥ 2 antibiotics use (37.3%); ≥ 2 invasive procedures (30.0%) and they were surgical patients (18.7%).

Very few programs have assessed antimicrobial resistance in Brazil and other Latin American countries. The SENTRY Program collects consecutive isolated microorganisms from clinically documented infections in more than 80 medical centers worldwide, including 10 in Latin America that has shown that resistance in Gram-negative bacteria seems to be much higher in these countries when compared to other regions of the world, especially North America and Europe [10]. On the other hand, some resistance problems related to Gram-positive cocci, such as glycopeptides resistance among enterococci are less frequently described in Latin America [11]. Possible reasons for the low prevalence of Gram-negative organisms (34%) compared to Gram-positives (66%) in this study were: predominance in primary bacteremia (57%) and the frequency of the patients with intra-abdominal surgery infections.

Surveillance of major resistance phenotypes among bacteria causing hospital-acquired infections are currently very common in ICUs in the USA [12] and Europe [13] as in all over the world including Latin America [14]. The most recent data from ICUs in Southern Europe revealed 80.0% of CoNS to be oxacillin-resistant against 74.0% at Nordic Centres [13], similar rates of oxacillin resistance (80.0%-86.7%) are found in US-ICUs [12]. In these series only 34.1% of the CoNS were associated to this phenotype.

Numerous studies have documented the importance of the *S. aureus* as the major agent of nosocomial bloodstream infection. In the USA, recent data from hospitals participating in SENTRY and NNIS have demonstrated that 45.0% and 54.5%, respectively, of nosocomial bloodstream isolations of *S. aureus* were oxacillin-resistant [12,15]. In the 25 European SENTRY centers, the mean MRSA prevalence was 39.0% [7]. Most Latin American tertiary hospitals have shown that 30.0% to 50.0% of *S. aureus* strains are resistant to oxacillin [14]. In this study, MRSA was responsible for 39.2% of *S. aureus* nosocomial bacteremias.

The VRE problem is more widespread in North American ICUs (25.9%) [12] than in European ones [13]. VRE isolation has been reported in some Brazilian hospitals [16] mainly in São Paulo [17]; but this kind of organism was not observed in the UFU-HC. On the other hand, the resistance of *Enterococcus* in a high level of aminoglycosides is not uncommon, with frequencies of 26.4% being resistant to

Table 1. Characteristics of patients with nosocomial bacteremia by Gram-negative and Gram-positive organisms in UFU-HC, between February/2000 and April/2001

Risk factors	Nosocomial bacteremia	
	Gram-negative N = 51 (%)	Gram-positive N = 99 (%)
Gender		
Female	27 (52.9)	43 (43.4)
Male	24 (47.0)	56 (56.6)
Time of hosp. (days)	33.5 ± 20.24	48.8 ± 108.26
Age (years)	40.4 ± 28.55	38.1 26.76
Antibiotics		
Yes	42/48 (87.5)	34 (34.3)
N ≥ 2	36/49 (73.5)	21 (21.2)
Invasive devices		
N ≥ 2	24 (47.0)	21 (21.2)
Central vascular catheter	9 (17.6)	44 (44.4)
Peripheral vascular catheter	33 (64.7)	90 (91.0)
Drain	9 (17.6)	33 (33.3)
Surgery	24 (47.0)	4 (4.0)
Overall mortality	10 (19.6)	21 (21.2)

Hosp.=hospitalization.

Table 2. Nature of nosocomial bacteremia by Gram-negative and Gram-positive organisms in UFU-HC, between February/2000 and April/2001

Bacteremia	Organisms	
	Gram-negatives N = 51 (%)	Gram-positives N = 99 (%)
Primary/Unknown[1]	18 (35.3)	67 (67.7)
Secondary[2]	33 (64.7)	32 (32.3)
Total	51 (100.0)	99 (100.0)

[1]Staphylococci coagulase negative was the most common agent. [2]*E. coli* was the most common agent.

Table 3. Sources of nosocomial bacteremia between February/2000 and April/2001

Source	Nosocomial bacteremia	
	Gram-negatives N = 51 (%)	Gram-positives N = 99 (%)
Intravascular devices	9 (17.6)	63 (63.6)
Surgical wound infection	15 (29.4)	10 (10.1)
Skin/abscess	1 (2.0)	7 (7.1)
Respiratory tract	12 (23.5)	11 (11.1)
Others	5 (9.8)	4 (4.0)
Unknown	9 (17.6)	4 (4.0)

Table 4. Occurrence of the major pathogens Gram-negative and Gram-positive isolated from bloodstream infections in UFU-HC, between February/2000 and April/2001

Rank ordering organisms	Bacteremia (N = 150)	
	N	%
Gram negatives	51	34.0
Enterobacteriaceae		
K. pneumoniae	12	23.5
K. oxytoca	2	3.9
E. coli	11	21.6
Enterobacter spp.	8	15.7
Serratia spp.	2	3.9
Citrobacter spp.	3	5.9
Morganella spp.	1	2.0
Non-fermented		
P. aeruginosa	9	17.6
Pseudomonas spp.	2	3.9
S. maltophilia	1	2.0
Gram positives	99	66.0
Staphylococcus aureus	49	49.5
Coagulase-negative staphylococci	43	43.4
Enterococcus spp.	7	7.1

Table 5. Resistance phenotypes of the Gram-positive and Gram-negative organismsrelated to nosocomial bacteremia in UFU-HC, between February/2000 and April/2001

Phenotypes	Nosocomial bacteremia (N = 153) N (%)
Staphylococcus spp.	95/153 (62.1)
S. aureus	51/95 (53.7)
MRSA[1]	20/51 (39.2)
Staphylococcus coagulase negative	44/95 (46.3)
MRCoNS[2]	15/44 (34.1)
Enterococcus spp.	7/153 (4.6)
HLRG[3]	1/7 (14.3)
Gram-Negative	51/153 (33.3)
ESBLs[4]	12/51 (23.5)
AmpC	9/51 (17.6)

[1]Methicillin-resistant *Staphylococcus aureus*. [2]Methicillin-resistant *Staphylococcus* coagulase negative. [3]High level resistant gentamicin. [4]Extended-spectrum beta lactamases.

gentamicin in São Paulo [18] and 24.8% in Porto Alegre [19]. In Uberlândia, it was detected 14.2% of isolates with high-level resistance just to gentamicin.

The two most important broad-spectrum resistance mechanisms to the newer b-lactam antibiotics are: the chromosomal gene, AmpC, encoding class C, type I inducible cephalosporins commonly expressed in *Enterobacter*, *Citrobacter*, *Serratia* and *Proteus* (CESP group), and expanded-spectrum β-lactamases which are plasmid-encoded

enzymes that confer resistance to oxymino-b-lactam antibiotics. *K. pneumoniae and E. coli* are the organisms most likely to be ESBL producers [20].

In the USA, ESBL-production rates are usually less than 5.0% for *K pneumoniae* [21], while in Europe the prevalence may be as high as 73.0% in Turkey or 7.0% in the UK [13]. The prevalence of strains producing ESBL in Latin America according to data from the SENTRY was 44. 4% for *Klebsiella* spp and 8.9% for *E. coli* strains, associated with bloodstream infection [14]. Sader et al. (2001) recently reported that in Brazil approximately 9.0% *of E. coli* and 50.0% of *K. pneumoniae* were characterized as ESBL producers. In this study, the prevalence of these ESBL producer isolates was 41.7% (*K. pneumoniae*) and 36.3% (*E. coli*). The frequency of this organism was even compatible with the observed in England, but a reevaluation is necessary, because this *E. coli* phenotype has not been told in the country. *Enterobacter* spp. have been recognized as the most important AmpC producer among Enterobacteriaceae. This kind of resistance varies widely among published reports in the US, and the percentage of resistant strains for ceftazidime ranges from 6.0% to 59.0% [22], while in Europe the percentage of AmpC producers ranges from 5.0% (Germany) to up to 30.0% (Poland) [5]. In these series, half of the *Enterobacter* strains were characterized as ESBL positive. This frequency is preoccupying considering the predominance of empirical therapy in the country, where the 3ª/4ª generation cephalosporins continue to be the main option.

Vascular catheter is the most important risk factor for hospital-acquired bacteremia with central venous catheter associated to up to 90.0% of these infections [8]. In these series, 89.3% of patients with Gram-positive bacteremia were using catheters, while only 28.0% of the patients with Gram-negative bacteremia were using this invasive device. The secondary bacteremia was more prevalent with Gram-negative bacilli (64.7%), and surgical site and lung were the most frequent foci with 29.4% and 23.5%, respectively. The greater role of staphylococci is the cause of primary nosocomial bacteremia that continues and is a nationwide phenomenon as illustrated elsewhere, and it was confirmed in our results (62.6%) being 40.0% of these organisms resistant to oxacillin.

Antibiotic resistance among bacteria causing hospital-acquired infections poses a threat and in order to control the spread of these bacteria, local, regional and national resistance surveillance data must be used to develop efficient intervention strategies [5]. Our results showed that the frequencies of major phenotypes among nosocomial pathogens were high.

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