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Major Article

Impact of the introduction of an automated microbiologic system on the clinical outcomes of bloodstream infections caused by *Enterobacteriaceae* strains

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ABSTRACT

Introduction: *Enterobacteriaceae* strains are a leading cause of bloodstream infections (BSI). The aim of this study is to assess differences in clinical outcomes of patients with BSI caused by *Enterobacteriaceae* strains before and after introduction of an automated microbiologic system by the microbiology laboratory. **Methods:** We conducted a retrospective cohort study aimed to evaluate the impact of the introduction of an automated microbiologic system (Phoenix™ automated microbiology system, Becton, Dickinson and Company (BD) - Diagnostic Systems, Sparks, MD, USA) on the outcomes of BSIs caused by *Enterobacteriaceae* strains. The study was undertaken at Hospital São Paulo, a 750-bed teaching hospital in São Paulo, Brazil. Patients with BSI caused by *Enterobacteriaceae* strains before the introduction of the automated system were compared with patients with BSI caused by the same pathogens after the introduction of the automated system with regard to treatment adequacy, clinical cure/improvement and 14- and 28-day mortality rates. **Results:** We evaluated 90 and 106 patients in the non-automated and automated testing periods, respectively. The most prevalent species in both periods were *Klebsiella* spp. and *Proteus* spp. Clinical cure/improvement occurred in 70% and 67.9% in non-automated and automated period, respectively (p=0.75). 14-day mortality rates were 22.2% and 30% (p=0.94) and 28-day mortality rates were 24.5% and 40.5% (p= 0.12). There were no significant differences between the two testing periods with regard to treatment adequacy, clinical cure/improvement and 14- and 28-day mortality rates. **Conclusions:** Introduction of the BD Phoenix™ automated microbiology system did not impact the clinical outcomes of BSIs caused by *Enterobacteriaceae* strains in our setting.

Keywords: *Enterobacteriaceae*. Bacteremia. Outcomes. Mortality.

INTRODUCTION

The *Enterobacteriaceae* family causes a significant number of bloodstream infections (BSIs) worldwide. The gradual emergence of antimicrobial resistance has led to difficulties in treating these infections[1,2].

It has been well documented that rapid and reliable blood culture results can significantly influence patient's treatment and reduce hospital costs[3,4]. Over the past 20 years, a variety of automated systems have been developed. Several factors have favored the use of these systems in microbiology laboratories, including reproducibility, ability to track results, reduction in contamination, automatic connection to computer lab software and opportunity for clinicians to obtain partial and final results more quickly[5]. An additional advantage is to be able to perform minimum inhibitory concentration (MIC) tests. However, as far as we know, there are no previous studies addressing the impact of an introduction of automated microbiologic systems on outcomes of *Enterobacteriaceae* infections.

This study was conducted to assess the impact of the introduction of an automated microbiologic system on the clinical outcomes of bloodstream infections caused by *Enterobacteriaceae* strains among hospitalized patients.

METHODS

Study design

This retrospective cohort study was conducted at Hospital São Paulo, a 750-bed university-affiliated hospital located in São Paulo, Brazil. The data recorded by the antimicrobial management team were used to identify patients hospitalized with BSI caused by *Enterobacteriaceae* strains between August 2006 and July 2009. The inclusion criteria were: patients ≥ 18 years and first episode of bacteremia. BSI episode was defined by the presence of Enterobacteriaceae strains cultured from one or more blood culture plus the following signs or symptoms: fever (>38^{0}C), chills, or hypotension. Central line associated bloodstream infection (CLABSI) follows the same criteria but the microorganism cultured from blood was not related to an infection at another site. Briefly, the patients were divided into two periods: the non-automated and the automated

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period. The non-automated period included the BSI episodes that occurred from August 2006 to July 2007. In this period, bacterial identification and antimicrobial susceptibility testing were performed using conventional biochemical tests and disk diffusion method, respectively. Conversely, in the automated period, from August 2008 to July 2009, the blood cultures were analyzed by the BD Phoenix™ automated microbiology system (Becton, Dickinson and Company (BD) - Diagnostic Systems, Sparks, MD, USA).

We did not include episodes of BSI occurred from August 2007 to July 2008 in the study because the automated microbiology system was not fully operational. We also excluded patients whose medical records could not be located, patients with community-acquired infections, patients who were not treated with antimicrobials and patients who died within 48 hours of the BSI diagnosis.

Variables and definitions

The analyzed variables included sex, age, severity scores, e.g. acute physiology and chronic health evaluation (APACHE) II score and McCabe score, comorbidities, neutropenia, use of immunosuppressive agents (e.g. corticosteroids, antineoplastic agents), previous surgery, previous hospitalization, use of antibiotics, exposure to invasive procedures (e.g. mechanical ventilation, central line), septic shock, hospital location [intensive care unit (ICU) or ward], length of hospitalization stay, *Enterobacteriaceae* species isolated, presence of extended-spectrum beta-lactamase (ESBL), presence of carbapenemase, polymicrobial infection, antimicrobial therapy, adequacy of antimicrobial therapy, change in antibiotic prescription, clinical response and 14-and 28-day mortality rates. Only antimicrobials used for more than 48 hours were considered. The use of antimicrobials was considered adequate if treatment was initiated with at least one antimicrobial to which the pathogen showed *in vitro* susceptibility within 48 hours of blood culture collection.

Microbiological procedures

All cultures were processed in the microbiology laboratory at Hospital São Paulo using the Bactec 9000 system (Becton Dickinson, Cockeysville, MD).

Until August 2007, bacterial identification and antimicrobial susceptibility testing were performed using biochemical tests and the disk diffusion method, respectively. After this date, bacterial identification and antimicrobial susceptibility testing were performed using the BD Phoenix™ automated microbiology system.

Statistical analysis

A student's t-test was performed to compare continuous variables. Chi-square or Fisher's exact tests were used to compare categorical variables. A p value < 0.05 was considered significant.

A multiple logistic regression technique was applied, and the variables that were significant in the univariate analysis were incorporated into the analysis. A p value < 0.05 was considered significant.

All of the statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) version 15.0 (Chicago, IL)

Ethical considerations

The study was approved by the local Ethics Committee, number 1149/09.

RESULTS

A total of 196 patients were included in the study, 90 patients in the non-automated testing period and 106 patients in the automated testing period.

The clinical and demographic characteristics of patients are shown in **Table 1**. There was a predominance of male sex in both testing periods (54.4% in the non-automated and 61.3% in the automated, respectively). The mean age was 59 years in the non-automated [standard deviation(SD) ± 16.8 years] and 64 years in the automated period (SD ± 15.9 years), respectively.

Table 1 shows the results of the univariate analysis. In the automated testing period, the patients had higher APACHE II scores (p <0.001), used more immunosuppressive agents (p <0.001) and had more central line-associated BSIs (p = 0.002).

There was a prevalence of infections caused by *Klebsiella* spp. and *Proteus* spp. in both testing periods and a reduced prevalence of infections caused by *Providencia* spp. (p = 0.01) in the automated testing period. During this same period, we observed a higher resistance rate to ciprofloxacin (p = 0.002) and piperacillin-tazobactam (p = 0.01) **(Table 2)**. The prevalence of ESBL-producing strains was similar in the two periods. *Klebsiella pneumoniae* and *Proteus mirabilis* were the most frequent ESBL-producing pathogens isolated. In the automated testing period, *Klebsiella pneumoniae* carbapenemase (KPC)-producing was identified in two blood cultures by polymerase chain reaction (PCR) method.

The treatment adequacy was similar in both periods and the appropriate antimicrobial treatment was initiated in 80% of episodes within the first 48 hours after infection. In approximately one-third of the cases a change in antimicrobial therapy was undertaken due to initial antimicrobial resistance. The antimicrobial management team recommended changes in approximately 40% of the episodes. There was a non-significant increase in antimicrobial descalation rate in the automated testing period when compared with the non-automated testing period (17.6% vs. 28.1%, respectively). There was no difference in clinical cure/improvement rates between the two periods. The clinical cure/improvement rate were 70% and 67.9% in the non-automated and automated period, respectively (p=0.75). In addition, no statistically significant difference was observed between the two periods with regard to mortality rates. The 14-day and 28-day mortality rates in the first period were 22.2% and 24.5% and they were 30% and 40.5% in the second period **(Table 3)**.

TABLE 1 - Demographic and clinical characteristics of bloodstream infections caused by **Enterobacteriaceae** *strains.*

	Non-automated period (n= 90)		Automated period (n= 106)			
Variables	**n**	**%**	**n**	**%**	**OR (CI 95%)**	**p**
Male sex	49	54.4	65	61.3	0.75 (0.43 - 1.33)	0.33
Age, mean, SD	59.63 ± 16.89		64.1 ± 15.9		- 0.06	
McCabe						
nonfatal	41	45.5	42	39.6	1.28 (0.72 - 2.25)	0.40
APACHE II, mean ,SD	17.31 ± 6.74		20.99 ± 7.57		- <0.001	
APACHE II ≥ 15	56	62.2	81	76.4	0.51 (0.27 - 0.94)	0.03
Two or more comorbidities	62	68.8	71	66.9	1.09 (0.6 - 1.99)	0.78
Neutropenia	4	4.4	2	1.8	2.42 (0.43 - 13.52)	0.30
Use of immunosuppressive agents	18	20	49	46.2	0.29 (0.15 - 0.55)	<0.001
Prior hospitalization	28	31.1	28	26.4	1.26 (0.68 - 2.34)	0.47
Prior surgery	33	36.6	30	28.3	1.47 (0.8 - 2.68)	0.21
Prior antibiotic use	65	72.2	85	80.1	0.64 (0.33 - 1.25)	0.19
Invasive procedures	77	85.5	91	85.8	0.98 (0.44 - 2.18)	0.95
LOS before BSI, median, SD	30.98 ± 29.41		32.55 ± 26.39		-	0.69
ICU admission at the time of BSI	62	68.8	72	67.9	1.05 (0.57 - 1.91)	0.88
Septic shock	19	21.1	24	22.6	0.91 (0.46 - 1.81)	0.12
Site of infection						
pulmonary	30	68.1	25	62.5	1.62 (0.87 - 3.03)	0.13
urinary tract	8	18.1	10	25	0.94 (0.35 - 2.48)	0.90
intra-abdominal	1	2.2	3	7.5	0.39 (0.04 - 3.77)	0.40
skin/soft tissue	4	9	2	5	2.42(0.43 - 13.52)	0.30
central line	13	14.4	35	33	0.34 (0.17 - 0.7)	0.002
other	21	23.3	15	14.1	1.85 (0.89 - 3.84)	0.10
Polymicrobial infection	15	16.6	16	15	1.13 (0.52 - 2.43)	0.76

SD: standard deviation; **APACHE II:** acute physiology and chronic health evaluation II; **LOS:** length of stay; **BSI:** bloodstream infection; **ICU:** intensive care unit; **OR:** odds ratio; **CI95%:** confidence interval 95%.

TABLE 2 - Etiologic agents and resistance profile of episodes of bloodstream infections caused by **Enterobacteriaceae** *strains.*

	Non-automated period (n = 90)		Automated period (n = 106)		
	n	**%**	**n**	**%**	**p**
Enterobacteriaceae					
Klebsiella spp.	33	36.6	46	43.3	0.34
Proteus spp.	12	13.3	20	18.8	0.30
Providencia spp.	11	12.2	3	2.8	0.01
Serratia spp.	9	10.0	16	15.0	0.30
Enterobacter spp.	12	13.3	16	15.0	0.73
Escherichia coli	12	13.3	6	5.6	0.06
Morganella morgannii	3	3.3	1	0.9	0.24
Citrobacter spp.	2	2.2	1	0.9	0.47
ESBL	28	31.1	45	42.4	0.10
Escherichia coli	3	10.7	3	6.6	0.83
Klebsiella pneumoniae	20	71.4	32	71.1	0.21
Proteus mirabilis	5	17.8	10	22.2	0.31
Resistance profile					
ciprofloxacin	28/78	35.8	60/103	58.2	0.002
ceftriaxone	55/88	62.5	40/55	72.7	0.21
cefepime	45/85	52.9	69/106	65	0.09
imipenem-cilastatin	2/89	2.2	2/106	1.8	0.86
meropenem	0/51	0.0	1/62	1.6	0.30
piperacillin-tazobactam	3/86	3.4	42/105	40.0	0.01

ESBL: extended-spectrum beta-lactamase.

TABLE 3 - Variables associated with clinical outcomes of episodes of bloodstream infection caused by **Enterobacteriaceae** *strains.*

	Non-automated period (n= 90)		Automated period (n= 106)			
Variables	**n**	**%**	**n**	**%**	**OR (CI 95%)**	**p**
Treatment adequacy	72	80.0	80	75.4	1.21 (0.62 - 2.38)	0.45
Antibiotic change	34	37.7	32	30.1	1.4 (0.77 - 2.54)	0.26
Reason						
antimicrobial resistance	18/34	52.9	12/32	37.5	1.88 (0.7 - 5.01)	0.21
lack of clinical improvement	8/34	23.5	10/32	31.2	0.68 (0.23 - 2.01)	0.48
antimicrobial descalation	6/34	17.6	9/32	28.1	0.55 (0.17 - 1.77)	0.31
Clinical cure/improvement	63	70.0	72	67.9	1.1 (0.6 - 2.02)	0.75
14th day mortality	20	22.2	26	24.5	0.97 (0.45 - 2.10)	0.94
28th day mortality	27	30.0	43	40.5	0.53 (0.24 - 1.19)	0.12

OR: odds ratio; **CI95%:** confidence interval 95%.

DISCUSSION

The importance of MIC results has been well documented in studies of methicillin-resistant *Staphylococcus aureus* (MRSA)[6,7]. However, there are few published studies demonstrating the impact of knowledge of MIC results on the outcomes of bloodstream infections caused by *Enterobacteriaceae* strains. Moreover, none of these studies has assessed the effect of the introduction of an automated microbiologic testing method on the clinical outcome of these infections.

We noticed an increase in ciprofloxacin and piperacillin-tazobactam resistance during the automated testing period. These results could be explained by the increased number of ESBL-producing strains, which usually are resistant to fluoroquinolones as well. Accordingly, a non-significant increase in the resistance to third- and fourth-generation cephalosporins was observed among those strains.

We observed higher MIC_{50} and MIC_{90} values to cefepime, ceftazidime and piperacillin-tazobactam among *Klebsiella* spp. strains. The MIC values determined in our study are higher than those described in the SENTRY antimicrobial surveillance program with Brazilian hospitals data[8]. In that study, the MIC_{50} values for cefepime, ceftazidime and piperacillin-tazobactam among *Klebsiella* strains (n=735) were 0.25, ≤ 1 and 4, respectively. Indeed, in our analysis, only imipenem-cilastatin demonstrated reasonable activity against *Klebsiella* strains. It is noteworthy that two cases of carbapenemase-producing *K. pneumoniae* were found among these strains.

The analysis of *Proteus* strains revealed that the MIC_{50} values for ceftazidime and piperacillin-tazobactam were lower than those of *Klebsiella* strains whereas the MIC_{50} values for cefepime and ciprofloxacin were similar. Despite the higher MIC_{50} and MIC_{90} of *Proteus* spp., their susceptibility to imipenem was 100%. These data highlight the high rates of antimicrobial resistance among *Enterobacteriaceae* strains in our setting.

Studies conducted in recent years have demonstrated that the inappropriate use of antibiotics is high[9]. Proper administration of antimicrobial drugs involves the appropriate use of antibiotics based on adequate selection of the dose, duration and route of administration. This strategy would minimize toxicity, costs related to treatment and limit the potential emergence of antibiotic resistant strains[9,11-14].

We found an eighty percent of treatment adequacy in both periods. This favorable adequacy rate is likely due to the use of broad-spectrum antibiotics at the beginning of treatment probably owing to the high levels of antimicrobial resistance rates in our hospital.

Antibiotic changes occurred at the same rate in both periods. These changes were recommended in less than 50% of the episodes by the antimicrobial management team. Indeed, there was no difference in antimicrobial switching rates by the antimicrobial management group after the introduction of the automated testing method (37.7% vs. 30.1%, p=0.26).

We found a non-significant increase in antimicrobial descalation rate (17.6% vs. 28.1%) after the introduction of the automated system. It would be interesting to observe whether this trend will continue in future analyses. Antimicrobial descalation after microbiological results is a key component in reducing antimicrobial resistance rates caused by the selective pressure of broad-spectrum antimicrobial therapy[10,15].

Despite the high treatment adequacy, the 14-day mortality was almost 25% in both periods. However, it is difficult to distinguish the outcomes associated with infection from those related to the severity of illness among those patients[16,17].

Unlike published reports for gram-positive bacteria (e.g. MRSA), the knowledge of MIC results did not have impact on mortality rates in our study[6,7]. Probably, the assistant physician is aware of the high level of antimicrobial resistance in our setting resulting in the prescription of broad-spectrum empirical antimicrobial treatments. Thus, as usually carbapenem antibiotics have been largely used, the knowledge of MIC results would have a lesser impact on outcomes in the automated period.

Our study had several limitations. First, the retrospective nature of the study contributed to loss of data including the lack of some MIC results during the automated testing period. Secondly, we could not analyze the time that the microbiological results became available to the attending physician in both testing periods. This point is very important because one of the major advantages of automated systems is the rapid time around when compared to conventional systems. However, according to information from the microbiology laboratory team this time has been substantially reduced after the automated system introduction. Thirdly, it was difficult to distinguish the mortality associated with the BSI infection from that caused by the severity of illness. Finally, although our study was performed in a large teaching hospital our results may not be applicable to other patient populations.

In summary, our study did not demonstrate impact of the introduction of an automated microbiologic system on clinical outcomes of patients with bloodstream infections caused by *Enterobacteriaceae* strains. Our data draw attention to the high rates of antimicrobial resistance among *Enterobacteriaceae* strains in our institution.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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