MARISTELA PINHEIRO FREIRE

**Aquisição de bactéria gram-negativa
multidroga resistente antes do transplante
de fígado : o impacto no desfecho**

Tese apresentada à Faculdade de Medicina da
Universidade de São Paulo para obtenção do título
de Doutora em Ciências

Programa de Doenças Infecciosas e Parasitárias

Orientador: Prof. Dr. Edson Abdala

São Paulo

2017

Dados Internacionais de Catalogação na Publicação (CIP)

Preparada pela Biblioteca da
Faculdade de Medicina da Universidade de São Paulo

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Freire, Maristela Pinheiro

Aquisição de bactéria gram-negativa multidroga resistente antes do transplante de fígado: o impacto no desfecho / Maristela Pinheiro Freire -- São Paulo, 2017.

Tese (doutorado)--Faculdade de Medicina da Universidade de São Paulo.

Programa de Doenças Infecciosas e Parasitárias.

Orientador: Edson Abdala.

Descritores: 1.Transplante de fígado 2.Bactéria multi-droga resistente
3.Fator de risco 4.Mortalidade 5.Bacilo Gram-negative 6.Resistência a carbapênico

USP/FM/DBD-244/17

MARISTELA PINHEIRO FREIRE

**Multidrug-resistant gram-negative bacteria
acquired before liver transplantation : the
impact on the outcome**

Thesis presented at the Faculdade de Medicina da
Universidade de São Paulo for acquisition of the
title of Philosophiae Doctor (PhD)

Infectious Diseases Program

Advisor: Prof. Dr. Edson Abdala

São Paulo

2017

This thesis is in accordance with the following rules, validity at the moment of the publication.

References: adapted from *International Committee of Medical Journals Editors* (Vancouver).

Universidade de São Paulo. Faculdade de Medicina. Divisão de Biblioteca e Documentação. *Guia de apresentação de dissertações, teses e monografias*.

Authors: Anneliese Carneiro da Cunha, Maria Julia de A. L. Freddi, Maria F. Crestana, Marinalva de Souza Aragão, Suely Campos Cardoso, Valéria Vilhena. 3^a edition. São Paulo: Divisão de Biblioteca e Documentação: 2011.

Abbreviations of periodic titles are in accordance with *List of Journals Indexed in Index Medicus*.

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Abbreviations and Acronyms List

SOT	–	Solid organ transplantation
CMV	–	Cytomegalovirus
EBV	–	Epstein-Barr virus
HAI	–	Healthcare-associated infection
MDR	–	Multidrug-resistant bacteria
LT	–	Liver Transplantation
MRSA	–	Methicillin-resistant <i>Staphylococcus aureus</i>
MELD	–	Model for End-Stage Liver Disease
VRE	–	Vancomycin-resistant enterococci
GNB	–	Gram-negative bacilli
ESBL	–	Extended spectrum beta-lactamase
CRE	–	Carbapenem-resistant <i>Enterobacteriaceae</i>
CRPA	–	Carbapenem-resistant <i>Pseudomonas aeruginosa</i>
CRKP	–	Carbapenem-resistant <i>Klebsiella pneumoniae</i>
CVC	–	Central venous catheter
BHI	–	Brain Heart Infusion
CLSI	–	Clinical Laboratory Standards Institute
PFGE	–	Pulsed-field Gel Electrophoresis

SSI-OE – Organ-space Surgical Site Infection

SSI-I – Incisional surgical site infection

BSI – Bloodstream infection

UTI – Urinary tract infection

RR – Relative risk

CI – Confidence interval

OR – Odds Ratio

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RESUMO

Freire MP. *Aquisição de bactéria gram-negativa multidroga resistente antes do transplante de fígado: o impacto no desfecho* [Tese]. São Paulo: Faculdade de Medicina, Universidade de São Paulo; 2017.

As infecções em pacientes submetidos a transplantes de órgãos sólidos são importante causa de morbidade, além de serem definidoras da sobrevida desta população. A maioria das infecções que ocorre nos dois primeiros meses pós-transplante é relacionada à assistência à saúde (IRAS). O objetivo deste trabalho é identificar fatores de risco para IRAS por bactérias Gram-negativas (BGN) multi-droga resistentes (MDR) em pacientes submetidos a transplante de fígado (TF), nos dois primeiros meses após o transplante. Os objetivos secundários são: identificar fatores de risco para aquisição por MDR GNB em pacientes submetidos a TF, e determinar o impacto das IRAS por MDR GNB na sobrevida desses pacientes. Foram avaliados os TF consecutivos realizados em pacientes adultos no Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HCFMUSP) no período de novembro de 2009 a novembro de 2011. A vigilância microbiológica foi realizada no dia do TF, e semanalmente até a alta hospitalar ou 60 dias após o transplante. Os sítios de coleta foram *swab* de orofaringe ou secreção traqueal, *swab* retal e *swab* axilar. Foram pesquisadas as seguintes bactérias: *A. baumannii*, *P. aeruginosa* e Enterobactérias resistentes a carbapenêmico, e *K. pneumoniae* e *E. coli* produtoras de betalactamase de espectro estendido (ESBL). Posteriormente, as amostras clínicas foram comparadas com as cepas da mesma espécie isoladas em culturas de vigilância por tipagem molecular. A análise de fatores de risco foi realizada por tipo de infecção e espécie de bactéria. Na análise estatística utilizou-se o teste qui-quadrado ou teste exato de Fisher para variáveis dicotômicas, e teste de Mann-Whitney para variáveis ordenáveis. A análise multivariada foi realizada por regressão logística. A análise de sobrevida foi realizada por regressão de Cox. O nível de significância de *P* considerado foi 0,05. Foram realizados, no período, 229 transplantes em 202 pacientes, e analisados 214 transplantes em 195 pacientes. O motivo de indicação do transplante mais frequente foi cirrose pelo vírus C, 33%. Foram identificados no período do estudo 110 pacientes (56,4%) com IRAS pós-TF, e um total de 201 infecções. Em 76,3% dos pacientes com IRAS (84/110) foi isolado MDR GNB em alguma amostra clínica relacionada à infecção. Os dois principais sítios de infecção foram infecção de sítio cirúrgico (32%) e infecção primária de corrente sanguínea (27%). Os dois microrganismos mais frequentemente isolados das IRAS foram *A. baumannii* e *K. pneumoniae*, e a proporção de infecções por cepas resistentes a carbapenêmico foi, respectivamente, 100% e 48,9%. Os fatores de risco para infecções por MDR GNB pós-TF foram: retransplante precoce, volume de concentrados de hemácias transfundidos no intra-operatório da cirurgia do TF, colonização por MDR GNB no pré-transplante, tempo prolongado de internação em UTI e tempo prolongado de

isquemia fria. Cento e cinco pacientes adquiriram algum MDR GNB nos 60 dias pós-TF, e o único fator de risco detectado para aquisição de MDR GNB no pós-TF foi tempo prolongado de sonda vesical de demora. A análise de clonalidade demonstrou que as cepas de MDR identificadas pré-TF eram fortemente relacionadas às cepas isoladas das infecções no pós-TF para *A. baumannii* e *K. pneumoniae* resistente a carbapenêmico. As infecções por MDR GNB apresentaram uma tendência a aumentar o risco de óbito nos 60 primeiros dias pós-TF, mas esta.

Descritores: transplante de fígado; bactéria multi-droga resistente; fator de risco, mortalidade; bacilo Gram-negativo; resistência a carbapenêmico

ABSTRACT

Freire MP. *Multidrug-resistant Gram-negative bacteria acquired before liver transplantation: the impact on the outcome* [Thesis]. São Paulo: "Faculdade de Medicina, Universidade de São Paulo"; 2017.

Bacterial infections among patients submitted to liver transplantation (LT) are an important cause of morbidity and have huge impact on patients' survival. The majority of infections in the first two months after LT are related to healthcare assistance. The aim of this study has been to identify risk factors for healthcare-associated infections (HAI) caused by multidrug-resistant Gram-negative bacteria (MDR GNB) in liver transplant patients in the first two months after LT. The secondary aims have been to identify risk factors for acquisition of MDR BGN among liver transplant patients and analyze the survival rate during the first two months after LT. We analyzed consecutive liver transplantations performed at Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HCFMUSP) from November 2009 to November 2011. Surveillance cultures were performed on a weekly basis, starting on the day of the LT until the hospital discharge or 60 days after the LT. We collected surveillance cultures through swab from oropharynx (or tracheal secretion), axillary and inguinal rectal sites. We surveyed the following bacteria: carbapenem-resistant *A. baumannii*, *P. aeruginosa*, Enterobacteriaceae, ESBL-producing *K. pneumoniae*, and *E. coli*. The strains isolated from surveillance culture were compared to strains isolated from clinical cultures through PFGE. The risk factor analysis was performed for each type of MDR bacterium for risk of colonization and infection. The statistical analysis was carried out for dichotomous variables using chi-square tests or Fisher's exact tests when appropriate; Mann-Whitney tests were used for continuous variable and step-wise logistic regression was used for multivariate analysis. The survival rate analysis was performed using Cox regression. The significant value of P was 0.05. During the study period, 229 liver transplantations were performed in 202 patients and we analyzed 214 LT performed in 195 patients. The main baseline disease that warranted LT was virus C cirrhosis, 33%. 110 (56.4%) patients developed healthcare-associated infections after the LT and a total of 201 infections were identified; 84 (76.3%) patients had MDR GNB isolated from clinical cultures related to HAI. Surgical wounds (31%) and primary bloodstream (27%) were the most prevalent infection sites. The risk factors for HAI by MDR GNB after the LT were: re-transplantation, volume of blood units transfused during the LT surgery, colonization by MDR GNB before the LT, prolonged time of ICU stay, and prolonged time of cold ischemia. 105 patients acquired MDR GNB during the first 60 days after the LT; the only risk factor identified was the prolonged use of urinary drain. The clonal analysis showed that strains isolated in the period before the LT were closely related to strains isolated from clinical culture after the LT for carbapenem-resistant *A. baumannii* e *K. pneumoniae*. The infections by MDR GNB have been shown to increase the risk of death in the first 60 days after LT.

Descriptors: liver transplantation, multidrug-resistant bacteria, risk factor, mortality, Gram-negative bacillus, carbapenem resistance

1 INTRODUCTION

Infections in patients undergoing solid organ transplant (SOT) are an important cause of morbidity and have a decisive role in survival of this population. Those infections may be didactically divided into three periods, in which the incidence of different pathogens varies according to the state of immunosuppression and environmental exposures: ⁽¹⁾

1. First or early period - comprises the first thirty days after the transplantation, when most infections occur. Risk factors are mainly related to the surgical procedure and the postoperative hospital stay. The most common agents are bacteria commonly related to healthcare-associated infection (HAI) and fungi that cause HAI, especially *Candida* species. Herpes simplex virus infections may already occur at this stage.
2. Second period - extends from the second to the sixth month post-transplantation, and the infections are in general related to the immunosuppression state. In this period there can occur opportunistic infections caused by cytomegalovirus (CMV), *Pneumocystis jiroveci* pneumonia and infections by *Aspergillus* species. Besides that, latent infections such as tuberculosis can be reactivated.

3. Third or late post-transplantation period - after six months of transplantation, the majority of the patients are prone to lower levels of immunosuppression. At this stage, infections are usually the same as those acquired in the community by the immunocompetent population. There are, however, some exceptions, such as those patients who develop late acute rejection or chronic rejection or other situations requiring an increase in immunosuppression, which increases the risk of opportunistic infections. Development of chronic infections such as hepatitis B and C and lymphoproliferative diseases associated with Epstein-Barr virus (EBV) can also occur. Currently, some late opportunistic infections such as CMV infection can occur in this period if prophylaxis has been given earlier.

The risk of opportunistic infections is much more dependent on the area under the curve of immunosuppression over time than on the dose of immunosuppressant drug. Thus, in the early period the majority of infections are not caused by opportunistic pathogens but are mainly related to patient care and the surgical procedure, i.e., HAI. ^{(2) (1)}

Most infections in patients undergoing SOT occurred in the early period. In 95% of the cases, the causative agent is a bacterium, and it is estimated that about 60% of the infections are caused by multidrug-resistant bacteria (MDR). ^{(1) (3)}

In this first period, patients undergoing liver transplantation (LT) are particularly susceptible to HAI compared to patients who have been

submitted to other SOT. There are certainly many factors that can contribute to this fact: the complexity of the procedure, prolonged hospital and intensive care stay, immunosuppression caused by baseline diseases, frequency of renal failure, malnutrition and hepatic failure by itself. It is expected that up to 68% of the patients will develop some kind of infection during the first months after LT compared with 47% of kidney transplant and 54% of lung transplant recipients. ^{(1) (2) (4)}

Among bacterial infections after LT, those caused by Gram-positive bacteria have been described as the most frequent ones in many studies. The most commonly isolated agent was *S. aureus*, with an average incidence of 25% and a high proportion of infections caused by methicillin-resistant strains(MRSA). ^{(5) (6)} The prevalence of MRSA varies from 1.3% to 22.7% in the post-LT period. ⁽⁷⁾ Among the patients undergoing LT at the Hospital das Clínicas of the University of São Paulo Medical School (HCFMUSP), MRSA have also been identified as the most frequent pathogens in the period from 2000 to 2005, accounting for 16% of the infections in the first month post-transplant, with 94% of methicillin resistance. ⁽⁸⁾ Risk factors for MRSA infection after LT are well established, and among them, the most important is the previous colonization, both pre- and post-LT. ^{(9) (10)} It is estimated that pre-transplant colonization increases the risk of infection by MRSA in the post-transplant period in more than five times. ⁽⁷⁾ Another risk factor identified in some studies has been the degree of hepatic impairment, measured by MELD (Model for End-Stage Liver Disease) or prothrombin time test. ⁽¹⁰⁾ Screening all patients, decolonization of carriers, and maintenance of straight

contact precautions measures for hospitalized, colonized patients have been shown to reduce the incidence of MRSA infections. Singh et al have shown a drop from 40% to 4% in the rate of MRSA infection with the implementation of this bundle of measures. ⁽¹¹⁾

Enterococcus spp. is a gram-positive bacterium with an increasing prevalence among patients submitted to LT, the incidence ranging from 1 to 16%, and vancomycin resistance in up to 40% depending on the epidemiological resistance profile of the transplant center. The prevalence of vancomycin-resistant *Enterococcus* (VRE) post-transplantation is on average 11.9%, ranging from 6.8% to 18.2%. ⁽⁷⁾ In our service this agent was identified in 11% of cases of the infections in the first month after LT, and vancomycin resistance was detected in 42% of the cases. ⁽¹²⁾ The predisposing factors to *Enterococcus* spp. infections are the prior use of antibiotics, biliary-digestive anastomosis and surgical complications related to the biliary tree. ^{(12) (13) (14)}. Colonization by VRE significantly increases the risk of infection by this pathogen, and it is estimated that the probability of VRE-colonized LT patients developing a VRE infection in the post-LT period is five times higher than that of non-colonized patients. ⁽¹⁴⁾

The prevalence of infections caused by Gram-negative bacilli (GNB) has been increasing in recent years. Singh et al have identified an increase in Gram-negative bacteremia cases, justified in part by the better management of fungal and viral infections surveillance. At our center, Gram-negative infections accounted for 47% of HAI in the first month post-LT. ^{(15) (8) (16)} Other studies have also pointed to GNB as the predominant causative pathogen

among post-LT bacteremic infections. Currently, Gram-negative infections may account for up to 65% of post-LT bacterial infections.⁽¹⁷⁾

In addition to the increase in the prevalence, certain species with reduced susceptibility to many classes of antimicrobial agents are on the increase in this group of bacteria.^{(18) (19) (20)} Singh et al have found that 54% of the Gram-negative isolates in post-LT infections are classified as MDR, and that there has been an increase in extended-spectrum beta-lactamase-producing (ESBL) strains over time. The one-year mortality rate was significantly higher for patients with infections caused by MDR bacteria.⁽²¹⁾

High morbidity and mortality rates of Gram-negative HAI can be associated with this frequent multidrug resistance profile, which increases the probability of errors in empiric treatment, and reduces the range of options available for effective antimicrobial therapy.⁽⁶⁾

Pseudomonas aeruginosa is often isolated after LT and is a frequent etiologic agent of pneumonia.^{(6) (17)} Some reports claim that patients submitted to LT are three times more susceptible to developing *P. aeruginosa* bacteremia than other SOT patients.⁽²²⁾ Among patients submitted to LT at HCFMUSP, *P. aeruginosa* was the fourth most prevalent HAI-causing Gram-negative bacillus, but remained the most common etiologic agent of pneumonia.⁽⁸⁾

Although *P. aeruginosa* is frequently associated with pneumonia, some studies have reported that this bacterium is also commonly isolated from intra-abdominal infections, and biliary intercurrents after LT are risk

factors for infection by that etiologic agent. ⁽²³⁾ ⁽²⁴⁾ Other risk factors described are: the intensive care unit (ICU) stay, re-transplantation and bloodstream infection (BSI) associated with health assistance. ⁽²⁵⁾

Solid Organ Transplantations were previously described as risk factors for infection by carbapenem-resistant *P. aeruginosa* (CRPA). ⁽²⁶⁾ Other risk factors often identified for HAI by CRPA in the general population are: prolonged hospital stay and antibiotic use, common situations found in Liver transplant patients. ⁽²⁷⁾ ⁽²⁸⁾ A Brazilian study has identified this microorganism as the most frequent MDR bacteria in LT. ⁽²⁹⁾ The resistance of *P. aeruginosa* to carbapenem in LT recipients is described in the literature as ranging from 30 to 83%. ⁽²¹⁾ ⁽³⁰⁾ A cohort study of 49 cases of *P. aeruginosa* bacteremia in SOT patients identified the following factors associated with CRPA: re-transplantation, septic shock, nosocomial acquisition. ⁽³¹⁾

Infections by *Acinetobacter baumannii* in LT recipients range widely in their incidence depending on the institution. Torre-Cisneros et al identified *A. baumannii* in 7.6% of BSI. ⁽⁵⁾ Among the patients submitted to LT at HCFMUSP, *A. baumannii* was the second most common Gram-negative agent causative of HAI in the first post-LT period, corresponding to 12% of the isolated microorganisms. ⁽⁸⁾ In that population, the *A. baumannii* was the most frequent pathogen isolated from surgical site infections, and 94% of the strains were resistant to carbapenem. ⁽³²⁾

The lung is the most common site of infection by this agent in SOT patients. However, particularly in LT patients, intra-abdominal infections are the predominant site in most series. ⁽³³⁾ ⁽³⁴⁾ ⁽³⁵⁾

Previously documented risk factors for HAI by *A. baumannii* in the general population are: disease severity, surgical procedures, immunosuppressive drug use, previous antibiotic use and ICU stay.⁽³⁶⁾ Those characteristics are usually identified in patients undergoing LT, and so we expect that in services with a high prevalence of *A. baumannii* infections, the LT population is an important group that develops HAI caused by this agent. Specific risk factors for *A. baumannii* infection in patients submitted to SOT described in the literature are: high pre-LT MELD score, prior use of carbapenem, use of central venous catheters and dialysis. Those studies, however, were not subjected to a multivariate analysis to identify risk factors due to the small number of cases.^{(33) (37)}

Klebsiella pneumoniae is the second most frequently identified Gram-negative after LT in the literature, and the most frequent microorganism identified at HCFMUSP.⁽⁸⁾ Wade and colleagues, in a study of 284 liver transplant patients identified infections by these microorganisms as a risk for increased length of hospital stay.⁽³⁸⁾ Liver transplant recipients are particularly susceptible to infections by Enterobacteriaceae.⁽³⁰⁾ In an outbreak of ESBL-producing *Escherichia coli* (*E. coli*), 67% of the patients were from an LT ward.⁽³⁹⁾ The intestine is the most frequent site of colonization by Enterobacteriaceae and Liver transplant patients have an increased risk of bacterial translocation due to frequent complications of biliary tract and Roux-en-Y hepatojejunostomy anastomosis. Besides that, LT patients are often exposed to antimicrobials, which increase the risk of infections caused by ESBL-producing strains.

Zhou et al identified 55 Enterobacteriaceae infections after LT. 32.4% of them were ESBL-producing *Enterobacter cloacae* strains and 24% of them were ESBL-producing AmpC. ⁽⁴⁰⁾

Infections caused by ESBL-producing Enterobacteriaceae are highly prevalent among SOT recipients, approximately 10 to 50%. The mean incidence is highly variable, and unlike other MDR bacterial infections, infections by ESBL-producing Enterobacteriaceae are also described in later periods after transplantation. ^{(6) (41) (42)} The single factor identified as a risk of infection by ESBL-producing Enterobacteriaceae in transplant patients is the need for post-transplant hemodialysis. ⁽⁶⁾

Currently, Enterobacteriaceae resistance to carbapenem (CRE) is being increasingly reported with a high worldwide prevalence of these agents. ^{(43) (44)} This resistance can occur due to several mechanisms: AmpC hyper-expression or class A ESBL (either TEM or SHV) associated with changes in the membrane (permeability of the outer membrane or hyper-regulation of efflux pumps), or the production of enzymes that hydrolyze carbapenem (carbapenemases). The carbapenemases identified in Enterobacteriaceae can be categorized as metallo- β -lactamases, extended-spectrum β -lactamase oxacillinases or carbapenemases inhibited by clavulanic acid. ⁽¹⁹⁾ Carbapenemases inhibited by clavulanic acid belong to Ambler's Class A, among which KPC are the most frequent enzymes. ⁽¹⁹⁾

The mortality rate associated with CRE infections ranges between 28% and 68%. The introduction of effective therapy usually suffers greater delay, with a consequent more prolonged hospital stay after diagnosis. ^{(44) (45) (46)}

For KPC-producing *K. pneumoniae* (KPKPC), there are reports of an increased risk of death by up to four times, and the risk factors associated with higher mortality rates are high Acute Physiology and Chronic Health Evaluation II (APACHE II) score, inadequate initial antibiotic therapy, age, and septic shock at diagnosis of infection. ^{(44) (47) (48) (46) (49) (50) (51)}

The risk factors identified for CRE infections are an increased number of invasive procedures, immunosuppression, use of cephalosporins, carbapenems, anti-pseudomonas penicillin and fluoroquinolone, high APACHE II score, mechanical ventilation, length of hospital stay, patient's severity rating, ICU stay, transplant of hematopoietic stem cells and SOT. ^{(48) (47) (52) (53) (54)}

Although patients submitted to SOT are at a higher risk for CRE infections, there is scarce data in the literature on this specific population. A KPKPC outbreak report in a hospital in São Paulo city described the evolution of 10 SOT recipients: four kidney transplants, four LT and two heart transplants, with an associated mortality rate of 50%. ⁽⁵⁵⁾

The incidence of KPKPC in patients undergoing LT varies from zero to 25% of the bacterial infections in the first year post transplant. Bloodstream infections and intra-abdominal infections are the two most common types of infection. These infections have a significant impact on the patient's survival, and an associated mortality rate that ranges from 42% to 71%. ^{(55) (56) (57) (58)}

In the Liver Transplant Service of the HCFMUSP, the carbapenem-resistant *K. pneumoniae* infections were more frequent in intra-abdominal

sites, mainly surgical site infections (SSI) with a high incidence of bacteremia, in 46% of the cases. Mortality rate was 27%, and the risk factors identified for carbapenem-resistant *K. pneumoniae* (CRKP) infection were previous colonization and the need for dialysis. In the literature the risk factors identified for infection by CRKP in LT recipients are: mechanical ventilation for more than 48 hours, recurrent hepatitis C virus infection, dialysis, high MELD score, Roux-en-Y biliary anastomosis, biliary fistula, and colonization by CRKP. ^{(59) (60)}

Apparently, infections caused by MDR-GNB in patients undergoing LT are strongly influenced by the frequency and sensitivity profile of the microorganisms found not only in the transplant facilities, but also in all the other hospital areas. Therefore, considering the growing incidence of MDR-GNB, it seems essential that the analysis of risk factors for MDR-GNB infections include active screening of colonization. The role and impact of colonization as a risk for infection by these agents are not yet definitely established in the literature. A better understanding of the epidemiology of infections by MDR-GNB can contribute to the development of more effective control strategies, as well as the most appropriate management of patients with suspected infection by these agents.

2 OBJECTIVES

2.1 Primary objective

The primary objective was to analyze the impact of MDR-GNB acquisition prior to LT on the incidence of healthcare-associated infections by MDR-GNB in the first two months after LT.

2.2 Secondary objectives

- To identify risk factors for healthcare-associated infection by MDR-GNB in the first two months after LT.
- To identify risk factors for MDR-GNB acquisition in the first two months after LT.
- To identify risk factors for acquisition and infection by the following species of MDR-GNB (carbapenem-resistant *P. aeruginosa*, *A. baumannii*, and Enterobacteriaceae) in the first two months after LT.
- To analyze the impact of MDR-GNB infection in the 60-day mortality rate after LT.

3 METHOD

3.1 Study Design

This was a prospective cohort study without intervention.

3.2 Participants

We evaluated consecutive liver transplants performed in LT patients at HCFMUSP from October 2009 to October 2011. The exclusion criterion was death in up to 48 hours after LT.

The liver transplantation service of HCFMUSP has been in activity since 1988. During the study period an average of 100 transplants a year were performed.

Data collection was prospective, using the following sources: the patient's medical record, the records of the LT infectious diseases team, infection control service database and the record of post-LT follow-up.

If the patient were discharged in less than two months after the liver transplantation, the monitoring was conducted in an LT outpatient care setting.

The HAI data and incidence of MDR bacteria were monitored monthly by the hospital's infection control service and surveillance cultures for CRE, VRE and carbapenem-resistant *Acinetobacter baumannii* (CRAB) were collected at admission and on a weekly basis for all patients in the liver transplant ward and ICU.

3.3 Prophylaxis

The surgical prophylaxis used during the study period consisted of a 48-hour administration of ampicillin plus cefotaxime. In the case of patients undergoing LT during the treatment of infections, we used the therapeutic antibiotic for prophylaxis provided its spectrum was similar to or wider than that of the scheme used for surgical prophylaxis. For prophylaxis in LT recipients whose donors were under infection treatment, we used the same antimicrobial as the one received by the donor, or a similar one, provided its spectrum was similar to or wider than that of the scheme used for surgical prophylaxis.

Antifungal prophylaxis was performed for all patients with fulminant hepatitis, dialysis indication, re-transplantation or treatment for acute cellular rejection. Patients with indication for antifungal prophylaxis received amphotericin B for 7 days.

3.4 Immunosuppression

The standard immunosuppression regimen was performed with tacrolimus associated with corticosteroids. The initial dose of tacrolimus was 0.3 mg/kg/day given every 12 hours (approximately 1 mg PO bid) for the first days post-transplant and was adjusted to achieve a blood concentration of 8-12 in the first three months. The induction immunosuppression was performed with 1g of methylprednisolone administered during the anhepatic phase.

3.5 Microbiology

3.5.1 Surveillance Culture

Cultures for identification of colonization by CRE, ESBL-producing Enterobacteriaceae, CRPA and CRAB were performed immediately before transplantation and weekly after LT while the patient was hospitalized. If the patient happened to be re-hospitalized within two months after LT, the collection of cultures was again performed at readmission and on a weekly base.

The materials for surveillance cultures were collected by oropharyngeal swab or tracheal aspirates (if the patient was on mechanical ventilation), and by axillary and inguinal-rectal swab.

The transport of the material was carried out in dry sterile bottles in the case of tracheal aspirates, or culture transport swab (Mueller Hinton) for oropharyngeal, axillary and inguinal-rectal samples.

The first surveillance culture (SC) was collected on the transplantation day. SC samples were collected twice a week, on Mondays and Thursdays, so that the second week SC was collected in an interval that varied from six to nine days from the first collection. The main researcher performed all the SC collections.

3.5.2 Processing of surveillance culture samples

The SC were processed at HCFMUSP's bacteriology and clinical research laboratory (LIM 54).

Surveillance cultures were plated on selective media containing either imipenem (1ml of Brain Heart Infusion broth (BHI) with a 10-µg-imipenem disk) or ceftriaxone (1,8ml of BHI with a 30-µg-Ceftriaxone disk).

For identification, the isolated bacterial colonies were subjected to optical microscopy and biochemical tests. The identification of the colonies was carried out after the analysis of bacterial growth in different culture media, observing its characteristics of size, edge, elevation, color, density, consistency and hemolysis on blood agar media.

The Enterobacteriaceae were identified by IAL medium (modified Rugai) and oxidase and citrate utilization tests.⁽⁶¹⁾ Nonfermenting Gram-negative bacilli were identified through biochemical tests.

Several colonies were used in identification and subsequently subjected to sensitivity tests for confirmation of their phenotype.

Suspected colonies were characterized by a commercial microorganism identification kit (API 20E; BioMérieux).

Disk-diffusion test (Kirby-Bauer method)

a) Preparation of the bacterial suspension and its inoculation on plates

A bacterial suspension at a scale of 0.5 McFarland ($\sim 1.5 \times 10^8$ CFU / ml) was prepared by spectrophotometry, with the optical density of 0.08-0.1 at 625nm. A cotton swab was used to inoculate a homogeneous bacterial suspension on a Petri dish containing Mueller-Hinton agar (diameter 150mm). The procedures followed in this step were performed according to standardized rules for the document M2-A7.⁽⁶²⁾

b) Application of the disks and incubation of the plates

After a period of 15 minutes, the antibiotic disks were applied to the plates. The samples were incubated at 35°C for 16 to 20 hours in the ambient atmosphere. The antibiotic discs were chosen according to the bacteria identified, following the recommendations of the Clinical Laboratory Standards Institute (CLSI).⁽⁶³⁾

c) Reading of the plates

The diameters (mm) of the inhibition halos were measured with the aid of a ruler and compared to the cutoff points proposed by CLSI.⁽⁶³⁾ The isolates were then characterized as either sensitive or resistant.

Confirmation of carbapenem sensitivity for *P. aeruginosa* and *A. baumannii* was checked using the disk-diffusion method (Kirby-Bauer method) and (mm) the inhibition zone diameters were measured with the aid of a ruler and compared to the cutoffs standardized by the CLSI⁽⁶⁴⁾. We used the E-test for confirmation of strains with MIC ≥ 2 mg / ml for carbapenem.

The detection of ESBL was carried out using the disk-diffusion method, Ceftazidime and Cefotaxime for screening, followed by a combination of Disc Test (with or without the addition of clavulanic acid), and E-test strips when necessary (Ceftazidime and Cefotaxime with or without clavulanic acid).

3.5.3 Culture for diagnosis of infection

Cultures for diagnosis of infection were performed as part of the health assistance routine and collected according to indication after evaluation by the LT infectious diseases team.

The identification and sensitivity tests of the isolated microorganisms were performed using the CLSI methodology described above.⁽⁶⁴⁾

The identification and initial susceptibility testing of microorganisms were performed by automated method (Vitek - BioMérieux Marcy l'Etoile, France).

The cultures of clinical samples were initially processed at HCFMUSP's Microbiology Laboratory of the Central Laboratory Division, and then forwarded to the LIM 54 for further investigation.

Cultures of interest for this project that were sent to the LIM 54 were isolated from ESBL-producing *Klebsiella pneumoniae*, ESBL-producing *E. coli*, cephalosporin-resistant *Enterobacter* spp., CRPA, CRAB and CRE. The sources of interest were surgical sites (wound secretion or ascites fluid), pneumonia (tracheal aspirate or bronchoalveolar lavage) and bloodstream (blood culture).

All carbapenem-resistant Enterobacteriaceae strains isolated via surveillance or clinical culture were submitted to polymerase chain reaction (PCR) for the *bla*_{KPC}.

3.5.4 Molecular typing – Pulsed Field Electrophoresis

The tests for molecular typing were performed at the LIM 54. Microorganisms phenotypically identified as CRKP, CRAB and CRPA were analyzed by pulsed-field gel electrophoresis (PFGE).

3.5.4.1 Protocol for molecular characterization by pulsed-field gel electrophoresis (PFGE)

Molecular analysis was performed by electrophoresis of pulsed-field gels, according to the protocols described by Ridley and Kaufmann. ^{(65) (66)}

Preparation of the bacterial suspension

Bacteria were subcultured on 5% sheep-blood agar medium and incubated for 18-24 hours at $35^{\circ}\text{C} \pm 2^{\circ}\text{C}$. Three to five colonies were transferred into tubes containing 3 ml of broth and incubated at $35^{\circ}\text{C} \pm 2^{\circ}\text{C}$, overnight. They were transferred into a previously weighed microtube, and extracted about 2 ml of the broth bacterial growth. The micro centrifuge tubes were centrifuged for 20 minutes at 11,000 rpm. The supernatant was discarded and the sediment was washed three times using 1 ml of sterile saline solution. After the last wash, the supernatant was discarded and the microtube was weighed on an analytical balance. The bacterial mass was calculated and then an appropriate volume of 25 mM EDTA pH 8.0 solution was added to give the bacterial suspension a resulting concentration of $100\mu\text{g}/\mu\text{L}$.

Preparation of agarose blocks

DNA blocks were obtained by mixing 225 μL of TEN buffer solution (100 mM of Tris, pH 7.5; 100 mM of EDTA; 150 mM of NaCl) plus 25 μL of the bacterial suspension with 250 μL of agarose 2% in 0.5X TBE buffer (0.089 M of Tris, 0.089 M of boric acid, 0.002 M of EDTA). This mixture was poured into specific molds and chilled at 4°C for 30 minutes.

Bacterial DNA extraction step

The agarose blocks were removed from the molds and incubated in EC 2ml of buffer (6 mM of Tris, pH7.5; 1 M of NaCl; 0.01 M of EDTA, 0.5% Sarkosyl, 0.2% deoxycholate) for 5 hours at 37°C under gentle agitation. After that time period, the buffer was removed and CHEF TE-buffer (2 mL) (0.1 M of Tris, pH 7.5; 0.1 M of EDTA) was added. The latter buffer was washed and then the blocks were treated with a solution with a final concentration of proteinase K (Invitrogen Life Technologies, Carlsbad, USA) at 1.0 mg / mL in ES buffer (0.4M of EDTA, pH9,3, 1.0% Sarkosyl), overnight at 50°C. After this incubation period, the blocks were then washed five times with 2 mL of CHEF-TE buffer at intervals of one hour under gentle agitation. After the last wash, the blocks were stored at 4°C.

Restriction enzyme step

Before performing the enzymatic treatment, agarose blocks were washed five times with DNS buffer (0.1 M of Tris pH 8.0, 5 mM magnesium chloride) with one-hour intervals between washes. For each species of bacteria isolated a specific restriction enzyme was used.

Gel preparation

The treated wafers were dipped in agarose gel at 1% (TBE 0.5X). Electrophoresis was performed using CHEF-DRII system (Bio-Rad, Richmond, USA).

The patterns of electric current variation (time switch) were established according to the microorganism studied. In the case of *K.*

pneumoniae, race conditions were 5 to 30 seconds (initial and final switch time), 6 V / cm (electric current) and 23 hours of run time. After the electrophoresis, the gel was stained with ethidium bromide solution (1µg/ml) for 40 minutes, bleached in distilled water for 40 minutes and photographed.

Image analysis

The PFGE images were processed and analyzed by Bioumerics software version 7.1 (Applied Maths, Sint-Martens-Latem, Belgium). The images were normalized using standard molecular markers and then the band patterns were compared. The genetic similarity analysis was performed using the Dice coefficient with a band position tolerance of 1.25% and 0.5% optimization. Isolates were separated by similarity and grouped by pair by the arithmetic mean method. Only the biggest bands of 45.5 kb were included in the analysis.

3.6 Definitions

3.6.1 Multidrug-resistant bacteria definition

Multidrug-resistant bacterium was defined considering the following resistance profiles:

- Carbapenem-resistant *P. aeruginosa*
- Carbapenem-resistant *A. baumannii*

- ESBL-producing *E. coli* and *K. pneumoniae*
- Carbapenem-resistant Enterobacteriaceae
- Methicillin-resistant *S. aureus*
- Vancomycin-resistant *Enterococcus*

3.6.2 Diagnostic criteria for bacterial colonization

Colonization was defined as proliferation of microorganisms detected on the patient's surface or internally, without necessarily resulting in detectable immune response, cellular damage or clinical symptoms.

The criteria used to define the patient only colonized by a MDR GNB was the isolation of the agent in one or more clinical specimen samples or surveillance cultures, and the exclusion of any active infection by this agent at the time of collection.

3.6.3 Criteria for diagnosis of healthcare-associated infection

The diagnoses of bacterial and fungal infectious diseases were performed through medical history, physical examination findings and laboratory data, based on the criteria defined by the Centers for Disease Control and Prevention (CDC / NHSN).⁽⁶⁷⁾

Post-LT healthcare-associated infections were those that occurred at least 24 hours after the LT procedure. The cases of superficial fungal infections and esophageal candidiasis were excluded.

The surgical site infections were categorized as superficial incisional, when infection affected the skin and subcutaneous tissue; deep incisional, when infection affected fascial or muscle layers; and organ space infection when the infection involved any part of the anatomy other than the incision that was opened or manipulated during the surgical procedure. ⁽⁶⁷⁾

The primary BSI was positive if patients had a recognized pathogen isolated in one or more blood samples and this microorganism was not associated with infection at another site. The BSI were defined as associated with central venous catheter (CVC) if the patient had been in use of the CVC for more than 2 days before the diagnosis of infection, considering the 1st day the day of the implant. Infections with a single agent in blood culture but with criteria for infection of other sites were defined as secondary BSI. ⁽⁶⁷⁾

3.6.4 Definition of acquisition of multidrug-resistant bacteria

The acquisition of MDR GNB was characterized in the case of patients who had not had either colonization or infection caused by a certain agent in over two years, and the MDR GNB was isolated through clinical culture or SC. during the two-month follow-up period after LT.

3.6.5 Criteria for diagnosis of acute cellular rejection

Acute cellular rejection was defined as graft inflammation caused by genetic mismatch between donor and recipient, affecting primarily interlobular bile duct and vascular endothelium, including portal vein, hepatic venules and occasionally hepatic artery and its branches.⁽⁶⁸⁾

The diagnosis of rejection was histologically determined. The criterion used was based on biopsy findings of at least two of the following: predominantly mononuclear portal infiltrate with lymphocytes in blast phase, neutrophils and frequent eosinophils and inflammation/injury of the bile ducts; subendothelial inflammation of the portal vein or hepatic venule terminals.⁽⁶⁸⁾ The classification used the Banff criteria.⁽⁶⁸⁾

3.6.6 Criteria for severity of liver failure

MELD was used as a prognostic index for the assessment of liver failure. The MELD was calculated immediately before the LT. The MELD is a score that was initially used for patients with cirrhosis undergoing transjugular intrahepatic portosystemic shunt, and then validated to assess survival in patients with end-stage liver disease. The formula for the calculation uses the total bilirubin levels, serum creatinine and International Normalized Ratio (INR).⁽⁶⁹⁾

3.7 Statistical treatment

3.7.1 Performance of surveillance culture

For each SC collection site, the positivity rate was calculated as follows: total number of positive SC tests for a given MDR GNB at a specific collection site divided by the total number of positive SC tests for that MDR GNB. For all patients, cultures were reviewed from three months before LT to three months after LT.

The sensitivity rates was calculated following the formula: number of patients with a positive SC test for a specific MDR GNB divided by the total number of patients with a specific MDR GNB isolated in a clinical or Surveillance Culture test.

3.7.2 Outcome variables

The outcome variables analyzed were:

- HAI by MDR in the first 60 days after LT;
- HAI by CRPA in the first 60 days after LT;
- HAI by CRAB in the first 60 days after LT;
- HAI by CRKP in the first 60 days after LT;
- Acquisition of MDR GNB in the first 60 days after LT;

- Acquisition of CRPA in the first 60 days after LT;
- Acquisition of CRAB in the first 60 days after LT;
- Acquisition of CRKP in the first 60 days after LT;
- Death in the first 60 days after LT.

3.7.3 Variables analyzed

The receptor-related independent variables were:

- Continuous: Length of pre-transplant hospital stay in days, age in years, pre-transplant functional MELD score, duration of central venous catheter use in days, duration of urinary catheter use in days, duration of mechanical ventilation use in days, duration of intra-abdominal drain use in days, days of parenteral nutrition, use of antimicrobial in the three months from pre-LT to the outcome or 60 days after LT for non-cases.
- Categorical: acute cellular rejection, use of Pulse corticosteroid therapy for treatment of rejection, infection within 10 days from previous LT, post-transplant bacterial infection, acquisition of MDR GNB after the transplant, acquisition of MDR GNB before LT, post-transplant fungal infection, use of therapeutic antibiotics in the three months prior to the LT, performing surgical procedures after LT, re-transplantation less than two months from the first transplant, performing of hemodialysis, type of immunosuppression used after

transplantation - defined as standard scheme, the use of two immunosuppressant drugs (tacrolimus and corticosteroids), fulminant hepatitis as indication of LT, the occurrence of CMV infection, biliary complications, and American Society Anesthesiology score (ASA).

- The variables related to transplant were:
- Continuous: duration of transplant surgery in minutes, cold and total ischemia time in minutes, numbers of blood cell units transfused during the LT.
- Categorical: Senior Surgeon (main surgeon who performed the transplant with over eight years' experience), type of antibiotic prophylaxis used in LT (standard prophylaxis was defined as ampicillin and cefotaxime for elective surgery and clindamycin, cefotaxime and amphotericin B for fulminant hepatitis), type of biliary anastomosis (defined as standard choledocho-choledochostomy).

Patients who had MDR GNB identified before LT were excluded from the analysis of risk of acquiring MDR GNB. Patients who underwent LT while under treatment for MDR GNB infection were excluded from the analysis of risk factor for post-LT MDR GNB infection.

Variables related to exposure time

For patients with positive outcome, we considered the exposure time up to the outcome. For patients with a negative outcome, we considered the total exposure time during the first 60 days after LT. For each outcome the exposure time was calculated separately.

The variables were ordered after the initial analysis, and all of them were converted into categorical. The cutoff point adopted was the median of the variable of the cases considered, except in cases of surgical time and cold ischemia time, where the cutoff was the 75th percentile of the total samples. For the multivariate analysis, the choice between the continuous and categorical variable of the same information was that with the less value of *P*.

3.7.4 Statistical analysis

Univariate analysis was performed using the chi-square test or Fisher's exact test for categorical variables when indicated, and the Mann-Whitney test for continuous variables, considering a significance level of $P = .05$. For the multivariate analysis we used stepwise logistic regression. Variables that reached a significance level of less than or equal to 0.2 in the univariate analysis were included in the model, following the ascending order of the *P* value. Variables were sustained in the model if they reached a $P < 0.05$ or reduced the log-likelihood ratio by -2. The mortality rate analysis and survival plots were performed using Cox regression. The following

variables were treated as time-depended variables: age, post-LT dialysis, acute cellular rejection, HAI, HAI by MDR and CMV infection.

The inclusion criterion in the mortality rate multivariate analysis was *P* value lower or equal to 0.2 in the univariate analysis. The inclusion of mortality in the multivariate analysis model was also by Stepwise. The statistical software used for performing the analysis was SPSS 17.0.

4 RESULTS

4.1 Characterization of the population

During the study period, 229 transplants were performed in 202 patients. Seven patients were excluded due to death in less than 48 hours. A total of 214 LT in 195 patients were evaluated.

The most common baseline disease that motivated LT was cirrhosis due to hepatitis C virus, in 33% of the cases. (Table 1)

Demographic and clinical characteristics of the 195 patients evaluated are shown in Table 2.

Table 1 – Baseline diseases of 195 patients submitted to liver transplantation

Baseline diseases	N	Proportion
Cirrhosis due to hepatitis C virus	51	26%
Alcoholic cirrhosis	26	13%
Cryptogenic cirrhosis	21	11%
Fulminant hepatitis	16	8%
Alcoholic and hepatitis C virus cirrhosis	13	7%
Cirrhosis due to hepatitis B virus	13	6%
Autoimmune hepatitis	6	3%
Primary biliary cirrhosis	5	3%
Hemochromatosis	5	3%
Budd-Chiari syndrome	4	2%
Non-alcoholic steatohepatitis	4	2%
Familial paramyloidosis	4	2%
Primary sclerosing cholangitis	3	2%
Ductopenic diseases	3	2%
Wilson diseases	3	2%
Biliary atresia	2	1%
Cholestatic syndrome	2	1%
Alfa-1-antitrypsin deficiency	2	1%
Schistosomiasis	2	1%
Intra-hepatic lithiasis	1	1%
Caroli's disease	1	1%
Graft dysfunction	1	1%
Glycogenosis type ii	1	1%
Cirrhosis due to C and B virus	1	1%
Hemangioendothelioma	1	1%
Thrombosis of hepatic artery	1	1%
Alcoholic and hepatitis B virus cirrhosis	1	1%
Alagille syndrome	1	1%
Secondary biliary cirrhosis	1	1%

Table 2 – Demographic and clinical characteristics of 195 patients submitted to liver transplantation

Characteristic	Proportion (N)	Median (min-max)
Gender (Female)	35% (69)	-
Live donor	12% (25)	-
Age (years)	-	49 (16-73)
Length of hospital stay (days)	-	32 (3-146)
Hepatocellular carcinoma	31% (61)	-
Early re-transplantation	13% (26)	-
Liver-kidney transplant	6% (12)	-
Reoperation after LT	32% (62)	-
Length of hospital stay before LT (days)	-	6 (0-67)
ASA score >3	54% (97)	-
Surgery duration (minutes)	-	488 (210-1330)
Choledocho-choledochostomy	94%(184)	-
MELD score	-	23 (6-53)
Total ischemia time (minutes)	-	428 (65-960)
Cold ischemia time (minutes)	-	397 (20-910)
Nº of blood units transfused in LT procedure	-	2 (0-15)
Serum creatinine on the day of LT (mg/dl)	-	1,1 (0.51-6.79)
Post-LT dialysis	58% (110)	-
Immunosuppression therapy with 3 drugs	45% (87)	-
Cytomegalovirus infection	23% (44)	-
Bacterial infection 10 days before LT	25% (49)	-
Acute rejection treatment *	24% (43)	-
Length of stay in ICU (days)	-	16 (1-74)
Colonization or infection by Vancomycin-resistant <i>Enterococcus</i> spp.	47% (92)	

*Standard treatment rejection was done with pulsed methylprednisolone therapy

LT – liver transplantation; ASA – American society of anesthesiology; MELD – model for end-stage liver disease, ICU- intensive care unit.

Forty-nine (25%) patients were diagnosed with bacterial infection 10 days before LT. Table 3 describes the agents and sites of infection of those infections. One patient had two different infections in less than 10 days before transplantation. The average number of days between infection and

the transplant was four days. Thirty patients (15.4%) had a diagnosis of infection within 48 hours before the transplantation.

Table 3 – Sites, microorganisms and sensitivity profile of 50 infections identified in up to ten days before liver transplantation

Site of infection	N (%)	N° Infections with causative agent identified	Microorganism	N°	N MDR (%)	Bacteremia
Intra-abdominal infection	21 (43%)	18 (86%)	<i>K. pneumoniae</i>	6	3 (50%)*	3 (50%)
			<i>Enterococcus</i> spp.	7	4 (57%)	3 (43%)
			<i>E. cloacae</i>	4	0	2 (50%)
			<i>E. coli</i>	3	1 (33%)**	2 (67%)
			<i>S. aureus</i>	3	3 (100%)	2 (67%)
			<i>C. tropicalis</i>	3	0	2 (67%)
			<i>Streptococcus</i> spp.	2	0	1 (50%)
			<i>P. aeruginosa</i>	2	0	1 (50%)
			Gram-negative bacillus	1	0	0
			<i>P. mirabilis</i>	1	0	0
			<i>A. baumannii</i>	1	1 (100%)	1 (100%)
			<i>C. freundii</i>	1	0%	0
Bloodstream Infection	20 (41%)	20 (100%)	<i>S. aureus</i>	4	3 (75%)	4 (100%)
			<i>Enterococcus</i> spp.	3	0%	3 (100%)
			<i>A. baumannii</i>	3	3 (100%)	3 (100%)
			<i>K. pneumoniae</i>	3	2 (67%)***	3 (100%)
			<i>E. coli</i>	2	0	2 (100%)
			SCN	2	0	2 (100%)
			<i>C. albicans</i>	2	0	2 (100%)
			<i>E. cloacae</i>	2	0	2 (100%)
			<i>C. glabrata</i>	1	0	1 (100%)
			<i>E. aerogenes</i>	1	0	1 (100%)
Pneumonia/Bronchitis	4 (8%)	4 (100%)	<i>K. pneumoniae</i>	1	1 (100%)***	0
			<i>A. baumannii</i>	1	1 (100%)	1 (100%)
			<i>E. aerogenes</i>	1	1 (100%)	1 (100%)
Urinary tract infection	4 (8%)	4 (100%)	<i>P. mirabilis</i>	1	0%	0%
			<i>S. aureus</i>	1	1 (100%)	0%
			<i>E. coli</i>	1	0%	0%
			<i>E. faecalis</i>	1	1 (100%)	0%
Soft tissues	1 (2%)	Zero				

* 1 strain resistant to carbapenem and 2 ESBL- producing strains

**resistant to carbapenem

***ESBL producer

CNS – Coagulase-negative *Staphylococcus* ; MDR – Multidrug-resistant bacteria

4.2 Surveillance cultures

A total of 4110 samples of SC were collected from three sites (inguinal-rectal, oropharyngeal and axillary). Fifteen patients had no samples collected by the protocol, but were not excluded because all had had at least one SC performed for *Enterobacteriaceae* and CRAB during hospital stay, which was processed in the routine microbiology laboratory. On average, patients had SC samples every three weeks, the period ranging between one and 14 weeks.

The site with the highest SC positivity rate for all MDR GNB was inguinal-rectal, followed by the oropharynx. (Table 4)

The MDR-GNB SC with the lowest positivity rate was CRAB, with 19% of patients with CRAB having negative SC; among patients with SC and positive clinical cultures, the positivity rate of SC was higher for carbapenem-resistant *Enterobacteriaceae* and lower for ESBL-producing *Enterobacteriaceae*. (Table 5)

Table 4 – Positivity rate of surveillance culture by site of harvest from the different Multidrug-resistant Gram-negative bacteria in 180 patients submitted to liver transplantation

MDR GNB	Total number of patients with MDR GNB	Sensitivity rate of inguinal-rectal site	Sensitivity rate of oropharyngeal site	Sensitivity rate of axillary site	non-identification rate if SC was collected only from rectal site
<i>Carbapenem resistant A. baumannii</i>	105	53.3%	47.6%	27.6%	35.3%
<i>Carbapenem resistant P. aeruginosa</i>	30	80.8%	57.7%	26.9%	30.7%
<i>Carbapenem resistant K. pneumoniae</i>	79	92.4%	53.0%	34.8%	7.6%
Others carbapenem resistant Enterobacteriaceae	38	80.6%	52.8%	41.7%	19.4%
Enterobacteriaceae ESBL-producing	78	84.0%	36.0%	17.3%	16.0%

MDR GNB – Multidrug-resistant Gram-negative bacteria; SC – surveillance culture; ESBL – extended spectrum betalactamases.

Table 5 – Performance of surveillance culture of different MDR GNB in 180 patients submitted to liver transplantation

MDR GNB		Total number of patients with MDR GNB	Total number of patients with positive SC	Positivity rate of SC among patients with positive MDR GNB clinical culture	Proportion of patients in whom SC was the first positive culture for MDR GNB
<i>Carbapenem-resistant baumannii</i>	A.	105	85 (81.0%)	54 (74.0%)	59 (56.2%)
<i>Carbapenem-resistant aeruginosa</i>	P.	30	26 (86.7%)	7 (66.7%)	26 (86.7%)
<i>Carbapenem-resistant pneumoniae</i>	K.	79	73 (92.4%)	20 (76.9%)	70 (88.6%)
Other carbapenem-resistant Enterobacteriaceae		38	36 (94.7%)	6 (85.7%)	5 (71.4%)
ESBL-producing Enterobacteriaceae		78	68 (87.2%)	11 (52.3%)	68 (87.2%)

MDR GNB – Multidrug-resistant Gram-negative bacteria; SC – surveillance culture; ESBL – extended spectrum betalactamases.

4.3 Identification of KPC-producing Enterobacteriaceae

Carbapenem-Resistant *Klebsiella Pneumoniae* (CRKP) was identified in 146 samples of SC isolated from 57 patients. The *bla_{KPC}* gene was positive in 19 (33.3%) of the patients tested. The positivity of the KPC gene among patients with CRKP identified in the 1st, 2nd, 3rd and from the 4th week was respectively 34.8% (8/23), 26.7% (4/15), 26.7% (4/15) and 75.0% (3/4). A tendency to increase the proportion of strains with *bla_{KPC}* among CRKP during the study period (Table 6) was observed.

Other non-*K. pneumoniae* CRE were identified in SC from 42 patients. The search *bla_{KPC}* gene was positive in 7 (16.7%) of the tested patients. The distribution of positive *bla_{KPC}* strains was uniform during the study period. (Table 6)

Among clinical samples, *bla_{KPC}* gene was isolated in nine patients with positive culture by CRKP. Among patients with clinical cultures for other carbapenem-resistant Enterobacteriaceae species, *bla_{KPC}* gene was not identified in any sample.

Table 6 – Distribution of *bla_{KPC}* gene positivity rate among strains of carbapenem-resistant Enterobacteriaceae isolated from surveillance culture

	2º sem 2009	1º sem 2010	2º sem 2010	1º sem 2011	2º sem 2011	P
<i>K. pneumoniae</i>	0% (0/8)	20.8% (11/53)	18.2% (2/11)	20.0% (2/10)	100% (4/4)	0.02
Other Enterobacteriaceae	0% (0/5)	45.5% (5/11)	6.3% (1/16)	9.1%(1/11)	0% (0/0)	0.28
• <i>E. cloacae</i>	0% (0/1)	50.0% (1/2)	0% (0/5)	33% (1/3)		
• <i>E. aerogenes</i>	0% (0/3)	0% (0/4)	0% (0/6)	0% (0/4)		
• <i>Enterobacter</i> spp	0% (0/1)	75.0% (3/4)	0% (0/4)	0% (0/4)		
• <i>E. coli</i>		100% (1/1)	100% (1/1)			

4.4 Analysis of risk factors for multidrug-resistant Gram-negative bacteria acquisition after liver transplantation

Among 195 patients included in this study, 90 of them had some MDR GNB identified in the pre-LT period, therefore the risk factor analysis for MDR GNB acquisition after LT was performed in 105 patients. (Tables 7, 8 and 9) After LT, 42 of the 105 (40.0%) patients acquired at least one MDR GNB.

Table 7 – Univariate analysis of risk factors for acquisition of MDR GNB after LT in 105 patients – categorical variables

Variable	Patients with MDR GNB after LT (N=63) N (%)	Patients without MDR GNB after LT (N=42) N (%)	P	RR (CI95%)
Pre-LT characteristics				
Bacterial infection before LT	13 (20.6%)	5 (11.9%)	0.25	1.11 (0.94-1.31)
Gender (F)	30 (69.8%)	13 (30.3%)	0.09	0.65 (0.39-1.09)
Hepatocellular carcinoma	15 (23.8%)	18 (42.9%)	0.04	0.75 (0.56-1.01)
Fulminant hepatitis	9 (14.3%)	1 (2.4%)	0.05	1.14 (1.02-1.27)
ASA score	-	-	0.79	-
LT surgical procedure characteristics				
Combined liver-kidney transplant	4 (6.3%)	2 (4.2%)	1.00	1.02 (0.93-1.12)
Living-donor transplant	9 (14.3%)	9 (21.4%)	0.34	0.92 (0.76-1.11)
Senior surgeon	32 (50.8%)	21 (50.0%)	0.94	1.02 (0.69-1.51)
Duct-to-duct biliary anastomosis	60 (95.2%)	39 (92.9%)	0.68	1.50 (0.32-7.08)
Post-LT Evolution				
Standard immunosuppressant therapy	29 (46.0%)	26 (61.9%)	0.11	0.71 (0.45-1.11)
Re-transplantation	10 (15.9%)	2 (4.8%)	0.12	1.13 (1.00-1.28)
Re-operation	11 (17.5%)	5 (11.9%)	0.58	1.07 (0.91-1.25)
CMV infection	3 (4.8%)	4 (9.5%)	0.43	0.95 (0.85-1.06)
Vancomycin-resistant <i>Enterococcus</i> colonization	30 (47.6%)	7 (16.7%)	0.01	1.59 (1.21-2.09)
Acute cellular rejection	9 (14.3%)	5 (11.9%)	0.78	1.03 (0.89-1.19)
Post-LT dialysis	28 (44.4%)	12 (28.6%)	0.10	1.29 (0.96-1.72)

MDR GNB – Multidrug-resistant Gram-negative bacteria; LT – liver transplantation; CMV - cytomegalovirus

Table 8 – Univariate analysis for risk factors for acquisition of MDR GNB after LT in 105 patients – continuous variables

Variable	Patients with MDR GNB after LT (N=63) Median (min-max)	Patient without MDR GNB after LT (N=42) Median (min-max)	P
Pre-LT characteristics			
Pre-LT length of hospital stay (days)	1 (0-22)	1 (0-46)	0.42
Age (years)	49 (16-70)	53 (21-73)	0.31
MELD score	23 (6-53)	17 (6-34)	0.03
Pre-LT serum creatinine (mg/dl)	1.15 (0.10-10.54)	0.94 (0.41-6.49)	0.2
ASA score			
LT surgical procedure characteristics			
Cold ischemia time (minutes)	400 (20-960)	389 (62-754)	0.42
Total ischemia time (minutes)	428 (81-910)	415 (100-730)	0.40
Duration of LT surgery (minutes)	460 (245-920)	492 (275-1330)	0.72
Units of blood transfused during LT surgery	2 (0-10)	0 (0-9)	0.02
Post-LT Evolution			
Carbapenem use (days)	0 (0-32)	0 (0-30)	0.08
Quinolone use (days)	0 (0-4)	0 (0-0)	0.30
Polymyxin use (days)	0 (0-8)	0 (0-4)	0.12
Cephalosporin use (days)	2 (0-20)	2 (0-7)	0.67
Piperacillin/Tazobactam use (days)	0 (0-12)	0 (0-21)	0.98
Penicillin use (in days)	1 (0-9)	2 (0-6)	0.61
Length of dialysis after LT (days)	0 (0-24)	0 (0-50)	0.06
Length of central venous catheter (days)	6 (0-31)	4 (1-50)	0.02
Length of mechanical ventilator (days)	1 (0-17)	0 (0-9)	0.05
Length of urinary catheter (days)	6 (0-19)	4 (1-13)	0.01
Length of abdominal drain (days)	7 (0-23)	6 (0-21)	0.78
Length of ICU stay (days)	7(1-25)	4 (2-14)	0.007

MDR GNB – Multidrug-resistant Gram-negative bacteria; LT – liver transplantation; MELD – model of end-stage liver disease, ICU – intensive care unit; ASA - American Society of Anesthesiologists (ASA) Physical Status classification.

Table 9 – Multivariate analysis of risk factors for acquisition of MDR GNB after LT in 105 patients

Variable	P	OR	CI95%
Fulminant hepatitis	0.11	5.74	0.67-49.28
Length of urinary catheter (days)	0.02	1.16	1.03-1.32

4.4.1 Analysis of risk factors for carbapenem-resistant *K. pneumoniae* acquisition after liver transplantation

CRKP acquisition was identified in 25 patients before LT, therefore those patients were excluded from this analysis. A total of 54 patients acquired CRKP after LT. The analysis of risk factors for CRKP acquisition is shown in tables 10, 11 and 12.

Table 10 – Univariate analysis of risk factors for acquisition of carbapenem-resistant *K. pneumoniae* after LT in 172 patients – categorical variables

Variable	Patients with carbapenem-resistant <i>K. pneumoniae</i> after LT (N=54) N (%)	Patients without carbapenem-resistant <i>K. pneumoniae</i> after LT (N=118) N(%)	P	RR (CI95%)
Pre-LT characteristics				
Bacterial infection before LT	15 (27.8%)	33 (28.4%)	0.93	0.97 (0.80-1.19)
Gender (F)	34 (61.5%)	76 (66.1%)	0.57	0.88 (0.58-1.35)
Hepatocellular carcinoma	14 (25.9%)	38 (32.8%)	0.29	0.89 (0.73-1.09)
Fulminant hepatitis	4 (7.4%)	12 (10.3%)	0.78	0.97 (0.88-1.08)
ASA score	-	-	0.54	-
LT surgical procedure characteristics				
Combined liver-kidney transplant	3 (5.6%)	6 (5.2%)	>0.99	1.01 (0.93-1.09)
Living-donor transplant	7 (13.0%)	16 (13.8%)	0.99	1.00 (0.89-1.14)
Senior surgeon	22 (40.7%)	61 (52.6%)	0.26	0.84 (0.62-1.13)
Duct-to-duct biliary anastomosis	51 (94.4%)	110 (94.8%)	1.00	0.88 (0.23-3.39)
Post-LT evolution				
Standard immunosuppressant therapy	24 (44.4%)	64 (55.2%)	0.19	0.76 (0.56-1.89)
Reoperation	16 (30.2%)	24 (20.7%)	0.12	1.16 (0.94-1.43)
Re-transplantation	5 (9.4%)	11 (9.5%)	0.78	0.98 (0.88-1.08)
Dialysis	32 (59.3%)	52 (44.8%)	0.08	1.65 (1.06-2.58)
Vancomycin-resistant <i>Enterococcus</i> colonization	28 (53.8%)	53 (44.9%)	0.28	1.19 (0.85-1.67)
Acute cellular rejection	6 (11.1%)	23 (19.8%)	0.20	0.91 (0.80-1.04)
CMV infection	5 (9.3%)	15 (13.0%)	0.48	0.97 (0.86-1.08)

LT – liver transplantation; ASA - American Society of Anesthesiologists (ASA) Physical Status classification; CMV - cytomegalovirus

Table 11 – Univariate analysis of risk factors for acquisition of carbapenem-resistant *K. pneumoniae* after LT in 172 patients – continuous variables

Variable	Patients with carbapenem-resistant <i>K. pneumoniae</i> after LT (N=52)	Patients without carbapenem-resistant <i>K. pneumoniae</i> after LT (N=118)	P
Pre-LT characteristics			
Age (years)	51 (17-70)	53 (16-73)	0.35
Length of hospital stay before LT (days)	1 (0-46)	1 (0-56)	0.69
Meld score	23 (6-53)	21 (6-52)	0.78
Pre-LT serum creatinine (mg/dl)	1.20 (0.51-6.57)	1.09 (0.40-10.54)	0.49
LT surgical procedure characteristics			
Cold ischemia time (minutes)	440 (47-695)	384 (20-960)	0.07
Total ischemia time (minutes)	462 (81-740)	413 (65-910)	0.07
Duration of LT surgery (minutes)	435 (245-910)	488 (210-1330)	0.03
Units of blood transfused during LT surgery	2 (0-10)	2 (0-15)	0.72
Post-LT evolution			
Quinolone use (days)	Zero	0 (0-6)	0.20
Piperacillin/Tazobactam use (days)	0 (0-21)	0 (0-33)	0.61
Cephalosporin use (days)	2 (0-20)	2 (0-21)	0.14
Penicillin use (days)	2 (0-9)	1 (0-21)	0.83
Polymyxin use (days)	0 (0-26)	0 (0-69)	0.47
Carbapenem use (days)	0 (0-40)	1 (0-64)	0.42
Length of mechanical ventilator (days)	1 (0-60)	1 (0-78)	0.68
Length of abdominal drain (days)	8 (0-26)	7 (0-49)	0.43
Length of central venous catheter (days)	9 (2-73)	6 (1-96)	0.20
Length of urinary catheter (days)	9 (0-66)	6 (1-89)	0.26
Length of dialysis after LT (days)	4 (0-58)	0 (0-91)	0.17
Length of ICU stay (days)	9 (1-72)	6 (1-71)	0.27

LT – liver transplantation; MELD – model for end-stage liver disease, CMV – cytomegalovirus

Table 12 – Multivariate analysis of risk factors for acquisition of carbapenem-resistant *K. pneumoniae* after LT in 172 patients

Variable	P	OR	CI (95%)
Dialysis after LT	0.02	2.33	1.17-4.64

4.4.2 Analysis of risk factors analysis of carbapenem-resistant *A. baumannii* acquisition after liver transplantation

CRAB was identified in 24 (12.3%) patients before LT. Among those, four developed infection in the pre-LT period: two primary BSIs, one pneumonia and one cholangitis. Two patients were submitted to LT with active infection by CRAB.

The risk factors analysis for post-LT acquisition of CRAB was performed on 171 patients.

Among the patients with CRAB, seven presented strains with reduced MIC for polymyxin.

Tables 13, 14 and 15 show the risk factor analysis for CRAB acquisition after LT.

Table 13 – Univariate analysis of risk factors for acquisition of carbapenem-resistant *A. baumannii* after LT in 171 patients – categorical variables

Variable	Patients with carbapenem-resistant <i>A. baumannii</i> after LT (N=81) N (%)	Patients without carbapenem-resistant <i>A. baumannii</i> after LT (N=90) N (%)	P	RR (CI95%)
Pre-LT characteristics				
Bacterial infection before LT	24 (30.0)	22 (24.2)	0.39	1.08 (0.91-1.32)
Gender (F)	50 (62.5)	61 (67.0)	0.53	0.89 (0.59-1.34)
Hepatocellular carcinoma	17 (21.3)	36 (39.6)	0.01	0.77 (0.63-0.94)
Fulminant hepatitis	11 (13.8)	2 (2.2)	0.004	1.15 (1.04-1.26)
ASA score	-	-	0.67	
LT surgical procedure characteristics				
Combined liver-kidney transplant	11 (13.8)	13 (14.3)	0.92	0.99 (0.88-1.12)
Living-donor transplant	4 (5.0)	5 (5.5)	>0.99	0.99 (0.93-1.07)
Senior surgeon	45 (56.3)	42 (46.2)	0.19	1.23 (0.87-1.65)
Duct-to-duct biliary anastomosis	77 (96.3)	86 (94.5)	0.73	0.98 (0.92-1.05)
Post-LT evolution				
Standard immunosuppressant therapy	39 (48.8)	52 (57.1)	0.27	0.84 (0.61-1.15)
Reoperation	17 (20.9)	19 (21.3)	0.95	1.05 (0.86-1.17)
Retransplantation	13 (16.3)	1 (1.1)	<0.001	1.19 (1.08-1.31)
Dialysis	47 (58.0)	34 (37.4)	0.005	1.52 (1.13-2.05)
Vancomycin-resistant <i>Enterococcus</i> colonization	9 (11.1)	17 (18.7)	0.18	0.92 (0.81-1.04)
Acute cellular rejection	44 (55.0)	30 (33.3)	0.004	2.44 (1.31 5.55)-
CMV infection	6 (7.4)	14 (15.4)	0.11	0.92 (0.82-1.02)

LT – liver transplantation; ASA - American Society of Anesthesiologists (ASA) Physical Status classification; CMV - cytomegalovirus

Table 14 – Univariate analysis of risk factors for acquisition of carbapenem-resistant *A. baumannii* after LT in 171 patients – continuous variables

Variable	Patients with carbapenem-resistant <i>A. baumannii</i> after LT (N=81) Median (max-min)	Patients without carbapenem-resistant <i>A. baumannii</i> after LT (N=90) Median (max-min)	P
Pre-LT characteristics			
Pre-LT length of hospital stay (days)	1.0 (0-42)	1.0 (1.0-46.0)	0.24
Age (years)	50.0(16-70)	53.5 (18.0-73.0)	0.29
MELD score	26.0(6-51)	19.0 (6.0-5.0)	0.02
Pre-LT serum creatinine (mg/dl)	1.05 (0.51-8.43)	0.84 (0.55-6.57)	0.26
LT surgical procedure characteristics			
Cold ischemic time (minutes)	445.0 (20-910)	390.0 (28-754)	0.27
Total ischemic time (minutes)	461.0 (70-960)	420 (65-768)	0.23
Duration of LT surgery (minutes)	465.0 (210-920)	465.0 (245-1330)	0.48
Units of blood transfused during LT surgery	2.0 (0-15)	0.5 (0-9)	0.006
Post-LT Evolution			
Carbapenem use (days)	2.5 (0-32)	0.0 (0-68)	<0.001
Quinolone use (days)	0.0 (0-5)	0.0 (0-0)	>0.99
Polymyxin use (days)	0.0 (0-15)	0.0 (0-26)	<0.001
Cephalosporin use (days)	2.00 (0-13)	0.0 (0-20)	0.98
Piperacillin/Tazobactam use (days)	0.0 (0-16)	0.0 (0-33)	0.005
Penicillin use (days)	1.00 (0-6)	2.0 (0-16)	0.21
Length of dialysis after LT (days)	2.00 (0-58)	.0.0 (0-52)	0.006
Length of central venous catheter (days)	8.0 (1.0-68.0)	4.0 (1.0-54.0)	0.01
Length of mechanical ventilator (days)	2.00 (0-25)	0.0 (0-29)	0.007
Length of urinary catheter (days)	7.0 (1.0-28.0)	5.0 (1.0-47.0)	0.08
Length of abdominal drain (days)	7.0 (0.0-83.0)	6.0 (0.0-49.0)	0.35
Length of ICU stay (days)	7.0 (1.0-28.0)	5.0 (2.0-44.0)	0.02

LT – liver transplantation; ICU – intensive care unit; MELD – model for end-stage liver disease

Table 15 – Multivariate analysis of risk factors for acquisition of carbapenem-resistant *A. baumannii* after LT in 171 patients

Variable	P	OR	CI (95%)
Hepatocellular carcinoma	0.05	0.46	0.22-0.98
Units of blood transfused during LT surgery	0.06	1.17	0.99-1.37
Re-transplantation	0.01	10.09	1.22-83.10
Previous use of carbapenem	0.001	3.31	1.66-6.60
Previous use of piperacillin / tazobactam	0.02	2.19	1.11-4.32

4.4.3 Analysis of risk factors analysis of carbapenem-resistant *P. aeruginosa* acquisition after liver transplantation

CRPA was identified in a total of 30 LT recipients. In five patients CRPA was identified before LT, and those patients were excluded from risk factors analysis for CRPA acquisition after LT. (Table 16, 17 and 18) No infections by CRPA were identified in the pre-LT period.

Table 16 – Univariate analysis of risk factors for acquisition of carbapenem-resistant *P. aeruginosa* after LT in 190 patients – categorical variables

Variable	Patient with carbapenem-resistant <i>P. aeruginosa</i> (N=25) N (%)	Patient without carbapenem-resistant <i>P. aeruginosa</i> (N=165) N (%)	P	RR (CI95%)
Pre-LT characteristics				
Bacterial infection before LT	8 (32.0%)	52 (31.5%)	0.96	1.01 (0.76-1.34)
Gender (F)	12 (48%)	109 (66.1%)	0.08	0.65 (0.42-1.01)
Hepatocellular carcinoma	5 (20%)	52 (31.5%)	0.24	0.86 (0.69-1.07)
Fulminant hepatitis	4 (16%)	14 (8.5%)	0.27	1.09 (0.91-1.30)
ASA score	-	-	0.11	-
LT surgical procedure characteristics				
Combined liver-kidney transplant	2 (8%)	23 (13.9%)	0.54	0.94 (0.82-1.07)
Living-donor transplant	2 (8%)	10 (6.1%)	0.66	1.02 (0.90-1.15)
Senior surgeon	11 (44%)	86 (52.1%)	0.45	0.86 (0.58-1.25)
Duct-to-duct biliary anastomosis	23 (92%)	156 (94.5%)	0.61	0.68 (0.16-2.98)
Post-LT Evolution				
Standard immunosuppressant therapy	12 (48%)	75 (45.5%)	0.81	1.05 (0.70-1.57)
Re-transplantation	12 (48%)	45 (27.4%)	0.04	1.40 (0.95-2.06)
Re-operation	6 (24%)	19 (11.7%)	0.09	1.16 (0.93-1.46)
CMV infection	6 (24%)	32 (19.6%)	0.61	1.06 (0.84-1.34)
Vancomycin-resistant <i>Enterococcus</i> colonization	17 (68%)	72 (43.6%)	0.02	1.76 (0.98-3.17)
Acute cellular rejection	6 (24%)	38 (23.2%)	0.93	1.01 (0.80-1.28)
Post-LT dialysis	20 (80%)	90 (54.5%)	0.02	2.27 (1.02-5.07)

LT – liver transplantation; ASA - American Society of Anesthesiologists (ASA) Physical Status classification; CMV - cytomegalovirus

Table 17 – Univariate analysis of risk factors for acquisition of carbapenem-resistant *P. aeruginosa* after LT in 190 patients – continuous variables

Variable	Patient with carbapenem-resistant <i>P. aeruginosa</i> after LT (N=25) Median (min-max)	Patient without carbapenem-resistant <i>P. aeruginosa</i> after LT (N=165) Median (max-min)	P
Pre-LT characteristics			
Pre-LT length of hospital stay (days)	6 (0-31)	1 (0-67)	0.02
Age (years)	55 (25-70)	53 (16-73)	0.64
MELD score	25 (6-45)	22 (6-53)	0.08
Pre-LT serum creatinine (mg/dl)	1.59 (0.5-4.7)	1.15 (0.4-10.5)	0.59
LT surgical procedure characteristics			
Cold ischemic time (minutes)	460 (65-713)	390 (20-960)	0.02
Total ischemic time (minutes)	502 (105-758)	420 (65-910)	0.004
Duration of LT surgery (minutes)	475 (210-910)	460 (245-1330)	0.80
Units of blood transfused during LT surgery	3.0 (0-15)	2.0 (0-12)	0.009
Post-LT evolution			
Piperacillin / tazobactam use (days)	0 (0-16)	0 (0-33)	0.27
Carbapenem use (days)	12 (0-45)	4 (0-87)	0.30
Polymyxin use (days)	0 (0-52)	0 (0-78)	0.17
Cephalosporin use (days)	2 (0-13)	2 (0-21)	0.68
Penicillin use (days)	1 (0-5)	1 (0-21)	0.23
Length of dialysis after LT (days)	6 (0-36)	2 (0-78)	0.04
Length of central venous catheter (days)	17 (2-68)	7 (1-85)	0.01
Length of mechanical ventilator (days)	5 (0-44)	1 (0-67)	0.01
Length of urinary catheter (days)	11 (2-72)	7 (0-90)	0.05
Length of abdominal drain (days)	9 (2-28)	7 (0-81)	0.12
Length of ICU stay (days)	14 (0-75)	7 (1-63)	0.02

LT – liver transplantation; ICU – intensive care unit; MELD – model for end-stage liver disease

Table 18 – Multivariate analysis of risk factors for acquisition of carbapenem-resistant *P. aeruginosa* after LT in 190 patients

Variable	P	OR	CI 95%
Length of dialysis (days)	0,12	0,97	0,94-1,01
Units of blood transfused during liver transplantation surgery	0,02	1,20	1,03-1,39
Length of intensive care unit stay (days)	0,02	1,04	1,01-1,07
Cold ischemic time (minutes)	0,07	1,01	1,00-1,01

4.5 Healthcare-associated infections (HAI) after liver transplantation

During the study period we identified 201 HAI in 110 (56.4%) patients after LT.

The two most frequent sites of infection were the surgical site and BSI (Table 19), and the two most frequent agents were *A. baumannii* e *K. pneumoniae*. (Table 20).

Table 19 – Distribution of the sites of 201 healthcare-associated infections after liver transplantation

Site of infection	N	%
Organ space surgical site	56	28%
Catheter related bloodstream	44	22%
Pneumonia	35	17%
Gastroenteritis	13	6%
Urinary tract infection	13	6%
Primary bloodstream	10	5%
Incisional surgical site	8	4%
Soft tissues	8	4%
Intra-abdominal	6	3%
Bronchitis	3	1%
Insertion catheter site	3	1%
Genital tract	1	0%
Total	201	100%

Table 20 – Distribution of microorganism isolated from 201 healthcare-associated infections after liver transplantation

Agent	N	Bacteremia (%)	Multidrug-resistance N(%)
<i>A. baumannii</i>	56	36 (64.3%)	56 (100%)
<i>K. pneumoniae</i>	47	26 (55.3%)	40 (85.1%)*
<i>E. faecium</i>	29	15 (51.7%)	29 (100%)
<i>P. aeruginosa</i>	17	9 (52.9%)	13 (76.4%)
<i>E. coli</i>	13	7 (53.8%)	7 (53.8%)**
<i>E. cloacae</i>	9	4 (44.4%)	2 (22.2%)
<i>E. aerogenes</i>	9	4 (44.4%)	3 (33.3%)
Coagulase- negative <i>Staphylococcus</i>	9	5 (55.6%)	0
<i>E. faecalis</i>	7	3 (42.9%)	1 (14.3%)
<i>S. aureus</i>	6	3 (50.0%)	5 (83.3%)
<i>M. morgannii</i>	5	3 (60.0%)	0
<i>C. albicans</i>	5	1 (20.0%)	-
<i>C. glabrata</i>	4	1 (25.0%)	-
<i>C. krusei</i>	3	2 (66.7%)	-
<i>C. parapsilosis</i>	2	1 (50.0%)	-
<i>P. mirabilis</i>	2	1 (50.0%)	1 (50.0%)**
<i>C. freundii</i>	2	1 (50.0%)	0
<i>A. faecalis</i>	1	1 (100.0%)	-
<i>B. ovalis</i>	1	1 (100.0%)	-
Gram-negative bacillus	1	1 (100.0%)	-
<i>C. dubliensis</i>	1	1 (100.0%)	-
<i>Enterococcus sp</i>	1	1 (100.0%)	0
<i>S. marcescens</i>	1	1 (100.0%)	0
<i>C. davisae</i>	1	0	0
<i>Aspergillus spp.</i>	1	0	-
<i>S. bovis</i>	1	1 (100.0%)	0
<i>P. stuartii</i>	1	1 (100.0%)	0
<i>C. pelliculosa</i>	1	1 (100.0%)	-
<i>S. viridans</i>	1	0	0
Total	237	132 (55.7%)	125 (52.7%)

*among the resistant isolates, 57.5% (23) were carbapenem-resistant and 42.5% (17) ESBL-producing; ** ESBL-producing; 1 strain resistant to carbapenem; *** ESBL-producing

4.5.1 Surgical site infections

Surgical site infection corresponded to 31.8% of the HAI after LT, and 87.5% of those were classified as organ-space (OESI). In one case of combined kidney-liver transplant, the infection was identified in the renal graft. The time period between LT and OESI was on average 13 days, and the median time period from LT to OESI was 10 days. In 24 (43.0%) cases of OESI more than one agent was identified.

Among bacteria that caused OESI, 38 (77.6%) were GNB and 29 (76.3%) were classified as MDR. Two cases had no etiological agent identified (3.6%). (Table 21)

Table 21 – Microorganisms isolated from post-transplant organ space surgical site infections, percentage of bacteremia and resistance profile.

Agent	N	Secondary bacteremia N(%)	Multidrug-resistance N(%)
<i>A. baumannii</i>	18	15 (83.3%)	18 (100%)
<i>K. pneumoniae</i>	16	9 (56%)	14 (88%)*
<i>E. faecium</i>	15	6 (40%)	15 (100%)
<i>E. cloacae</i>	6	3 (50%)	2 (33%)
<i>E. aerogenes</i>	5	2 (40%)	2(40%)
<i>E. coli</i>	4	2 (50%)	2 (50%)**
<i>M. morgannii</i>	4	2 (50%)	0%
<i>P. aeruginosa</i>	4	3 (75%)	3 (75%)
<i>C. glabrata</i>	3	1 (33%)	-
<i>S. aureus</i>	3	2 (67%)	2 (67%)
<i>S. Coagulase-negative</i>	3	1 (33%)	0%
<i>C. krusei</i>	2	2 (100%)	-
<i>E. faecalis</i>	2	0%	0%
<i>A. faecalis</i>	1	1 (100%)	-
<i>B. ovalis</i>	1	1 (100%)	-
Gram-negative bacillus	1	1 (100%)	-
<i>C. albicans</i>	1	1 (100%)	-
<i>C. dubliensis</i>	1	1 (100%)	-
<i>C. parapsilosis</i>	1	0%	-
<i>Enterococcus sp</i>	1	1 (100%)	0%
<i>S. marcescens</i>	1	1 (100%)	0%
Total Geral	94	55 (59%)	-

* Among resistant isolates, 78.6% (11) were carbapenem-resistant and 21.4% (3) ESBL-producing; ** ESBL-producing SCN – coagulase-negative *Staphylococcus* ; BSI – bloodstream infection

In the case of incisional surgical site infections (ISSI), an etiological agent was identified in only half of them. No case of secondary bacteremia was observed. The identified agents were: two coagulase-negative *Staphylococcus*, one susceptible *E. faecalis*, one VRE and one CRAB.

4.5.2 Bloodstream Infections after liver transplantation

Among BSI, 44 (68.7%) were classified as catheter-related BSI and 10 (18.5%) were classified as primary BSI.

In eight (15.0%) BSI more than one agent was identified. Among isolated microorganisms, 44 (68.8%) were GNB. The most common Gram-positive bacterium was *Enterococcus* in 12 (60.0%) cases. Among BSI bacterial infections, 69% were classified as MDR, and among infections caused by *K. pneumoniae*, 31.3% (5) were resistant to carbapenem. (Table 22)

Table 22 – Microorganisms isolated from post-transplant bloodstream infections and resistance profile

Agent	N	Multidrug-resistance N(%)
<i>K. pneumoniae</i> *	16	11 (69%)
<i>A. baumannii</i>	14	15 (100%)
<i>E. faecium</i>	9	9 (100%)
<i>E. coli</i> **	5	3 (60%)
<i>P. aeruginosa</i>	4	2 (50%)
<i>E. faecalis</i>	3	1 (33%)
<i>S. epidermidis</i>	2	0%
<i>S. haemolyticus</i>	2	0%
<i>C. freundii</i>	1	0%
<i>C. parapsilosis</i>	1	-
<i>C. pelliculosa</i>	1	-
<i>E. aerogenes</i>	1	0%
<i>E. cloacae</i>	1	0%
<i>P. mirabilis</i> ***	1	1 (100%)
<i>P. stuartii</i>	1	0%
<i>S. aureus</i>	1	1 (100%)
<i>S. bovis</i>	1	0%
Total	64	-

*four strain were carbapenem-resistant and seven ESBL-producing ; ** one strain was carbapenem-resistant and two ESBL-producing; ***ESBL-producing

The average between LT and BSI diagnosis was 28 days, and the median was 19 days.

4.5.3 Lower respiratory tract infection after liver transplantation

During the study period 35 pneumonia cases were identified, in one case the causative agent was not identified, and in 13 (37.1%) cases more than one microorganism was identified. Three cases of tracheobronchitis were identified in the study period and none presented secondary bacteremia. In one case, *C. glabrata* was implicated as the etiological agent of empyema, this microorganism was isolated from pleural liquid with no evidence of intra-abdominal infection. (Tabela 23)

Table 23 – Microorganisms isolated from lower respiratory tract infection, secondary bacteremia and resistance profile.

Agent	N	Secondary bacteremia	Multidrug-resistance N(%)
<i>A. baumannii</i>	20	6 (30.0%)	20 (100%)
<i>P. aeruginosa</i>	7	2 (29%)	7 (100%)
<i>K. pneumoniae</i> *	6	1 (17%)	6 (100%)
<i>E. cloacae</i>	2	0%	0%
<i>E. aerogenes</i>	2	1 (50.0%)	1 (50%)
<i>C. glabrata</i>	1	0%	-
<i>E. coli</i> **	1	0%	1 (100%)
<i>E. faecalis</i>	1	0%	0%
<i>C. davisae</i>	1	0%	0%
<i>M. morgannii</i>	1	1 (100%)	0%
<i>Aspergillus</i> spp.	1	0%	-
<i>S. aureus</i>	2	0%	2 (100%)
<i>S. viridans</i>	1	0%	0%
Total	47	11 (23%)	-

* three strains ESBL producing and three strains resistant to carbapenem; **ESBL producing; BSI-bloodstream infection

Twenty-four cases of pneumonia (68.6%) and two (66.7%) cases of tracheitis were associated to ventilator use.

The average length of time between LT and pneumonia was 18 days, and the median of this interval was 15 days.

4.5.4 Other types of infection after liver transplantation

The urinary tract infection (UTI) was the fourth most frequent infection. No case of secondary bacteremia was identified. (Table 9) Among bacterial infections, 86% of the agents were considered MDR. The mean time between LT and UTI was 17 days. In two infection cases more than one agent was identified. Four (30.8%) UTI cases were related to urinary catheter use.

Table 24 – Microorganisms isolated from post-transplant urinary tract infections and resistance profile.

Agent	N	Multidrug-resistant N(%)
<i>K. pneumoniae</i> *	5	5 (100%)
<i>C. albicans</i>	3	-
<i>E. faecium</i>	3	3 (100%)
<i>A. baumannii</i>	1	1 (100%)
<i>C. freundii</i>	1	0%
<i>C. krusei</i>	1	-
<i>E. coli</i>	1	0%
<i>P. aeruginosa</i>	1	1 (100%)
Total	16	-

*four strains were resistant to carbapenem and one was ESBL-producing

One patient had balanoposthitis by CRKP.

During the study period 13 *Clostridium difficile* strains were identified, the average time between LT and *Clostridium* gastroenteritis was 15 days and the median was 10 days.

We identified eight soft tissue infections and the causative agent was identified in only 38%; there were two cases of polymicrobial infection and one case of secondary bacteremia by CRAB. (Table 10) 57% percent of bacterial agents were classified as MDR.

Table 25 – *Microorganisms isolated from soft tissue infections and resistance profile*

Agent	N	Multidrug-resistant N(%)
<i>E. coli</i> *	2	1 (50%)
<i>A. baumannii</i>	1	1 (100%)
<i>C. albicans</i>	1	-
<i>E. aerogenes</i>	1	0%
<i>E. faecium</i>	1	1 (100%)
<i>K. pneumoniae</i> *	1	1 (100%)
<i>P. aeruginosa</i>	1	0%
Total	8	4 (50,0%)

*ESBL – producing

4.6 Analysis of risk factors for healthcare-associated infection by MDR GNB after liver transplantation

HAI were identified in 110 (56.4%) patients after LT. Among the patients who developed HAI, 84 (76.3%) had an MDR GNB isolated from a clinical culture related to that infection. The analysis of risk factors for developing HAI by MDR GNB after LT is described in Tables 26, 27, 28.

Table 26 – Univariate analysis of risk factors for healthcare-associated infection by MDR GNB after liver transplantation in 195 patients – categorical variables

Variable	Patient with HAI by MDR GNB (N=84) N (%)	Patient without HAI by MDR GNB (N=111) N (%)	P	RR (CI 95%)
Pre-LT characteristics				
Bacterial infection within 10 days before LT	32 (38.1%)	29 (26.1%)	0.07	1.19 (0.98-1.46)
Pre-LT acquisition of MDR GNB	47 (56.0%)	43 (38.7%)	0.02	1.39 (1.05-1.85)
Gender (M)	56 (66.7%)	70 (63.1%)	0.60	1.11 (0.75-1.63)
Hepatocellular carcinoma	23 (27.4%)	38 (34.2%)	0.31	0.91 (0.75-1.09)
Fulminant hepatitis	12 (14.3%)	6 (5.4%)	0.03	1.10 (1.00-1.22)
ASA score	-	-	0.13	-
LT surgical procedure characteristics				
Living-donor transplant	8 (9.5%)	17(15.3%)	0.23	0.94 (0.84-1.04)
Combined kidney-liver transplant	6 (7.1%)	6 (5.4%)	0.62	1.02 (0.95-1.10)
Senior surgeon	39 (53.2%)	59 (46.4%)	0.35	0.87 (0.66-1.16)
Duct-to-duct biliary anastomosis	78 (92.9%)	106 (95.5%)	0.43	0.63 (0.20-2.00)
Post-LT Evolution				
Standard immunosuppressant therapy	44 (52.4%)	62 (55.9%)	0.63	0.93 (0.68-1.26)
Re-transplantation	22 (26.5%)	22 (19.8%)	0.27	1.09 (0.93-1.28)
Re-operation	14 (16.9%)	4 (3.6%)	0.002	1.16 (1.05-1.27)
CMV infection	11 (13.1%)	16 (14.7%)	0.75	0.98 (0.88-1.10)
Vancomycin-resistant <i>Enterococcus</i> colonization	52 (61.9%)	40 (36.0%)	<0.001	1.68 (1.24-2.28)
Post-LT dialysis	59 (70.2%)	44 (39.6%)	<0.001	2.03 (1.41-2.91)
Acute cellular rejection	11 (13.1%)	20 (18.2%)	0.34	0.94 (0.83-1.06)

LT – liver transplantation, MDR GNB – Multidrug-resistant Gram-negative bacteria; HAI – healthcare-associated infection; ASA - American Society of Anesthesiologists (ASA) Physical Status classification; CMV - cytomegalovirus

Table 27 – Univariate analysis of risk factors for healthcare-associated infection by MDR GNB after liver transplantation in 195 patients – continuous variables

Variable	Patient with HAIs by MDR GNB (N=84) Median (min- max)	Patient without HAIs by MDR GNB (N=111) Median (min- max)	P
Pre-LT characteristics			
Pre-LT length of hospital stay (in days)	2 (0-46)	1 (0-67)	0.004
Age (years)	52 (16-70)	53 (16-73)	0.93
MELD score	24 (6-52)	19 (6-53)	0.002
Pre-LT serum creatinine (mg/dl)	1.58 (0.40-6.79)	1.07 (0.41-10.54)	0.11
LT surgical procedure characteristics			
Cold ischemia time (minutes)	441 (47-960)	365 (65-730)	0.002
Total ischemia time (minutes)	466 (81-910)	405 (65-730)	0.002
Duration of LT surgery (minutes)	470 (210-850)	450 (245-1330)	0.56
Units of blood transfused during LT surgery	3 (0-15)	1 (0-9)	<0.001
Post-LT Evolution			
Carbapenem use (days)	6 (0-40)	0 (0-42)	0.001
Polymyxin use (days)	0 (0-21)	0 (0-31)	0.003
Cephalosporin use (days)	2 (0-15)	2 (0-21)	0.11
Duration of piperacilin / tazobactam use (days)	1 (0-21)	0 (0-33)	0.18
Duration of penicillin use (days)	1 (0-12)	2 (0-21)	0.23
Duration of dialysis after LT (days)	5 (0-50)	0 (0-45)	<0.001
Duration of use of central venous catheter (days)	10 (1-50)	5 (1-67)	<0.001
Duration of use of mechanical ventilator (days)	4 (0-30)	0 (0-10)	<0.001
Duration of use of urinary catheter (days)	9 (0-35)	5 (0-35)	<0.001
Duration of use of abdominal drain (days)	7 (0-60)	7 (0-34)	0.78
Duration of ICU stay (days)	10 (1-42)	5 (1-52)	<0.001

LT – liver transplantation; MELD – model for end-stage liver disease, HAI – Healthcare-associated infection; MDR GNB – Multidrug-resistant Gram-negative bacilli; ICU – intensive care unit

Table 28 – Multivariate analysis of risk factors for healthcare-associated infection by MDR GNB after LT in 195 patients

Variable	P	OR	CI 95%
Bacterial infection within 10 days before LT	0,33	0,68	0,31-1,48
Re-transplantation	0,04	3,85	1,06-14,03
Number of blood units transfused in LT surgery	0,005	1,25	1,07-1,46
Acquisition of MDR GNB before LT	0,05	1,98	0,99-3,93
Length of ICU stay (days)	0,04	1,05	1,02-1,11
Cold ischemia time (minutes)	0,06	1,02	1,01-1,04

LT – liver transplantation; MDR GNB – Multidrug-resistant Gram-negative bacilli; ICU – intensive care unit

4.6.1 Analysis of risk factors analysis of healthcare-associated infection by carbapenem-resistant *K. pneumoniae* after liver transplantation

A total of 23 patients developed HAI by CRKP after LT. One patient was submitted to LT while under treatment for a CRKP active infection and was excluded from the risk factors analysis. The risk factors analysis for CRKP after LT is described in Tables 29, 30 e 31.

Table 29 – Univariate analysis of risk factors for healthcare-associated infection by carbapenem-resistant *K. pneumoniae* after liver transplantation in 194 patients – categorical variables

Variable	Patients with HAIs by carbapenem-resistant <i>K. pneumoniae</i> after LT (N=23) N (%)	Patients without HAIs by carbapenem-resistant <i>K. pneumoniae</i> after LT (N=171) N (%)	P	RR (CI95%)
Pre-LT characteristics				
Bacterial infection within 10 days before LT	10 (43.5%)	51 (29.7%)	0.18	1.25 (0.86-1.80)
Pre-LT acquisition of CRKP	7 (30.4%)	18 (10.5%)	0.007	1.29 (0.98-1.70)
Gender (M)	16 (69.6%)	110 (64.0%)	0.60	1.18 (0.62-2.27)
Hepatocellular carcinoma	8 (34.8%)	53 (30.8%)	0.70	1.06 (0.77-1.45)
Fulminant hepatitis	1 (4.3%)	17 (9.9%)	0.70	0.94 (0.85-1.04)
ASA score	-	-	0.38	-
LT surgical procedure characteristics				
Living-donor transplant	4 (17.4%)	21 (12.2%)	0.51	1.06 (0.87-1.29)
Combined kidney-liver transplant	2 (8.7%)	10 (5.8%)	0.64	1.03 (0.90-1.18)
Senior surgeon	14 (60.9%)	84 (48.8%)	0.28	1.31 (0.77-2.22)
Duct-to-duct biliary anastomosis	20 (87.0%)	164 (95.3%)	0.10	0.36 (0.10-1.25)
Post-LT Evolution				
Standard immunosuppressant therapy	14 (60.9%)	91 (53.2%)	0.49	1.20 (0.70-2.04)
Re-operation	7 (31.8%)	37 (21.5%)	0.28	1.15 (0.86-1.55)
Re-transplantation	2 (9.1%)	16 (9.4%)	1.00	1.00 (0.87-1.15)
Post-LT dialysis	16 (69.6%)	87 (50.6%)	0.09	1.62 (0.86-3.07)
Acute cellular rejection	2 (8.7%)	29 (17.0%)	0.54	0.91 (0.79-1.05)
Colonization by vancomycin-resistant <i>Enterococcus</i>	10 (43.5%)	82 (47.7%)	0.71	0.93 (0.63-1.36)
CMV infection	4 (17.4%)	23 (13.5%)	0.54	1.05 (0.86-1.27)

LT – liver transplantation; HAI – healthcare-associated infection; CRKP – carbapenem-resistant *K. pneumoniae*; ASA – American Society of Anesthesiologists (ASA) Physical Status classification; CMV – cytomegalovirus

Table 30 – Univariate analysis of risk factors for healthcare-associated infection by carbapenem-resistant *K. pneumoniae* after LT in 194 patients – continuous variables

Variable	Patients with HAIs by carbapenem-resistant <i>K. pneumoniae</i> after LT (N=23)	Patients without HAIs by carbapenem-resistant <i>K. pneumoniae</i> after LT (N=171)	P
Pre-LT characteristics			
Age (years)	50.0 (18.0-70.0)	53.0 (16.0-73.0)	0.81
Duration of hospital stay before LT (days)	1.0 (0.0-46.0)	1.0 (0.0-67.0)	0.15
MELD score	23.0 (7.0-52.0)	22.0 (6.0-53.0)	0.86
Serum creatinine at LT (mg/dl)	1.77 (0.57-4.26)	1.09 (0.40-10.54)	0.28
LT surgical procedure characteristics			
Duration of cold ischemia (minutes)	480.0 (81.0-758.0)	435.0 (65.0-960.0)	0.08
Duration of total ischemia (minutes)	445.0 (47.0-713.0)	385.0 (20.0-910.0)	0.04
Duration of LT surgery (minutes)	460.0 (250.0-850.0)	470.0 (210.0-1330.0)	0.61
Units of blood transfused during LT surgery	3.0 (0.0-12.0)	2.0 (0.0-15.0)	0.67
Post-LT Evolution			
Duration of use of quinolone (days)	0.0 (0.0-4.0)	0.0 (0.0-6.0)	0.45
Duration of use of piperacilin / tazobactam (days)	2.0 (0.0-22.0)	0.0 (0.0-33.0)	0.28
Duration of use of cephalosporins (days)	1.0 (0.0-15.0)	2.0 (0.0-21.0)	0.48
Duration of use of penicillin (days)	1.0 (0.0-9.0)	1.0 (0.0-21.0)	0.25
Duration of use of polymyxin (days)	12.0 (0.0-86.0)	0.0 (0.0-77.0)	0.002
Duration of use of carbapenem (days)	20.0 (0.0-39.0)	4.0 (0.0-72.0)	0.003
Duration of use of ventilator (days)	5.0 (0.0-101.0)	1.0 (0.0-107.0)	0.06
Duration of use of abdominal drain (days)	9.0 (0.0-39.0)	7.0 (0.0-83.0)	0.49
Duration of use of central venous catheter (days)	25.0 (2.0-98.0)	8.0 (0.0-102)	0.02
Duration of use of urinary catheter (days)	15.0 (2.0-86.0)	7.0 (0.0-110.0)	0.003
Duration of use of dialysis after LT (days)	21.0 (0-91.0)	3.0 (0.0-101.0)	0.009
Duration of ICU stay (days)	18.0 (1.0-98.0)	7.0 (0.0-110.0)	0.008

LT – liver transplantation; HAI – healthcare-associated infection; MELD - model for end-stage liver disease; ICU – intensive care unit

Table 31 – Multivariate analysis of risk factors for healthcare-associated infection by carbapenem-resistant *K. pneumoniae* after liver transplantation in 194 patients

Variable	P	OR	CI (95%)
Pre-LT acquisition of CRKP	0.003	4.70	1.72-12.85
Carbapenem use (days)	0.09	1.02	1.00-1.05

LT – liver transplantation; CRKP – carbapenem-resistant *K. pneumoniae*

4.6.2 Analysis of risk factors for healthcare-associated infection by carbapenem-resistant *A. baumannii* after liver transplantation

Fifty-six cases of HAI by CRAB were identified during the study period. Two patients had active infection by CRAB at the moment of LT and were excluded from the risk factors analysis of infection after LT. The analysis of risk for developing HAI by CRAB after LT is shown in Tables 32, 33 and 34.

Table 32 – Univariate analysis of risk factors for healthcare-associated infection by carbapenem-resistant *A. baumannii* after liver transplantation in 193 patients – categorical variables

Variable	Patients with HAI by carbapenem-resistant <i>A. baumannii</i> after LT (N=56) N (%)	Patients without HAI by carbapenem-resistant <i>A. baumannii</i> after LT (N=137) N (%)	P	RR (CI95%)
Pre-LT characteristics				
Bacterial infection within 10 days before LT	22 (40.0)	37 (26.8)	0.07	1.22 (0.95-2.30)
Pre-LT acquisition of CRAB	11 (19.6)	11 (8.0)	0.09	1.48 (0.95-2.30)
Gender (M)	37 (67.3)	88 (63.8)	0.65	1.11 (0.74-1.80)
Hepatocellular carcinoma	14 (25.5)	47 (34.3)	0.25	0.89 (0.72-1.05)
Fulminant hepatitis	9 (16.4)	8 (5.8)	0.02	1.13 (1.01-1.30)
ASA score	-	-	0.63	-
LT surgical procedure characteristics				
Living-donor transplant	5 (9.1)	20 (14.5)	0.31	0.94 (0.84-1.04)
Combined kidney-liver transplant	3 (5.5)	8 (5.8)	>0.99	0.99 (0.92-1.07)
Senior surgeon	26 (47.3)	72 (52.2)	0.54	0.91 (0.70-1.29)
Duct-to-duct biliary anastomosis	54 (98.2)	130 (94.3)	0.24	3.19 (0.43-26.00)
Post-LT Evolution				
Standard immunosuppressant therapy	25 (45.5)	81 (58.7)	0.10	0.76 (0.55-1.04)
Re-operation	16 (29.1)	35 (25.4)	0.60	1.05 (0.86-1.25)
Re-transplantation	11 (20.0)	10 (7.2)	0.01	1.16 (1.00-1.32)
Post-LT dialysis	41 (74.5)	62 (44.9)	<0.001	2.16 (1.37-3.42)
Acute cellular rejection	9 (16.4)	25 (18.2)	0.77	0.98 (0.85-1.11)
Colonization by vancomycin-resistant <i>Enterococcus</i>	35 (63.6)	55 (39.9)	0.003	1.65 (1.14-2.41)
CMV infection	6 (10.9)	26 (19.0)	0.18	0.91 (0.80-1.02)

LT – liver transplantation; HAI – healthcare-associated infection; ASA - American Society of Anesthesiologists (ASA) Physical Status classification; CRAB – carbapenem-resistant *A. baumannii*; CMV - cytomegalovirus

Table 33 – Univariate analysis of risk factors for healthcare-associated infection by carbapenem-resistant *A. baumannii* after liver transplantation in 193 patients – continuous variables

Variable	Patients with HAI by carbapenem-resistant <i>A. baumannii</i> after LT (N=56) Median (max-min)	Patients without HAI by carbapenem-resistant <i>A. baumannii</i> after LT (N=137) Median (max-min)	P
Pre-LT characteristics			
Pre-LT length of hospital stay (days)	2.0 (0.0-42.0)	1.0 (0.0-56.0)	0.19
Age (years)	52.0 (16-70)	53.0 (16.0-73.0)	0.78
MELD score	27.0 (6-52)	21.0 (6.0-53.0)	0.002
Pre-LT serum creatinine (mg/dl)	1.59 (0.40-6.79)	1.07 (0.41-10.54)	0.22
LT surgical procedure characteristics			
Duration of cold ischemia (minutes)	441.0 (50-910)	375.0 (20.0-754.0)	0.01
Duration of total ischemia (minutes)	467.5 (95-960)	410.0 (65-798)	0.006
Duration of LT surgery (minutes)	467.5 (210.0-829.0)	460.0 (245.0-1330.0)	0.78
Units of blood transfused during LT surgery	2.0 (0.0-15.0)	1.0 (0.0-9.0)	0.004
Post-LT Evolution			
Duration of use of carbapenem (days)	6.0 (0.0-40.0)	0.0 (0-72)	0.07
Duration of use of quinolone (days)	0.0 (0-0)	0.0 (0-6)	0.94
Duration of use of polymyxin (days)	0.0 (0-21)	0.0 (0-26)	0.18
Duration of use of cephalosporin (days)	2.0 (0-15)	2.0 (0-21)	0.25
Duration of use of piperacilin / tazobactam (days)	1.0 (0-19)	0.0 (0-33)	0.25
Duration of use of penicillin use (days)	0.0 (0-9)	2.0 (0-21)	0.003
Duration of dialysis after LT (days)	7.0 (0-90)	0.0 (0-78)	0.001
Duration of use of central venous catheter (days)	10.0 (1.0-49.0)	6.0 (1.0-91.0)	0.008
Duration of use of ventilator (days)	4.0 (0-30)	0.0 (0-29)	<0.001
Duration of use of urinary catheter (days)	7.5 (3.0-35.0)	6.5 (3.0-56.0)	0.09
Duration of use of abdominal drain (days)	7.0 (1.0-35.0)	7.0 (0.0-49.0)	0.99
Duration of ICU stay in days	9.5 (1.0-42.0)	6.0 (2.0-44.0)	0.01

LT – liver transplantation; HAI – healthcare-associated infection; ICU – intensive care unit; MELD – model for end-stage liver disease

Table 34 – Multivariate analysis of risk factors for healthcare-associated infection by carbapenem-resistant *A. baumannii* after liver transplantation in 193 patients – categorical variables

Variable	P	OR	CI (95%)
Fulminant hepatitis	0.02	3.41	1.19-9.77
Pre-LT acquisition of CRAB	0.06	2.57	0.94-7.05
Cold ischemia time (minutes)	0.01	1.03	1.01-1.06
Post-LT dialysis	0.003	3.08	1.46-6.49
Carbapenem use (days)	0.25	0.98	0.96-1.01

CRAB – carbapenem-resistant *A. baumannii*; LT – liver transplantation;

4.6.3 Analysis of risk factors for healthcare-associated infection by carbapenem-resistant *P. aeruginosa* after liver transplantation

No patient had an infection by CRPA before LT, and 12 developed infection by this agent after LT. The analysis of risk factors associated to CRPA HAI after LT is demonstrated in Tables 35, 36 and 37.

Table 35 – Univariate analysis of risk factors for healthcare-associated infection by carbapenem-resistant *P. aeruginosa* after liver transplantation in 195 patients – categorical variables

Variable	Patients with HAIs by carbapenem-resistant <i>P. aeruginosa</i> after LT (N=12) N (%)	Patients without HAIs by carbapenem-resistant <i>P. aeruginosa</i> after LT (N=183) N (%)	P	RR (CI95%)
Pre-LT characteristics				
Bacterial infection within 10 days before LT	5 (41.7)	56 (30.6)	0.42	1.19 (0.73-1.94)
Pre-LT acquisition of CRPA	2 (16.7)	3 (1.6)	0.03	1.18 (0.92-1.52)
Gender (M)	9 (75.0)	117 (63.9)	0.55	1.44 (0.53-3.92)
Hepatocellular carcinoma	2 (16.7)	59 (32.2)	0.35	0.81 (0.62-1.07)
Fulminant hepatitis	0	18 (9.8)	0.61	0.90 (0.86-0.95)
ASA score	-	-	0.84	-
LT surgical procedure characteristics				
Living-donor transplant	0	25 (13.7%)	0.37	0.86 (0.82-0.92)
Combined kidney-liver transplant	1 (8.3)	11 (6.0)	0.54	1.03 (0.86-1.22)
Senior surgeon	4 (33.3)	94 (51.4)	0.25	0.73 (0.48-1.12)
Duct-to-duct biliary anastomosis	11 (91.7)	173 (94.5)	0.51	0.66 (0.09-4.71)
Post-LT Evolution				
Standard immunosuppressant therapy	5 (41.7)	101 (55.2)	0.36	0.77 (0.46-1.27)
Re-operation	5 (41.7)	54 (29.5)	0.52	1.21 (0.74-1.97)
Re-transplantation	3 (25.0)	23 (12.6)	0.20	1.17 (0.84-1.62)
Post-LT dialysis	12 (100)	101 (55.2)	0.001	-
Acute cellular rejection	3 (25.0)	44 (24.2)	>0.99	1.01 (0.72-1.42)
Colonization by vancomycin-resistant <i>Enterococcus</i>	9 (75.0)	83 (45.3)	0.07	2.19 (0.81-5.88)
CMV infection	4 (33.3)	38 (21.0)	0.30	1.19 (0.79-1.78)

LT – liver transplantation; CRPA – carbapenem-resistant *P. aeruginosa*; ASA - American Society of Anesthesiologists (ASA) Physical Status classification; CMV - cytomegalovirus

Table 36 – Univariate analysis of risk factors for healthcare-associated infection by carbapenem-resistant *P. aeruginosa* after liver transplantation in 195 patients – continuous variables

Variable	Patients with HAIs by carbapenem-resistant <i>P. aeruginosa</i> after LT (N=12) Median (min-max)	Patients without HAIs by carbapenem-resistant <i>P. aeruginosa</i> after LT (N=183) Median (min-max)	P
Pre-LT characteristics			
Pre-LT length of hospital stay (days)	9.5 (0-31)	1.0 (0-67)	0.004
Age (years)	58.0 (25-70)	53.0 (16-73)	0.12
MELD score	23.5 (16-44)	22.0 (6-53)	0.17
Serum creatinine at LT (mg/dl)	2.14 (0.82-3.99)	1.12 (0.40-10.54)	0.08
LT surgical procedure characteristics			
Duration of cold ischemia (minutes)	450.5 (335-713)	385.0 (20-910)	0.02
Duration of total ischemia time (minutes)	525 (380-758)	435.0 (65-960)	0.009
Duration of LT surgery (minutes)	457.5 (277-660)	460.0 (210-1330)	0.79
Units of blood transfused during LT surgery	3.5 (0-15)	2.0 (0-12)	0.01
Post-LT Evolution			
Duration of use of piperacilin/tazobactam (days)	0.0 (0-6)	0.0 (0-33)	0.09
Duration of use of carbapenem (days)	29 (12-71)	4.0 (0-87)	<0.001
Duration of use of polymyxin (days)	11.5 (3-80)	0.0 (0-81)	<0.001
Duration of use of cephalosporin (days)	2.0 (0-9)	2.0 (0-21)	0.71
Duration of use of penicillin (days)	0.0 (0-6)	1.0 (0-21)	0.05
Duration of dialysis after LT (days)	27.0 (6-45)	3.0 (0-74)	<0.001
Duration of use of central venous catheter (days)	35 (8-67)	8.0 (1-92)	<0.001
Duration of mechanical ventilation (days)	20.5 (3-44)	1.0 (0-78)	<0.001
Duration of use of urinary catheter (days)	18.0 (5-71)	7.0 (0-98)	<0.001
Duration of use of abdominal drain (days)	12.0 (2-37)	7.0 (0-83)	0.05
Duration of ICU stay (days)	28.5 (13-83)	7.0 (1-75)	<0.001

LT – liver transplantation; HAI – healthcare-associated infection; MELD – model for end-stage liver disease, ICU – intensive care unit

Table 37 – Multivariate analysis of risk factors for healthcare-associated infection by carbapenem-resistant *P. aeruginosa* after liver transplantation in 195 patients – categorical variables

Variável	P	OR	CI95%
Units of blood transfused in LT surgery	0.06	1.22	0.99-1.52
Length of ICU stay (days)	<0.001	1.08	1.04-1.12
CRPA acquisition before LT	0.007	34.02	2.59-416.14
Total ischemia time (minutes)	0.08	1.005	1.00-1.01

LT – liver transplantation; CRPA – carbapenem-resistant *P. aeruginosa*; ICU – intensive care unit

4.7 Evaluation of strains clonality through PFGE

We analyzed the genetic similarity between CRAB and CRKP strains through PFGE.

Eighty-five strains of CRAB from 51 (62.2%) patients were analyzed. Seven pulsotypes were identified during the study period: pulsotype A 5 (9.6%); pulsotype B 34 (65.4%); pulsotype C 3 (5.8%); pulsotype D 5 (9.6%); pulsotype E 3 (5.8%); pulsotype F 1 (1.9%); pulsotype G 1 (1.9%). In one patient, two different pulsotypes were identified. Pulsotype B was the most prevalent and had a homogeneous distribution during the study period. (Figure 1)

Figure 1 – Distribution of PFGE pulsotype of carbapenem-resistant *A. baumannii* strains from November 2009 to November 2011

Eleven patients acquired CRAB before LT and developed infection by this agent after LT. The paired analysis of isolates before LT and from infection after LT was possible in 8 patients. Among those eight paired

isolates, five pulsotypes were identified, and in seven patients the strain isolated after and before LT belonged to the same cluster (Figure 2)

Figure 2 – Dendrogram of carbapenem-resistant *A. baumannii* strains from eight patients who acquired CRAB before LT and developed infection by CRAB after LT

Twenty-six strains from 18 (75.0%) *bla*_{KPC}-positive-CRKP patients were analyzed, 16 from SC and 10 from clinical cultures. Seven clusters and nine PFGE pulsotypes were identified: cluster A 11 (42.3%); cluster B 7 (26.9%); cluster E 4 (15.4%); cluster F 1 (3.8%); cluster O 1 (3.8%); cluster S 1 (3.8%); cluster X 1 (3.8%). Cluster A was the most prevalent one in 2010 and cluster E was more common in 2011. (Figure 3) In three *bla*_{KPC}-positive-CRKP patients it was possible to analyze the paired cultures (before and after LT), and all strains belonged to cluster A.

Figure 3 – Distribution of PFGE cluster of carbapenem-resistant *bla*_{KPC}-positive *K. pneumoniae* strains from January 2010 to November 2011

Among *bla*_{KPC}-negative-CRKP strains, the analysis of genetic similarity was performed in 36 strains from 29 (52.7%) patients. Among the 36 strains analyzed, 24 were isolated from SC and 12 from clinical cultures. Nineteen PFGE clusters were identified: cluster C 8 (22.2%); cluster D 5 (13.9%); cluster F 1 (2.8%); cluster G 2 (5.6%); cluster H 2 (5.6%); cluster I 2 (5.6%); cluster J 3 (8.3%); cluster K 2 (5.6%); cluster L 1 (2.8%); cluster M 1 (2.8%); cluster N 1 (2.8%); cluster P 1 (2.8%); cluster Q 1 (2.8%); cluster R 1 (2.8%); cluster T 1 (2.8%); cluster U 1 (2.8%); cluster W 1 (2.8%); cluster Y 1 (2.8%); cluster Z 1 (2.8%). Paired isolates (before and after LT) were analyzed in two patients, and in those two cases the strains belonged to the same PFGE cluster. (Figure 4)

Figure 4 – Dendrogram of carbapenem-resistant *K. pneumoniae* isolated from Liver transplant patients from November 2009 to November 2011

4.8 Survival analysis

During the study period, 67 (34.4%) patients evolved to death in the first 60-days after LT. The risk factors for 60-day mortality after LT are described in Tables 38, 39 and 40.

Table 38 – Univariate analysis of risk factors for death in the 60 first days after liver transplantation, among 195 patients – categorical variables

Variable	Patients who did not survive within 60-days of LT (N=67) N (%)	Patients who survived within 60-days of LT (N=128) N (%)	P	HR (IC95%)
Pre-LT characteristics				
Bacterial infection within 10 days before LT	23 (34.3)	37 (29.1)	0.42	1.23 (0.75-2.04)
Gender (M)	46 (68.7)	79 (62.2)	0.33	0.76 (0.46-1.30)
Hepatocellular carcinoma	20 (29.9)	41 (32.3)	0.82	0.94 (0.56-1.59)
Fulminant hepatitis	11 (16.4)	7 (5.5)	0.02	2.21 (1.16-4.22)
ASA score	-	-	0.05	1.54 (1.01-2.36)
Acquisition of MDR GNB before LT	35 (52.2)	54 (42.5)	0.23	1.34 (0.83-2.17)
Acquisition of carbapenem-resistant GNB before LT	26 (38.8)	32 (25.2)	0.04	1.66 (1.02-2.72)
Acquisition of CRAB before LT	12 (17.9)	11 (8.7)	0.03	1.99 (1.06-3.71)
Acquisition of CRKP before LT	12 (17.9)	12 (9.4)	0.06	1.82 (0.97-3.40)
LT surgical procedure characteristics				
Living-donor LT	7 (10.4)	18 (14.2)	0.43	0.73 (0.33-1.59)
Kidney-liver combined transplant	5 (7.5)	6 (4.7)	0.29	1.64 (0.66-4.07)
Senior surgeon	35 (52.2)	63 (49.6)	0.73	1.09 (0.67-1.76)
Duct-to-duct anastomosis	63 (94.0)	121 (95.3)	0.81	1.13 (0.41-3.11)
Post-LT Evolution				
Standard immunosuppressant therapy	26 (38.8)	62 (48.8)	0.14	0.69 (0.42-1.13)
Reoperation	28 (41.8)	33 (26.0)	0.09	1.52 (0.94-2.48)
Re-transplantation	18 (26.9)	8 (6.3)	<0.001	0.34 (0.20-0.59)
Post-LT dialysis	61 (91.0)	51 (40.2)	<0.001	10.0 (4.32-23.15)
Acute cellular rejection	20 (30.3)	27 (21.6)	0.33	1.30 (0.77-2.19)
Colonization by vancomycin-resistant <i>Enterococcus</i>	32 (47.8)	59 (46.5)	0.95	0.99 (0.61-1.59)
Healthcare-associated infection after LT	50 (74.6)	59 (46.5)	0.001	2.53 (1.46-4.38)
HAI by MDR bacteria after LT	45 (67.2)	42 (33.1)	<0.001	2.82 (1.69-4.70)
HAI by carbapenem-resistant GNB after LT	42 (62.7)	33 (26.0)	<0.001	3.12 (1.90-5.13)
HAI by CRAB after LT	32 (47.8)	24 (18.9)	<0.001	2.63 (1.62-4.25)
HAI by CRKP after LT	13 (19.4)	11 (8.7)	0.06	1.78 (0.97-3.27)
Surgical site infection	26 (38.8)	27 (21.3)	0.04	1.67 (1.02-2.73)
Pneumonia	19 (28.4)	14 (11.0)	0.003	2.24 (1.31-3.81)
Bloodstream infection	20 (29.9)	28 (22.0)	0.33	1.30 (0.77-2.20)
CMV infection	9 (13.4)	35 (27.6)	0.02	0.43 (0.22-0.88)

LT – liver transplantation; MDR – Multidrug-resistant; GNB – Gram-negative bacilli; ASA - American Society of Anesthesiologists (ASA) Physical Status classification; HAI – healthcare-associated infection; CMV - cytomegalovirus

Table 39 – Univariate analysis of risk factors for death in the 60 first days after liver transplantation, among 195 patients – continuous variables

Variable	Patients who not survival in 60-days after LT (N=67) Median (min-max)	Patients who survival in 60-days after LT (N=128) Median (min-max)	P	HR (CI95%)
Pre-LT characteristics				
length of Pre-LT hospital stay (days)	2.0 (0-56)	1.0 (0-46)	0.16	1.02 (0.99-1.04)
Age (years)	55.0 (16-70)	52.0 (16-73)	0.10	1.02 (1.00-1.04)
MELD score	27.0 (6-52)	21.0 (6-53)	0.004	1.03 (1.01-1.05)
Serum creatinine at LT (mg/dl)	1.56 (0.40-6.79)	1.04 (0.44-10.54)	0.03	1.15 (1.01-1.31)
LT surgical procedure characteristics				
Duration of cold ischemia (minutes)	415 (20-910)	390.0 (20-740)	0.07	1.001 (1.000-1.003)
Duration of total ischemia (minutes)	460 (84-960)	420.0 (65-754)	0.05	1.002 (1.000-1.003)
Duration of LT surgery (minutes)	490.0 (250-1330)	445.0 (210-915)	0.03	1.002 (1.000-1.003)
Units of blood transfused during LT surgery	2.0 (0-15)	1.0 (0-10)	0.006	1.11 (1.03-1.20)

LT – liver transplantation, MELD – model for end-stage liver disease

Table 40 – Multivariate analysis of risk factor for death in the 60 first days after liver transplantation, among 195 patients

Variable	P	HR	CI 95%
Post-LT dialysis	<0.001	7.91	3.33-18.94
Duration of LT surgery (minutes)	0.05	1.002	1.001-1.003
HAI by carbapenem-resistant Gram-negative bacilli	0.06	1.65	0.98-2.76

LT – liver transplantation; HAI – healthcare associated infection;

The 60-day survival curve is shown in Figure 5.

Figure 5 – 60-day survival curve adjusted according to LT surgery duration and post-LT dialysis, categorized by type of microorganism isolated from HAI after LT.

The P value was calculated based on group without HAI; HAI by CRAB - <0.001 ; HAI by CRE – 0.06; HAI by more than one carbapenem-resistant GNB – 0.002; HAI by non-MDR agent – 0.13.

5 DISCUSSION

This study was performed in a scenario of high prevalence of MDR GNB, especially CRAB and CRKP. Although CRKP was very frequent among LT patients in the present study, the incidence of *bla*_{KPC}-producing strains was 14.9%, which is a moderate incidence when compared to other studies in liver transplant patients.^{(70) (71)} Having said that, we were able to observe a significant increase in the prevalence of *bla*_{KPC}-producing-CRKP strains during the period of this study, which was not observed for the other CRE. The dissemination of *bla*_{KPC}-producing-CRKP strains is a phenomenon described worldwide^{(72) (73)}, therefore this epidemiological find was probably a reflection of the general occurrence in our hospital and our city.

The incidence of CRE after liver transplantation ranges widely among transplant centers, from 3 to 23%, and recent reports have described higher rates of colonization and infection by CRE.^{(60) (71) (59)} The SC for CRKP showed a high performance, with a good sensitivity and negative predictive value (NPV). The SC positivity rate for CRKP was highest (nearly 93%) for samples collected from the inguinal-rectal area, a finding that is in agreement with those of previous reports^{(70) (74)}. An important aspect described in this study is the difference in SC sensitivity rate when CRKP is compared with the other CRE. In the literature the sensitivity rate of CRE

SC ranges between 87.5 and 100%, similar to the rate found in the present study.^{(74) (70)} However, the SC sensitivity rate for the other CRE in our study was substantially lower. The comparison of SC performance between the species of CRE is scarce in literature^{(74) (75)} and the most plausible explanation for this difference observed in the sensitivity rate between CRKP and other CRE is the lowest incidence of the latter.

At our facility, CRAB was the most common MDR GNB colonization pathogen and in early post-LT infections. Other authors have shown that CRAB is the MDR GNB that most often colonizes LT recipients before and after transplant.⁽³²⁾ Although the prevalence of CRAB colonization was quite high in our patient sample, there are, to our knowledge, no comparative data in literature regarding the prevalence of CRAB colonization in the context of active, weekly surveillance after LT.

This high prevalence of MDR GNB may influence the overall rate of SC sensitivity, which was found to be higher than that from other reports in literature, especially for non-fermenting GNB. The sensitivity of SC for CRAB described in literature ranges between 55 and 72%, compared to 80.6% in the present study;^{(76) (77) (78)} Another possible reason for the impressive results obtained in this study is that immunocompromised patients have higher loads of MDR microorganisms colonizing the sites sampled.

For CRAB, the perineum-rectal site proved to be the site with the best SC positivity rate, followed by oropharynges.^{(77) (79)} Other studies have found that the oropharynx site had a better positivity rate than the rectal site for CRAB isolation, however neither of these studies collected swabs from

groin and rectal sites at the same time.⁽⁷⁸⁾ One study that analyzed different anatomic sites of skin for CRAB found that the groin site and the perirectal site had better sensitivity when compared to the perirectal site alone, which allows us to infer that the groin is an important region of CRAB colonization and therefore carrying out simultaneous SC of this region and the rectal site may enhance the chances of identifying colonized patients.⁽⁷⁶⁾ On the other hand, according to previous findings in literature and confirmed by data in the present study, the use of only one site implicates in an important loss of SC sensitivity for CRAB.⁽⁷⁷⁾

Another important MDR GNB related to HAI in LT recipients is CRPA. The prevalence of this agent in our service was lower when compared to other MDR GNB. Also, when compared to other MDR GNB in this study, CRPA had a lower proportion of colonized patients who developed infection. Other study that evaluated living-donor LT found that the rate of pre-LT carriers that developed infection in post-LT period was 13%;⁽²⁴⁾ In another population this rate is described as 19.0% in HSCT recipients and 38.5% in ICU patients.^{(80) (81)} The SC site with the best positivity rate for CRPA was the perineum-rectal site. Other studies that analyzed the CRAP SC positivity rate for different sites reported that the best site was the rectal site; however the positivity rate for this site ranged between 47% and 54%, compared to 84% in the present study.^{(82) (77)} Among patients with CRPA isolated in clinical cultures, 76.9% had positive SC; this finding has been described before in HCST patients, in whom only 53.8% of the patients with CRPA infection had CRPA detected in SC.⁽⁸¹⁾ Therefore SC for CRPA seems to

be an inefficient tool for direct empirical therapy, especially in the immunocompromised population.

Our study found a low rate of infection by ESBL-producing Enterobacteriaceae, the proportion rate of colonized patients who developed infection was 16.3% for ESBL-producing *K. pneumoniae* and 16.7% for the other ESBL-producing Enterobacteriaceae. One study that screened recipients for ESBL-producing Enterobacteriaceae in the pre-LT period found that 48% of LT recipients with positive SC developed infection.⁽⁸³⁾ In HSCT patients this proportion was even higher, 61%.⁽⁸⁴⁾ One possible explanation for our findings is that ESBL-producing Enterobacteriaceae are less virulent than CRE, and so patients who were colonized by those two types of Enterobacteriaceae had a higher probability of developing infection by CRE⁽⁸⁵⁾. Based on this reasoning, we could infer that the same is applicable for CRBA and ESBL-producing Enterobacteriaceae, since CRBA has been described as an impressively virulent bacterium in LT recipients.^{(86) (35)}. The sensitivity of SC for ESBL-producing Enterobacteriaceae was quite low, particularly for *K. pneumoniae*. The site with the best positivity rate was the perineum-rectal site, however if this had been the only site surveyed, more than 15% of *K. pneumoniae* ESBL carriers would have been left out. Previous reports of sensitivity for ESBL-producing Enterobacteriaceae SC range largely between 48.8 and 100%, and in most studies the only site surveyed was the rectal site, therefore it was not possible to establish whether or not SC for ESBL-producing Enterobacteriaceae is a good strategy for identifying the asymptomatic carriers^{(87) (88) (76)}

Therefore this study confirms that SC was a sensitive tool in detecting LT recipients colonized by carbapenem-resistant GNB. The SC for non-fermenting GNB should include more than one body site. Moreover, the active surveillance should be considered in transplant services with moderate and high incidence of MDR GNB once the proportion of colonized patients who developed infection is impressively high.

In the present study the only risk factor identified for acquisition of MDR GNB was the duration of use of urinary drain counted in days. The group of patients who acquired MDR GNB was very heterogeneous, with different GNB species and different resistance profiles, and this may be the major reason for the fact that few acquisition risk factors were identified. The use of invasive devices is often described as a risk factor for MDR bacteria acquisition in the general population and also among LT recipients.^{(89) (36) (28) (72)} Besides that, the use of a urinary drain is an indirect marker of renal function impairment and also a marker of patients with less independence in self-care activities.

In the present study, post-LT CRAB acquisition was also dependent on the degree to which hepatic function was impaired. Patients submitted to re-transplantation usually recover hepatic function more slowly and more often develop post-operative complications such as biliary leaks and thrombosis.⁽⁹⁰⁾ In contrast, patients submitted to LT for treatment of hepatocellular carcinoma typically present with more preserved hepatic function before LT, lower MELD score at LT, and fewer postoperative complications.⁽⁹¹⁾

Another major finding of the present study was that the use of broad-spectrum antibiotics, such as carbapenem and piperacillin-tazobactam, increased the risk for CRAB acquisition. Antibiotic use is frequently related to the emergence of MDR bacteria and has previously been correlated with CRAB colonization in immunocompetent individuals.^{(92) (93)} In addition, the use of such antibiotics before or after SOT has been associated with a greater risk for MDR GNB infection in the recipients.^{(37) (18) (89)}

Dialysis was found to be a risk factor for CRKP acquisition after LT in this study. Renal failure is a well-known risk for infection after LT.⁽⁹⁴⁾ Patients under dialysis usually need a central venous catheter, are more frequently submitted to kidney-liver transplant and have a longer duration of stay in ICU, all conditions associated with an increased risk for MDR bacteria acquisition.^{(18) (89)}

CRPA was, in this cohort study, the MDR GNB with the lowest incidence. The risk factors associated to CRPA acquisition were length of ICU stay and number of blood units transfused in LT surgery. These two conditions may be related to an increased risk of acquiring MDR bacteria in general in the hospital and not specifically in the LT unit.^{(27) (28)} Patients in ICU clearly have an increased risk of acquiring MDR bacteria, as they usually have more invasive devices, more severe conditions and need surgical procedures more frequently. Volume of intra-operative blood transfusion was an indirect marker of a more complex procedure and was previously associated to increase risk of infection after LT.^{(32) (95)} Besides that, transfusion itself plays an immunosuppressive role.

A liver transplant surgery is a complex and prolonged procedure, therefore surgical site infection is the most common infection reported in the early post-LT period.⁽²⁾ In the present study SSI were identified in 32% of the patients. The majority of these infections were classified as organ-space and more than half had secondary bacteremia associated. The data in literature are very similar to our findings, with a reported incidence of SSI up to 37%, the proportion of intra-abdominal commitment ranges between 67% and 79% and bacteremia was reported in 10-46% of the cases.^{(96) (95) (97)}

The most impressive data of our study about the epidemiology of post-LT infection was the high incidence of GNB with a high percentage of MDR strains. Liver transplant recipients have been described as a group of risk for MDR bacterial infection.^{(98) (89) (6)} The postoperative care is associated to frequent manipulation, use of invasive devices, prolonged length of ICU stay and frequent renal replacement therapy; also these patients receive a high amount of broad-spectrum antibiotic in pre-transplant and early post-transplant period. All these factors increase the risk for MDR bacteria acquisition.

As infections in early post-LT period are in their majority HAI, the microbial agent profile is closely related to the overall epidemiology of the institution where transplantation occurs.⁽²⁾ The most recent studies report a tendency of an increase in MDR GNB as agents of post-LT early infections.^{(98) (89)} Bert et al in a cohort study of 704 LT recipients reported that the most frequent agents of BSI in the first year after LT were Enterobacteriaceae species.⁽³⁰⁾ Shi et al in a cohort study of 475 LT recipients described that 32% developed bacteremia due to GNB in the first

six months after LT, and 56.3% of those GNB isolated were categorized as MDR; the most frequent MDR GNB was CRAB. ⁽¹⁸⁾ In contrast a Spanish study that analyzed all bacteremia cases occurring in hospitalized SOT recipients during a five-year period, found that CRPA was the most frequent MDR bacteria in LT recipients. ⁽⁶⁾

The CRPA is a common agent related to HAI after LT; the most common site of CRPA infection is pneumonia, CRPA being isolated in up to 18% of this type of infection. ^{(31) (99) (24) (100)} CRPA can be the causative agent in 9% of BSI. ^{(6) (89)} In this cohort study CRPA infection was identified in 6.2% of patients after LT, and the most common site was also pneumonia and was the agent isolated in 18.4% of the infections in the lower respiratory tract.

Risk factors identified in the present study associated to MDR GNB infection were: prolonged ICU stay, large amount of blood transfusion during LT surgery, re-transplantation and colonization by MDR GNB prior to LT.

The amount of blood transfusion during LT surgery and re-transplantation were two variables associated with graft complication. It was expected that variables associated with an increased risk for SSI would be identified in the risk factor analysis for MDR GNB infection, once SSI represented a great number of infections in our cohort study and as described in literature infections by MDR bacteria were more commonly isolated from graft infection than from SSI. ^{(71) (35) (89)}

As discussed for CRAB acquisition, re-transplantation is associated to slower recovery of hepatic function and more frequent postoperative

complications such as biliary leaks and thrombosis, which also increases the risk for post-LT infection.⁽⁹⁰⁾ A Spanish cohort study of 276 episodes of bacteremia in SOT recipients described that patients with prior transplantation had an increased risk for current bacteremia by MDR bacteria.⁽⁶⁾ A study designed to identify risk for SSI after LT also found that re-transplantation was associated with higher number of SSI.⁽⁹⁶⁾

Amount of blood transfused during LT surgery, as discussed earlier, is a marker of complication during this procedure. In literature patients who received more blood during transplant surgery had an increased risk of developing SSI; this finding was described in LT but also in other types of SOT.⁽¹⁰¹⁾

Patients in ICU have an increased risk for developing infection, due to the severity of the patient and the degree of invasiveness they are submitted to. In other populations ICU stay was frequently associated with risk for MDR GNB infection, such CRKP and CRAB.^{(85) (28)} One study that evaluated bacteremia by *P. aeruginosa* in transplant patients identified that previous ICU stay was a risk for bacteremia by CRPA in non-lung transplant recipients.⁽³¹⁾

The incidence of post-LT CRE infection also varies among transplant centers, ranging from 3% to 23%, and recent studies have reported higher rates of CRE colonization and infection.^{(71) (60) (59)} At our facility, the incidence of CRE infection is high, as evidenced by the number of colonized patients in the present study, as well as by the progressive increase in the proportion of KPC-producing strains over the study period.

An interesting aspect described in this study is that despite the fact that the rate of colonized patients who developed infection in a relatively short period of follow-up was high (27.3%), this proportion is lower than was described in other reports of LT recipients, which ranges between 39.0% and 88.9%.^{(70) (59)} One possible explanation for that finding is that *bla*_{KPC}-producing CRKP are described as more virulent than the strains that harbor other types of carbapenem resistance mechanisms and in this cohort the majority of CRE were *bla*_{KPC} negative.⁽⁸⁵⁾

There have been few studies analyzing the risk of CRE acquisition and infection in Liver transplant patients in a context of weekly screening. Giannella et al. analyzed 237 LT recipients who were screened weekly for CRE starting at LT and they identified CRE acquisition at any time (pre- or post-LT) as the variable most strongly correlated with post-LT CRE infection.⁽⁵⁹⁾ In our study, pre-LT acquisition was also the only risk factor identified for developing CRE infection.

The reported prevalence of post-transplant CRAB infection among LT recipients varies widely across facilities, ranging between 2.8% and 16.8%.^{(6) (18) (102)} In the present study, the prevalence of CRAB infection was high, 27.7%. In our analysis, we identified other factors associated with an increased risk of post-LT CRAB infection: post-LT need for dialysis, prolonged cold ischemia time, and LT performed due to fulminant hepatitis. Cold ischemia time longer than 400 minutes has previously been identified as a risk factor for SSI after LT,⁽³²⁾ and in the present study a prolonged total ischemia time was associated with an increased risk for infection by

CRPA. In general, it can be assumed that longer ischemia time is associated with slower post-LT recovery of the systemic inflammatory response, as well as, indirectly, longer ICU stays, increased use of invasive devices, and delayed recovery of renal function. ^{(103) (104)}

The need for dialysis after LT is a well-known risk factor for early postoperative bacterial infections. ⁽⁹⁴⁾ Patients requiring dialysis are more often subjected to the use of invasive devices and are more often admitted into ICU, thus having a higher incidence of bacterial infection. ^{(89) (18)} Renal failure itself plays an immunosuppressive role and can be an indirect marker of graft malfunction. Acute kidney injury can also be seen in LT recipients with severe preoperative hepatic impairment. Gouvêa et al. reported that hemodialysis after SOT is more common among recipients who develop infection with CRAB than among those who develop infection with carbapenem-susceptible *A. baumannii*. ⁽³⁷⁾

Patients undergoing LT due to fulminant hepatitis typically show high MELD scores at the time of transplantation, and such patients also often have longer ICU stays and need dialysis before LT. ⁽³²⁾ A high MELD score at LT has been associated with a greater risk of bacterial infection, specifically post-LT bacteremia caused by Enterobacteriaceae. ⁽¹⁰⁵⁾ In a univariate analysis, Hsieh et al. demonstrated that a high MELD score is a risk factor for *A. baumannii* infection after LT. ⁽³³⁾

This study has demonstrated that the acquisition of MDR GNB in pre-LT can increase the risk for developing infection by these agents in post-LT period. This was also true when specific MDR GNB were separately

analyzed, namely CRKP, CRAB and CRPA. One possible explanation for this finding is that pre-LT MDR GNB acquisition could be a marker of poorer clinical conditions, which would increase the risk of developing post-LT complications. Another possibility is that the standard antibiotic prophylaxis might have been less effective in the patients colonized by MDR GNB.

There is little knowledge regarding outcomes in SOT recipients colonized by MDR GNB before transplant, and the impact of modifying the surgical prophylaxis has not been established.^{(99) (101)} In the present study, modifying the surgical prophylaxis did not prevent the occurrence of post-LT GNB MDR infection. However, because of the small number of cases in which the prophylaxis was adjusted, it was not possible to draw a conclusion about the efficacy of that measure. At our facility, surgical prophylaxis is not systematically modified on the basis of colonization of the recipient by MDR bacteria, but rather only on the basis of preoperative infection. It is therefore likely that the patients in whom the surgical prophylaxis was modified were those who were the sickest. In addition, the total number of patients recognized as colonized before LT was too small to demonstrate the benefit of this strategy.

In addition, the PFGE analysis showed that most of the patients developed post-LT infection from the same strain that they harbored in the pre-LT period. That was also true for the patients who harbored strains other than the predominant clone. The impact that pre-LT colonization by MDR GNB has on infectious outcomes after LT has not been well defined. Kim et al. reported that, in a small number of LT recipients, colonization by

A. baumannii, identified in sputum on the day of LT, was associated with the risk of *A. baumannii*-associated pneumonia in the post-transplant period.⁽⁹⁹⁾ In another study, involving 475 LT recipients, microbiologically proven pre-LT infection was found to be a risk factor for post-LT death from MDR GNB infection.⁽¹⁸⁾

In one study, surveillance samples were collected to culture for extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae before LT⁽¹⁹⁾. The authors also found that pre-LT acquisition substantially increased the risk of infection in the post-LT period; 48% of the patients colonized in the pre-LT period developed infection with ESBL-producing Enterobacteriaceae after LT, compared with 6.7% of the patients not colonized⁽¹⁹⁾.

One study in living-donor liver transplant analyzed the impact of pre-LT colonization by CRPA in risk of infection after LT by this agent. This study did not find any correlation, however there was no active screening for detection of CRPA colonization in any time during pre and post LT period.⁽²⁴⁾

We performed an analysis for 60-day mortality risk factors in order to investigate the role of MDR GNB infection in LT recipient surveillance. The infection by carbapenem-resistant GNB showed a considerable trend toward significance as risk for 60-day mortality.

In our study infection by carbapenem-resistant GNB after LT was related to increased 60-day mortality after LT. Although the p-value showed a trend toward significance, this finding has a high biological plausibility.

Other studies that analyzed infection by MDR GNB in LT recipients usually found a high associated mortality.^{(70) (71) (35) (3)} In the survival analysis, infection by CRGNB infection had the best fitness in the final model compared to other MDR GNB infections. In spite of that, in survival plot it is possible to observe that CRAB had the highest mortality rate when compared to patients with other MDR GNB infections.

Post-SOT infection with *A. baumannii* is associated with high mortality, reportedly as high as 80.0%, and carbapenem resistance appears to increase the risk of death significantly. In literature, the mortality associated with CRE infection in LT recipients' ranges between 41% and 72%.^{(59) (70) (56) (71) (106)} Because SOT recipients infected with MDR bacteria are more likely to receive initial antibiotic therapy that is inappropriate, to have longer ICU stay, and to require mechanical ventilation, mortality rates are higher among such patients than among SOT recipients infected with bacteria that are drug-susceptible and among those without infection.^{(6) (102) (98)}

Other risk factors identified in our 60-day risk factor analysis were to have performed dialysis after LT and prolonged LT surgical time. Renal impairment was consistently associated to a lower survival in post-LT. A national wide study in Finland that included 631 LT recipients found that glomerular filtration rate is inversely proportional to patient survival.⁽¹⁰⁷⁾ Another large population based cohort study from Taiwan that included 2938 patients submitted to LT described that dialysis increase the mortality rate in up to 100%.⁽¹⁰⁸⁾

Surgical time length is a very good marker for surgery complexity. Longer surgical times were associated to increased mortality once they have a worse systemic inflammatory response syndrome, increase in the risk of infection, and are more associated to technical surgeries complication as bleeding and biliary lesions.^{(109) (110)}

In conclusion, we found that patients who acquired MDR GNB prior to LT were at a greater risk for post-LT MDR GNB infection, which has a negative impact on recipient's survival. We also found that the risk of MDR GNB infection was higher among patients submitted to re-transplantation, patients who received large amount of blood units during LT surgery and had a prolonged ICU stay. Patients who were colonized by MDR GNB when they underwent LT should be carefully evaluated for the best moment for LT, and measures for minimizing that risk, such as adjusting the antibiotic prophylaxis or decolonization treatment, should be investigated and implemented.

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7 APPENDIX

Manuscripts derived from this thesis published in index medical journals:

- A. Surveillance culture for multidrug-resistant gram-negative bacteria: Performance in liver transplant recipients.
Freire MP, Villela Soares Oshiro IC, Bonazzi PR, Pierrotti LC, de Oliveira LM, Machado AS, Van Der Heijden IM, Rossi F, Costa SF, Carneiro D'Albuquerque LA, Abdala E.
Am J Infect Control. 2017 Mar 1;45(3):e40-e44. doi: 10.1016/j.ajic.2016.12.010.
- B. Carbapenem-Resistant Enterobacteriaceae Acquired Before Liver Transplantation: Impact on Recipient Outcomes.
Freire MP, Oshiro IC, Pierrotti LC, Bonazzi PR, de Oliveira LM, Song AT, Camargo CH, van der Heijden IM, Rossi F, Costa SF, D'Albuquerque LA, Abdala E.
Transplantation. 2017 Apr;101(4):811-820. doi: 10.1097/TP.0000000000001620
- C. Carbapenem-resistant Acinetobacter baumannii acquired before liver transplantation: Impact on recipient outcomes.
Freire MP, Pierrotti LC, Oshiro IC, Bonazzi PR, Oliveira LM, Machado AS, Van Der Heijden IM, Rossi F, Costa SF, D'Albuquerque LA, Abdala E.
Liver Transpl. 2016 May;22(5):615-26. doi: 10.1002/lt.24389
- D. Polymyxin use as a risk factor for colonization or infection with polymyxin-resistant Acinetobacter baumannii after liver transplantation.
Freire MP, Van Der Heijden IM, do Prado GV, Cavalcante LS, Boszczowski I, Bonazzi PR, Rossi F, Guimarães T, D'Albuquerque LA, Costa SF, Abdala E.
Transpl Infect Dis. 2014 Jun;16(3):369-78. doi: 10.1111/tid.12210. Epub 2014 Apr 12.

Appendix A

American Journal of Infection Control 45 (2017) e40–e44

Contents lists available at ScienceDirect

American Journal of Infection Control

journal homepage: www.ajicjournal.org

Major Article

Surveillance culture for multidrug-resistant gram-negative bacteria: Performance in liver transplant recipients

Maristela Pinheiro Freire MSc ^{a,*}, Isabel Cristina Villela Soares Oshiro MSc ^a,
 Patrícia Rodrigues Bonazzi PhD ^b, Ligia Câmara Pierrotti PhD ^c,
 Larissa Marques de Oliveira MSc ^d, Anna Silva Machado PhD ^a,
 Inneke Marie Van Der Heijden PhD ^d, Flavia Rossi PhD ^e, Silvia Figueiredo Costa PhD ^{c,d},
 Luiz Augusto Carneiro D'Albuquerque PhD ^b, Edson Abdala PhD ^{b,c}

^a Infection Control Team, University of São Paulo School of Medicine Hospital das Clínicas, São Paulo, Brazil

^b Liver and Intestinal Transplant Division, Department of Gastroenterology, University of São Paulo School of Medicine, São Paulo, Brazil

^c Department of Infectious Diseases, University of São Paulo School of Medicine, São Paulo, Brazil

^d Microbiology Research Laboratory—LIM54, University of São Paulo School of Medicine Hospital das Clínicas, São Paulo, Brazil

^e Microbiology Laboratory, University of São Paulo School of Medicine Hospital das Clínicas, São Paulo, Brazil

Key Words:

Acinetobacter baumannii
Klebsiella pneumoniae
Pseudomonas aeruginosa
 Infection
 Extended spectrum β -lactamase
 Risk factor for infection

Background: The prevalence of infection with multidrug-resistant gram-negative bacteria (MDR-GNB) after solid-organ transplantation is increasing. Surveillance culture (SC) seems to be an important tool for MDR-GNB control. The goal of this study was to analyze the performance of SC for MDR-GNB among liver transplant (LT) recipients.

Methods: This was a prospective cohort study involving patients who underwent LT between November 2009 and November 2011. We screened patients for extended spectrum β -lactamase-producing *Escherichia coli*, extended spectrum β -lactamase-producing *Klebsiella pneumoniae*, and carbapenem-resistant Enterobacteriaceae, carbapenem-resistant *Pseudomonas aeruginosa* (CRPA), and carbapenem-resistant *Acinetobacter baumannii* (CRAB). We collected SC samples immediately before LT and weekly thereafter, until hospital discharge. Samples were collected from the inguinal-rectal area, axilla, and throat. The performance of SC was evaluated through analysis of its sensitivity, negative predictive value, and accuracy.

Results: During the study period, 181 patients were evaluated and 4,110 SC samples were collected. The GNB most often identified was CRAB, in 45.9% of patients, followed by CRKP in 40.3%. For all microorganisms, the positivity rate was highest among the inguinal-rectal samples. If only samples collected from this area were considered, the SC would fail to identify 34.9% of the cases of CRAB colonization. The sensitivity of SC for CRKP was 92.5%. The performance of SC was poorest for CRAB (sensitivity, 80.6%).

Conclusions: Our data indicate that SC is a sensitive tool to identify LT recipients colonized by MDR-GNB.

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The prevalence of multidrug-resistant gram-negative bacteria (MDR-GNB) as agents of infection after solid-organ transplantation (SOT) is increasing. It is estimated that 10%–20% of SOT recipients become infected with MDR-GNB.^{1–3}

* Address correspondence to Maristela Pinheiro Freire, MSc, Infection Control Service, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, Rua Dr Eneas de Carvalho Aguiar, 255, São Paulo, SP, 05403-900, Brazil.

E-mail address: maristelapf@uol.com.br (M.P. Freire).

Financial support was provided by the Fundação de Amparo à Pesquisa do Estado de São Paulo (grant No. 2010/02267-6).

Conflicts of interest: None to report.

Colonization by MDR-GNB has been described as an important risk factor for infection by those same bacteria. Gianella et al⁴ reported that liver transplant (LT) recipients colonized by carbapenem-resistant Enterobacteriaceae (CRE) were 13.9 times more likely to develop CRE infection than were their noncolonized counterparts. Another cohort study involving pre-LT screening for extended-spectrum β -lactamase (ESBL)-producing Enterobacteriaceae found that colonized patients were 7 times more likely to progress to infection.⁵

The identification of patients harboring MDR-GNB is essential for the prevention of in-hospital cross-infection with such bacteria. The guidelines for the prevention and control of MDR-GNB

infection recommend active surveillance for certain MDR-GNB, such as CRE and carbapenem-resistant nonfermenting GNB, in health care settings where their prevalence is high.⁶ The recommendation for routine pretransplant CRE screening of all SOT recipients should also be considered.⁷

The sensitivity of surveillance cultures (SCs) varies widely among MDR-GNB species, as does the positivity rate for different SC collection sites. In addition, the natural history of MDR-GNB colonization in SOT recipients has been poorly described in the literature. The sensitivity and negative predictive value of SC in LT recipients remain unclear, as does the best site from which to collect the culture samples.

It seems that SC is an important tool for MDR-GNB control and provides relevant information for the management of empirical therapy in infected LT recipients. We designed a study to analyze the performance of SC for MDR-GNB among LT recipients in the early posttransplant period and to determine the proportion of patients who evolve to invasive infection with MDR-GNB.

METHODS

This was a prospective cohort study involving all patients who underwent LT at the University of São Paulo School of Medicine Hospital das Clínicas, São Paulo, Brazil, between November 2009 and November 2011. Patients were followed from hospital admission until the end of the second month after transplantation. We excluded patients who died within the first 72 hours after transplantation. We screened all patients for ESBL-producing *Escherichia coli* (ESBL-EC), ESBL-producing *Klebsiella pneumoniae* (ESBL-KP), CRE, carbapenem-resistant *Pseudomonas aeruginosa* (CRPA), and carbapenem-resistant *Acinetobacter baumannii* (CRAB).

We collected SC samples immediately before LT and weekly thereafter, until hospital discharge or day 60 of the hospital stay. For patients who were readmitted within the first 60 days after LT, samples were also collected at readmission and on a weekly basis thereafter. Samples were obtained, by swabbing, from 3 collection sites—the inguinal-rectal area (swab collected for perineal followed by the rectal area), the axilla, and the throat—and stored in Stuart's transport medium. In patients on mechanical ventilation, we collected tracheal aspirate rather than swabbing the throat. For each patient, we obtained 6 swab samples per collection. Half of the samples were directly inoculated into brain heart infusion broth containing imipenem (10 mg/L), the other half were directly inoculated into brain heart infusion broth containing ceftriaxone (10 mg/L). All of the samples were then cultured overnight, after which they were plated on MacConkey agar. Suspected colonies were characterized through a commercial microorganism identification kit (API 20NE or API 20E; bioMérieux, Marcy l'Étoile, France). Antimicrobial susceptibility was tested by the disc diffusion method, and minimum inhibitory concentrations were interpreted according to the breakpoints established by the Clinical and Laboratory Standards Institute.⁸

During the follow-up period, clinical samples were collected for culture when any infection was suspected. Organisms were identified and antimicrobial susceptibility was tested through an automated system (VITEK 2; bioMérieux). Minimum inhibitory concentrations were again interpreted according to the breakpoints established by the Clinical and Laboratory Standards Institute.⁸ Enterobacteriaceae isolates found to be carbapenem resistant were submitted to polymerase chain reaction for the *bla_{KPC}* gene.

All patients were followed until 2 months after LT, and health care-associated infections (HAIs) were identified through active surveillance. The criteria used to identify and classify HAIs were those outlined by the National Healthcare Safety Network.⁹

We analyzed all of the cultures assuming that the process of microorganism identification (API and VITEK 2) had 100% specificity. The gold standard test was the combination of the results of SC and clinical cultures. The SC performance was evaluated through analysis of sensitivity rates (number of patients with a positive SC for specific MDR-GNB/total number of patients with specific MDR-GNB isolated in clinical or SC), negative predictive value (number of patients with negative SCs and clinical cultures for specific MDR-GNB/total number of negative SCs for specific MDR-GNB) and accuracy (number of patients with a positive SC for specific MDR-GNB plus the number of patients with a true negative SC for specific MDR-GNB/total number of patients tested). To calculate those figures, we assumed that the total number of patients testing positive for a specific MDR-GNB was the total number of patients in whom the MDR-GNB had been identified in a clinical culture or SC. For each SC collection site, the positivity rate was calculated as follows: the total number of SCs testing positive for a given MDR-microorganism at a specific collection site/the total number of SCs testing positive for that MDR-microorganism. For all patients, cultures were reviewed from 3 months before LT to 3 months following LT. We used χ^2 tests to compare categorical variables and the Kruskal-Wallis test to compare continuous variables.

In our analysis of risk factors for HAI by MDR-GNB in the first 60 days following LT, we evaluated variables related to the LT process, variables related to the LT recipient, and those variables related to hospitalization, and the type of immunosuppression therapy (standard vs tacrolimus, prednisone, and mycophenolate mofetil). The variables related to exposure time were recorded from hospital admission to the first MDR-GNB HAI diagnosis, and for the remaining patients those variables were recorded during the first 60 days following transplantation, which is considered to be the total time of risk for a given patient.

For dichotomous variables, we performed univariate analysis using the χ^2 test or Fisher exact test, according to the case. For continuous variables, we used the Mann-Whitney *U* test. Multivariate analysis was performed by stepwise binary logistic regression. The criterion for inclusion in the multivariate analysis was $P < .2$ in the univariate analysis. Variables that reduced the -2 log likelihood or showed $P < .05$ were retained in the model.

RESULTS

During the study period, 181 patients were recruited and 4,110 SC samples were collected. The patients were submitted to screening over a mean period of 3 weeks (range, 1–9 weeks). As can be seen in Table 1, CRAB was the most common GNB identified in the SCs, in 83 (45.9%) of patients, followed by carbapenem-resistant *K pneumoniae* (CRKP) in 72 patients (39.7%), and ESBL-KP in 45 patients (24.9%). Among 72 patients in whom CRKP was isolated, the polymerase chain reaction for *bla_{KPC}* was positive in 25 patients (34.7%). Among 46 patients in whom other CRE were isolated, 9 patients (19.6%) were positive for *bla_{KPC}*.

Sixty-nine patients underwent LT after testing positive for an MDR-GNB in the first SC, just before the transplant procedure. The microorganism most often identified at that point in time was CRKP, in 36 patients (19.9%). Among the patients who were colonized after LT, the median time from LT to first positive SC was 10 days and CRAB was isolated earlier than were other MDR-GNB, although the difference was not statistically significant ($P = .25$). However, among the 103 patients testing positive for CRAB, the pathogen was first identified in an SC (ie, before being identified in a clinical culture) in 67 patients (65.0%).

For all the microorganisms evaluated, the positivity rate was highest among the samples collected from the inguinal-rectal area, followed by those collected from the throat (Table 2). However, if

Table 1

Isolation of various multidrug resistant gram-negative bacteria (MDR-GNB) species in surveillance cultures and time to first positive culture

Microorganism	Among liver transplant recipients				
	MDR-GNB identified	Positive SC	Pretransplant colonization	Identification of MDR-GNB by SC*	Time from transplant to first positive SC†
	n (%)	n (%)	n (%)	n (%)	Median (range)
CRAB	103 (56.9)	83 (45.9)	21 (11.6)	67 (65.0)	9 (4–59)
CRPA	30 (16.6)	27 (14.9)	5 (2.8)	27 (90.0)	15 (7–58)
CRKP	77 (42.5)	72 (39.8)	36 (19.9)	70 (92.1)	10 (5–62)
Other CRE	47 (26.0)	42 (23.2)	16 (8.8)	42 (91.3)	13 (6–31)
ESBL-KP	54 (29.8)	45 (24.9)	25 (13.8)	43 (81.1)	15 (6–56)
ESBL-EC	33 (18.2)	30 (16.6)	16 (8.8)	30 (96.8)	12 (6–54)

CRAB, carbapenem-resistant *Acinetobacter baumannii*; CRE, carbapenem-resistant Enterobacteriaceae; CRKP, carbapenem-resistant *Klebsiella pneumoniae*; CRPA, carbapenem-resistant *Pseudomonas aeruginosa*; ESBL-EC, extended-spectrum β -lactamase-producing *Escherichia coli*; ESBL-KP, extended-spectrum β -lactamase-producing *Klebsiella pneumoniae*; SC, surveillance culture.

*Number of patients who had the identification of MDR-GNB by SC rather than clinical culture.

†Excluding patients who tested positive before transplant.

Table 2

Positivity rates of surveillance cultures, by collection site, together with sensitivity, negative predictive value, and accuracy

Microorganism	SC collection site			Statistic [‡]		
	Inguinal-rectal area	Axilla	Throat			
	Positivity [*]			Sensitivity	NPV	Accuracy
	% (n)	% (n)	% (n)	% (range)	% (range)	% (range)
CRAB	65.1 (54)	36.1 (30)	63.9 (53)	80.6 (72.9–88.2)	79.6 (71.6–87.6)	89.0 (84.4–93.5)
CRPA	84.0 (21)	25.9 (7)	55.5 (15)	90.0 (79.9–100.0)	98.1 (95.9–100.0)	98.3 (96.5–100.0)
CRKP	88.9 (64)	31.9 (23)	58.3 (42)	93.5 (89.8–99.8)	95.4 (91.5–99.3)	97.2 (94.8–99.6)
Other CRE	76.2 (32)	40.5 (17)	52.4 (22)	89.4 (83.2–99.4)	96.4 (93.3–99.5)	97.2 (94.8–99.6)
ESBL-KP	82.2 (37)	17.8 (8)	35.6 (16)	83.3 (73.4–93.3)	92.7 (88.3–97.1)	94.5 (91.9–98.2)
ESBL-EC	83.3 (25)	16.7 (5)	40.0 (12)	90.9 (81.1–100.0)	98.0 (95.8–100.0)	98.3 (96.5–100.0)

CRAB, carbapenem-resistant *Acinetobacter baumannii*; CRE, carbapenem-resistant Enterobacteriaceae; CRKP, carbapenem-resistant *Klebsiella pneumoniae*; CRPA, carbapenem-resistant *Pseudomonas aeruginosa*; ESBL-EC, extended spectrum β -lactamase-producing *Escherichia coli*; ESBL-KP, extended-spectrum β -lactamase-producing *Klebsiella pneumoniae*; NPV, negative predictive value; SC, surveillance culture.

*Positivity rate = total number of surveillance culture positive in a given site/total number of positive surveillance culture.

†Considering all 3 sites of collection.

we had considered only the SC samples collected from the inguinal-rectal area, the test would have failed to identify 34.9% of the cases of patients with CRAB, as well as 23.8%, 17.8%, 16.7%, 16.0%, and 11.1% of those with CRE, ESBL-KP, ESBL-EC, CRPA, and CRKP, respectively.

The sensitivity of SC was highest (93.5%) for CRKP, followed by ESBL-EC (90.9%). The negative predictive value of SC was highest (98.1%) for CRPA colonization, followed by colonization with ESBL-EC (98.0%). The performance of SC was poorer for patients colonized with CRAB than for any other MDR-GNB.

Analyzing only the patients with positive clinical cultures, we found the sensitivity of SC to be as follows: 71.0% (49 out of 69) for CRAB, 76.9% (10 out of 13) for CRPA, 90.0% (36 out of 40) for CRKP, 63.6% (7 out of 11) for CRE, 63.6% (14 out of 22) for ESBL-KP, and 90.0% (9 out of 10) for ESBL-EC.

The proportion of LT recipients previously colonized by a given MDR-GNB and subsequently developing infection by the same microorganism was as follows: 38.8% (26 out of 67) for CRAB, 27.3% (6 out of 22) for CRPA, 41.6% (30 out of 72) for CRKP, 44.0% (11 out of 25) for *bla*_{KPC}-positive CRKP, 14.3% (6 out of 42) for other CRE, 16.3% (7 out of 43) for ESBL-KP, and 16.7% (5 out of 30) for ESBL-EC. The proportion of patients with a negative SC who subsequently developed infection was 19.4% (19 out of 98) for CRAB, 1.8% (2 out of 109) for CRKP, 1.9% (3 out of 154) for CRPA, 0.7% (1 out of 139) for CRE, 5.1% (7 out of 136) for ESBL-KP, and 1.3% (2 out of 151) for ESBL-EC. Patients colonized by CRKP were statistically more likely to develop such infection than were those colonized by other MDR-GNB (relative risk, 1.28; 95% confidence interval, 1.04–1.58; $P = .009$).

We identified 127 post-LT HAI in 82 patients. The most common infection took place in the surgical site in 46 patients (36.2%), followed by the lower respiratory tract in 41 patients (32.3%). Thirty-one

patients (24.4%) had catheter-related bloodstream infections, 7 patients (5.5%) had urinary tract infections, and 2 patients (1.6%) had intra-abdominal infections. Risk factors for post-LT MDR-GNB HAI identified in multivariate analysis were prolonged time of cold ischemia, increased amount of transfusion blood during LT procedure, mechanical ventilation for more than 24 hours, and previous colonization by the same MDR-GNB that caused the HAI (Table 3).

DISCUSSION

This study was performed in a region where the prevalence of infection with MDR-GNB, especially CRAB and CRKP, is high. Among the LT recipients evaluated in the present study, CRKP infection was quite common, although the incidence of infection with *bla*_{KPC}-positive CRKP was only 14.9%, which is moderate when compared with that reported in other studies of LT recipients.^{10–12}

The post-LT incidence of CRE varies widely among transplant centers, ranging from 3%–23%, and recent studies have reported higher rates of CRE colonization and infection.^{4,10,11} In the present study, the SC showed good performance for identifying CRKP, with high sensitivity and a high NPV. The SC positivity rate for CRKP was highest (nearly 89%) for samples collected from the inguinal-rectal area, a finding that is in agreement with those of previous reports.^{12,13} Two important aspects related to CRKP were described in the present study. First, despite the fact that the rate of conversion from colonization to infection in a relative short period of follow-up was high (27.3%), that proportion is lower than the 39.0%–88.9% reported in other studies of LT recipients.^{4,12} One possible explanation for that finding is that *bla*_{KPC}-positive CRKP strains, which have been described as being more virulent than strains

Table 3
Univariate and multivariate analyses of risk factors for posttransplant multidrug resistant gram-negative bacteria (MDR-GNB) infection in 181 liver transplant (LT) recipients

Variable	Post-LT CRAB HAI		Univariate analysis		Multivariate analysis	
	Yes	No	Relative risk (95% confidence interval)	P value	Odds ratio (95% confidence interval)	P value
	(n = 82)	(n = 99)				
Pre-LT characteristics						
Male gender	55 (67.1)	62 (62.6)	1.14 (0.76–1.69)	.53		
Age (y)	52.5 (16–70)	53 (16–68)		.72		
Fulminant hepatitis	11 (13.4)	6 (6.1)	1.09 (0.98–1.20)	.09		
Hepatocellular carcinoma	22 (26.8)	32 (32.3)	0.93 (0.77–1.12)	.42		
Length of hospital stay before LT (d)	2 (0–46)	1 (0–67)		.08		
Bacterial infection in the last 10 days before LT	31 (37.8)	27 (27.3)	1.17 (0.95–1.44)	.13		
MELD score at LT	24 (6–52)	21 (6–53)		.004		
Surgical characteristics						
Living-donor LT	7 (8.5)	13 (13.1)	0.95 (0.86–1.05)	.33		
Combined transplantation	6 (7.3)	6 (6.1)	1.01 (0.94–1.10)	.74		
Cold ischemia time (min)	440.5 (47–960)	365 (20–754)		.002	1.03 (1.01–1.05)	.02
ASA score > III	50 (61.7)	40 (45.5)	1.43 (1.02–1.99)	.03		
Senior surgeon	38 (46.3)	53 (53.5)	0.87 (0.65–1.16)	.34		
Surgical time (min)	470 (210–850)	450 (245–1,330)		.60		
Blood transfusion during LT (units)	3 (0–15)	1 (0–9)		<.001	1.20 (1.03–1.40)	.02
Duct-to-duct biliary anastomosis	78 (95.1)	95 (96.0)	0.83 (0.21–3.21)	>.99		
Post-LT characteristics						
Reoperation*	35 (42.7)	24 (24.2)	1.32 (1.06–1.64)	.08		
Retransplantation*	14 (17.1)	3 (3.1)	1.17 (1.05–1.30)	.002		
Immunosuppression therapy with 3 drugs	39 (47.6)	43 (43.4)	1.08 (0.82–1.41)	.58		
Length of ICU stay (d)	9.5 (1–42)	5 (1–52)		<.001		
Post-LT dialysis	67 (81.7)	40 (40.4)	3.26 (2.01–5.29)	<.001		
Mechanical ventilation >24 h	67 (81.7)	42 (42.4)	3.15 (1.93–5.13)	<.001	4.20 (1.99–8.85)	<.001
Duration of CVC use (d)	10 (1–50)	5 (1–78)		<.001		
Duration of urinary catheter use (d)	9 (0–35)	5 (1–35)		.01		
Duration of abdominal drain use (d)	7 (0–44)	7 (0–34)		.74		
Duration of previous carbapenem use (d)	5.5 (0–40)	0 (0–38)		.001		
Duration of previous cephalosporin use (d)	2 (0–15)	2 (0–21)		.05		
Duration of previous piperacillin/tazobactam use (d)	1 (0–21)	0 (0–33)		.23		
Cytomegalovirus disease	10 (12.2)	13 (13.4)	0.99 (0.88–1.10)	.81		
Acute cellular rejection	10 (12.2)	19 (19.4)	0.92 (0.81–1.04)	1.19		
MDR-GNB colonization	77 (93.9)	71 (71.7)	4.64 (1.88–11.47)	<.001	5.03 (1.70–14.89)	.004

NOTE. Values for post-LT CRAB HAI are presented as n (%) or median (range).

ASA, American Society of Anesthesiologists (physical status classification); CRAB, carbapenem-resistant *Acinetobacter baumannii*; CVC, central venous catheter; HAI, health care-associated infection; MELD, Model of End-Stage Liver Disease.

*During the first 60-days after LT.

harboring other types of carbapenem resistance,¹⁴ were not commonly isolated in our patient sample; the majority of CRE evaluated in the present study tested negative for *bla_{KPC}*. The second important aspect is the difference between CRKP and the other CRE evaluated in terms of the sensitivity of the SC. In the literature, the sensitivity of SC for CRE ranges from 87.5%–100%, which is similar to that found in the present study for CRKP, although we have found the sensitivity of SC for CRE other than CRKP to be substantially lower.^{12,13} Comparisons of SC performance among CRE species are scarce in the literature.^{13,15} The most plausible explanation for the difference we found between SC sensitivity for CRKP and SC sensitivity for other CRE is the lower prevalence of the latter.

The high prevalence of MDR-GNB infection in our region might have influenced the overall rate of SC sensitivity, which was higher than that reported in other studies in the literature, especially for nonfermenting GNB. The reported sensitivity of SC for CRAB ranges from 11%–55%. In the present study it was 80.6%.^{16,17} Another possible explanation for the impressive results obtained in our study is that immunocompromised patients (such as LT recipients) have higher loads of MDR microorganisms colonizing the collection sites.

For CRAB, the SC collection site with the best positivity rate was the inguinal-rectal area, followed by the throat. Other studies have found that CRAB positivity rates are higher for throat cultures than for rectal cultures, although such studies did not collect combined samples from the groin and rectum.^{17–19} One study that analyzed different cutaneous collection sites for CRAB isolation found that

the culture sensitivity was better for samples collected from the groin–perirectal area than for those collected from the perirectal area alone, which allows us to infer that the groin is an important region for CRAB colonization and that sampling the inguinal and rectal areas at the same time might therefore increase the chance of identifying colonized patients.¹⁶ However, according to data in the literature and our findings in the present study, the use of a single SC collection site results in a significant loss of sensitivity for the detection of CRAB.¹⁹

Another important MDR-GNB related to HAIs in LT recipients is CRPA. At our facility, the prevalence of CRPA colonization was lower than the prevalence of colonization with other MDR-GNBs. In addition, the proportion of colonized patients who subsequently developed infection was lower for CRPA than for the other MDR-GNBs evaluated in the present study. In another study that evaluated living-donor LT recipients, the rate of pre-LT colonization evolving to infection in the posttransplant period was 13%.²⁰ In other populations, that rate has ranged from 19.0% (among hematopoietic stem cell transplant recipients) to 38.5% (among intensive care unit patients).^{21,22} The SC positivity rate for CRPA was highest for samples collected from the inguinal-rectal area. Other studies analyzing the rate of SC positivity for CRPA have suggested that the rectal area is the best collection site, reporting positivity rates of 47%–54%, compared with 84% in the present study.^{19,23} Among the patients for whom CRPA was isolated in clinical cultures, 76.9% had a positive SC. That was previously described in hematopoietic stem cell

transplant recipients, among whom SC identified CRPA in 53.8% of those with CRPA infection.²¹ In the present study, only approximately one-third of patients who evolved to CRPA infection had been found positive for CRPA by SC. Therefore, SC for CRPA seems to be an inefficient tool for direct empirical therapy when the result is negative, although a positive SC for CRPA should be given weight, especially in immunocompromised populations.

In the present study, we observed a low rate of infection with ESBL-producing Enterobacteriaceae. The proportion of colonized patients who developed infection was 16.3% for ESBL-KP and 16.7% for other ESBL-producing Enterobacteriaceae. One study involving screening for ESBL-producing Enterobacteriaceae in the pre-LT period found that 48% of LT recipients with a positive SC evolved to infection.⁵ In hematopoietic stem cell transplant recipients, that proportion is even higher (61%).²⁴ One possible explanation is that ESBL-producing Enterobacteriaceae are less virulent than are CRE, and the likelihood of developing infection is greater among patients colonized by the latter.¹⁴ Following this rationale, the same may be applicable to ESBL-producing Enterobacteriaceae and CRAB, given that CRAB has been described as a highly virulent pathogen in LT recipients.^{25,26} In the present study, samples collected from the inguinal-rectal area had the highest positivity rate when compared with other sites of collection; however, if SC had been performed only at the inguinal-rectal site, more than 15% of cases of ESBL-KP colonization would have been missed. The sensitivity of SC for ESBL-KP was quite low. Previous studies have reported that SC sensitivity for ESBL-producing Enterobacteriaceae ranges from 48.8%–100%, the majority of such studies evaluating only samples collected from the rectal area.^{24,27,28} Therefore, it was not possible to determine whether using SC to screen for ESBL-producing Enterobacteriaceae is a good strategy for identifying asymptomatic colonization.

In the present study SC was efficient in earlier detection of colonized patients, which may be helpful in prevention of cross-transmission and also in the choice of empirical antibiotic therapy. However, routinely screening for MDR-GNB has consequences in cost and increased number of patients with contact precautions. Therefore the policy of screening for MDR-GNB as well as which MDR-GNB screened for should be customized based on the MDR-GNB prevalence of each institution.

Our findings indicate that SC is a sensitive tool for identifying LT recipients colonized by carbapenem-resistant GNB. However, we recommend that the inguinal area be swabbed together with the rectal area in the collection of SC samples. In addition, for SCs used in screening for non-fermenting GNB, multiple anatomic sites should be sampled. Active surveillance should be implemented at transplant centers where the incidence of MDR-GNB infection is moderate to high, given that a considerable proportion of colonized patients evolve to infection.

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Appendix B

Original Clinical Science—Liver

Carbapenem-Resistant *Enterobacteriaceae* Acquired Before Liver Transplantation: Impact on Recipient Outcomes

Maristela Pinheiro Freire, MSc,¹ Isabel C. V. S. Oshiro, MSc,¹ Ligia C. Pierrotti, PhD,² Patricia R. Bonazzi, PhD,³ Larissa M. de Oliveira, PhD,⁴ Alice T. W. Song, PhD,³ Carlos H. Camargo, PhD,⁵ Inneke M. van der Heijden, PhD,⁴ Flavia Rossi, PhD,⁶ Silvia F. Costa,^{2,4} Luiz A. C. D'Albuquerque, PhD,³ and Edson Abdala, PhD^{2,3}

Background. Carbapenem-resistant *Enterobacteriaceae* (CRE) is an emergent microorganism of infections after liver transplant (LT). The aim of this study was to analyze the risk factors for CRE acquisition and infection after LT. **Methods.** This was a prospective cohort study involving patients who underwent LT in the 2010 to 2014 period. Surveillance cultures for CRE were collected immediately before LT and weekly thereafter until hospital discharge. **Results.** We analyzed 386 patients undergoing a total of 407 LTs. Before LT, 68 (17.6%) patients tested positive for CRE, 11 (16.2%) of those patients having CRE infection, whereas 119 (30.8%) patients acquired CRE after LT. Post-LT CRE infection was identified in 59 (15.7%) patients: *Klebsiella pneumoniae* was isolated in 83.2%; surgical site infection was the most common type of infection (46.7%). Multivariate analysis showed that post-LT dialysis was the only risk factor for post-LT CRE acquisition. Eighty-two percent of patients who underwent 3 or more post-LT dialysis sessions and acquired CRE before LT evolved with post-LT CRE infection. Other risk factors for CRE infection were acquisition of CRE post-LT, Model for End-Stage Liver Disease score greater than 32, combined transplantation, and reoperation. Patients who acquired CRE before LT had a high risk of developing CRE infection ($P < 0.001$). **Conclusions.** Measures for minimizing that risk, including altering the antibiotic prophylaxis, should be investigated and implemented.

(*Transplantation* 2017;101: 811–820)

The prevalence of multidrug-resistant gram-negative bacteria (MDR-GNB) as agents of infection after solid organ transplantation (SOT) is increasing. It is estimated that 10% to 20% of SOT recipients become infected with MDR-GNB.^{1–3} Such infections occur most often in the early posttransplant period, and the site of infection is usually related to the transplant process. Various studies have reported decreased survival in SOT recipients infected with MDR-GNB.^{2–6} Among the emergent MDR-GNB are carbapenem-

resistant *Enterobacteriaceae* (CRE), and the incidence of infection with CRE has increased worldwide.⁷

In liver transplantation (LT) recipients, CRE infections occur in the early posttransplant period, the median time from LT to CRE infection ranging from 12 to 24 days.^{8–10} Such infections usually present with bacteremia, and intra-abdominal infection is identified in 55% to 79% of the cases.^{8,9} Because of the high associated mortality, CRE infection has a significant impact on first-year survival.^{8,10} Knowledge of the risk factors for developing CRE infection in LT recipients is crucial to the development of effective prevention strategies. In the general population,

Received 9 August 2016. Revision received 11 October 2016.

Accepted 7 November 2016.

¹ Infection Control Team, University of São Paulo School of Medicine Hospital das Clínicas, São Paulo, Brazil.

² Department of Infectious Diseases, University of São Paulo School of Medicine, São Paulo, Brazil.

³ Liver and Intestinal Transplant Division, Department of Gastroenterology, University of São Paulo School of Medicine, São Paulo, Brazil.

⁴ Microbiology Research Laboratory LIM54, University of São Paulo School of Medicine Hospital das Clínicas, São Paulo, Brazil.

⁵ Centro de Bacteriologia, Instituto Adolfo Lutz, São Paulo, Brazil.

⁶ Microbiology Laboratory, University of São Paulo School of Medicine Hospital das Clínicas, São Paulo, Brazil.

This study received financial support from the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, São Paulo Research Foundation; Grant no. 2010/02267-6).

The authors declare no conflicts of interest.

M.P.F. participated in the performance of the research, in the writing of the article and in data analysis. I.C.V.S.O. participated in the performance of the research and

in the data collection. L.C.P. participated in the performance of the research and in the writing of the article. P.R.B. participated in the performance of the research. L.M.D.O. participated in the performance of the research. A.W.S. participated in the performance of the research. C.H.C. participated in the performance of the research. I.M.v.d.H. participated in the performance of the research. F.R. participated in the performance of the research. S.F. C. participated in the performance of the research and in the revised the article. L.A.C.D'A. participated as general supervision of transplant unit and in the performance of the research. E.A. participated in research design, in the performance of the research and in the writing of the article.

Correspondence: Maristela Pinheiro Freire, Infection Control Team, University of São Paulo School of Medicine Hospital das Clínicas, São Paulo, Brazil. (maristela@uol.com.br).

Supplemental digital content (SDC) is available for this article. Direct URL citations appear in the printed text, and links to the digital files are provided in the HTML text of this article on the journal's Web site (www.transplantjournal.com).

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ISSN: 0041-1337/17/10104-811

DOI: 10.1097/TP.0000000000001620

previous colonization by CRE is one of the major risks for subsequent infection.¹¹ However, there have been few studies involving active surveillance of LT recipients. Prospective studies designed to analyze risk factors for CRE infection should include such screening, especially in areas where the incidence of such infection is high.

The aim of this study was to analyze the risk factors for CRE acquisition and infection after LT in a population submitted to weekly screenings for CRE. We also analyze the impact that pre-LT acquisition of CRE has on the risk of post-LT CRE infection and early mortality.

MATERIALS AND METHODS

Study Design and Patient Sample

This was a prospective cohort study involving all patients who underwent LT between January 2010 and December 2014 at the University of São Paulo School of Medicine, in Brazil. Patients were followed up from hospital admission until the end of the second month after transplantation. We excluded patients who died within the first 48 hours after transplantation.

Infections were identified through active surveillance on the LT ward and through the review of outpatient records. The criteria used in order to identify and classify healthcare-associated infections (HAIs) were those outlined by the National Healthcare Safety Network,¹² the only exception being that surgical site infections (SSIs) meeting the criteria were reported for up to 60 days after LT. All patients with CRE detected were isolated in a separate room and maintained in contact precaution during all hospital stay and subsequent admissions. Colonization was defined as CRE being isolated in cultures of surveillance or clinical specimens, with no evidence of infection. We defined CRE acquisition as having a surveillance or clinical specimen that was positive for CRE, with no evidence of CRE isolated in any culture within the last 2 years. We defined CRE as strains of Enterobacteriaceae resistant to at least one carbapenem. We defined effective treatment as having been treated for 48 hours or longer with at least 1 antimicrobial agent proven to display *in vitro* activity against CRE.

The standard surgical prophylaxis in use during the study period was a 48-hour course of ampicillin with cefotaxime. For cases in which patients underwent LT during treatment for an infection, as well as for those in which the donor had a suspected or confirmed infection, the prophylaxis protocol was modified. In such cases, the antibiotic used for prophylaxis was the same as that used for the treatment of the infection in question. The standard immunosuppression regimen was tacrolimus plus a corticosteroid, and immunosuppression was induced by administration of methylprednisolone.

During the follow-up period, clinical samples were collected for culture when any infection was suspected. To identify organisms and perform antimicrobial susceptibility testing, we used an automated system (VITEK 2; bioMérieux, Marcy l'Étoile, France). Minimum inhibitory concentrations (MICs) were interpreted according to the breakpoints established by the Clinical and Laboratory Standards Institute.¹³ Enterobacteriaceae for which carbapenem resistance were classified using CLSI criteria older than those published in June 2010 were reclassified using the newer breakpoints. All CRE strains were submitted to polymerase chain reaction for the *bla_{KPC}* and *bla_{CTXM}* gene.

Surveillance Cultures

Surveillance cultures (SC) were collected immediately before LT (independently of results of previous cultures) and weekly thereafter, until hospital discharge. Samples were obtained with perirectal swabs and stored in Stuart's transport medium. The samples were directly inoculated into brain heart infusion broth containing imipenem (1 µg/mL) and were cultured overnight, after which they were plated on MacConkey agar and all suspect. As in the case of the clinical samples, we used the VITEK 2 system (bioMérieux) to identify organisms and perform antimicrobial susceptibility testing, and we interpreted MICs according to the breakpoints established by the Clinical and Laboratory Standards Institute.¹³ For all strains isolated in clinical or SCs, polymyxin resistance was defined as an MIC > 2 mg/L in broth microdilution.¹³

Pulsed-Field Gel Electrophoresis Analysis

After extraction and digestion of whole bacterial DNA with XbaI restriction enzyme (50 units; New England Biolabs, UK), we performed pulsed-field gel electrophoresis (PFGE), as described by Gautom et al,¹⁴ using an electric field system (CHEF-DR III; Bio-Rad Laboratories, Hercules, CA), in accordance with the CDC protocol.¹⁵ The running parameters were as follows: initial switch time, 2.2 seconds; final switch time, 54.2 seconds; total run time, 19 hours; temperature, 14°C; and voltage, 6 V/cm. The PFGE images were processed and analyzed with BioNumerics software, version 7.1 (Applied Maths, Sint-Martens-Latem, Belgium). The images were normalized through the use of standard molecular markers, and banding patterns were compared. A similarity analysis was performed using Dice coefficients, with a band position tolerance of 1.5% and an optimization of 1.5%. Isolates were separated into similarity clusters by the unweighted pair group method with arithmetic mean. Only bands larger than 45.5 kb were included in the analysis.

Statistical Analysis

In our analysis of risk factors for CRE acquisition, infection, and mortality, we included each patient only once, regardless of the number of transplants they underwent, analyzing only the first LT performed during the study period. We evaluated the following variables related to the LT process: cold ischemia time, number of units of blood transfused intraoperatively, reoperation, retransplantation in the first 60 days after LT, level of experience of the lead surgeon (more than 5 years), American Society of Anesthesiologists physical status classification, surgical time, donor type (living or deceased), extensiveness of the procedure (liver-only or combined liver-kidney transplantation), the type of surgical prophylaxis, biliary complication (stricture, leak, sphincter of Oddi dysfunction), and type of biliary anastomosis. We also evaluated variables related to the LT recipient: age; sex; presence or absence of fulminant hepatitis, hepatocellular carcinoma; Model for End-Stage Liver Disease (MELD) score; and pretransplant serum creatinine level. In addition, we evaluated variables related to hospitalization: length of hospital stay before LT; post-LT need for dialysis; duration of abdominal drainage; and infection in the last week before transplantation. For the patients who evolved to CRE acquisition or infection, the variables related to exposure time were recorded from hospital admission to the first CRE-positive culture or from hospital admission to infection, respectively.

Dichotomous variables were considered positive if the event occurred before the first CRE-positive culture (for acquisition) or before the patient developed CRE infection. For the remaining patients, those variables were recorded for the total time at risk during the first 60 days after transplantation.

The main outcome measure was CRE infection during the first 60 days after transplantation (post-LT CRE infection). A secondary outcome measure was CRE acquisition during that same period (post-LT CRE acquisition). In our analysis of the risk of post-LT CRE acquisition, we excluded patients in whom CRE was detected before LT. In analyzing the risk of post-LT CRE infection, we excluded patients who underwent LT with active CRE infection, although we included patients in whom CRE had previously been identified but who showed no evidence of infection.

For dichotomous variables, we performed univariate analysis using the χ^2 test or Fisher exact test, as appropriate. For continuous variables, we used the Mann-Whitney test. Continuous variables were transformed into dichotomous variables through cluster analysis. Multivariate analysis was performed by stepwise binary logistic regression. The criterion for inclusion in the multivariate analysis was *P* value less than 0.2 in the univariate analysis. Variables that then reduced the -2 log likelihood or showed *P* value less than 0.05 were retained in the model. We also used decision tree analysis—specifically the classification and regression tree (CART) analysis method—to identify risk factors for infection with CRE. The CART analysis was performed on the entire dataset, with chi-square automatic interaction detection. We performed internal validation using tenfold cross-validation. Each predictor variable was tested within the overall cohort, and the statistically significant predictor with the largest odds ratio (OR) was selected manually to form the split. Each split created 2 nodes; ORs were recalculated, and the process was repeated within each node. This process proceeded iteratively until either or both of the following conditions were satisfied: there were no remaining variables with a statistically significant OR ($P < 0.05$); and a further split yielded a subgroup with fewer than 15 total patients. For recursive partitioning, we used CART in the program R (<http://www.R-project.org/>).

For the survival analysis, the outcome measure used was death within the first 60 days after the first LT. Univariate and multivariate analysis were performed by Cox regression. Data were processed and analyzed with the SPSS Statistics software package, version 22.0 (IBM Corporation, Armonk, NY).

This study was approved by ethical review board of University of São Paulo School of Medicine.

RESULTS

Clinical and Epidemiological Characteristics

During the study period, 401 patients underwent LT. Of those 401 patients, 15 (3.7%) died within the first 48 hours after LT. Therefore, we analyzed 386 patients, who collectively underwent a total of 407 LTs. In the first LT, 355 (92.0%) of the patients received a deceased donor organ. Of the 386 LT recipients, 240 (62.2%) were male. The median age was 53 years (range, 14–72 years). The most common underlying disease, observed in 149 (38.6%) of the recipients, was HCV-related cirrhosis (Table 1).

TABLE 1.

Demographic characteristic of patients undergoing LT

Characteristics	N = 386
Male sex, n (%)	240 (62.2)
Age: median (range), y	53 (14–72)
Diagnosis, n (%) ^a	
HCV infection	149 (38.6)
Hepatocellular carcinoma	126 (32.6)
Alcoholic cirrhosis	78 (20.2)
Cryptogenic cirrhosis	37 (9.6)
Fulminant hepatitis	37 (9.6)
HBV infection	32 (8.3)
Autoimmune hepatitis	15 (3.9)
Nonalcoholic steatohepatitis	11 (2.8)
Primary sclerosing cholangitis	10 (2.6)
Budd-Chiari syndrome	9 (2.3)
Primary biliary cirrhosis	8 (2.1)
Wilson disease	5 (1.3)
Other	34 (8.8)
Living donor in first LT, n (%)	31 (8.0)
Liver-kidney transplantation, n (%)	23 (6.0)
Cold ischemia time: median (range), min	410 (20–960)
Early retransplantation, ^b n (%)	46 (11.9)
Reoperation, n (%)	111 (28.8)
Post-LT dialysis, n (%)	186 (48.2)

^a Patients could have more than 1 diagnosis.

^b Performed in the first 60 days after LT.

HCV, hepatitis C virus; HBV, hepatitis B virus.

Pretransplant CRE Acquisition

In 68 (17.6%) patients, CRE species were identified before LT, 2 different species being isolated in 4 (5.9%) patients. Therefore, there were a total of 72 isolates. The median time between the last positive culture and LT was 1 day (range, 0–42 days). Of the 72 isolates, 70 (97.2%) were tested for *bla*_{KPC}, of which 33 (47.1%) tested positive. Eleven (16.2%) of the 68 patients had CRE infection in the pre-LT period: 8 (73.0%) of those patients had CRE isolated in blood; and the most common type of infection was spontaneous peritonitis, in 4 (36.5%). Among those 11 patients, the median time between CRE infection and LT was 3 days. Of those, 8 patients underwent LT with active infection. However, even patients who had no evidence of infection were given CRE treatment until LT. Of 57 patients who did not develop infection in pre-LT period, only 2 (3.5%) received antibiotic prophylaxis that included an antimicrobial agent with proven activity against CRE. Of the 68 patients in whom CRE were identified in the pre-LT period, 28 (36.8%) evolved to post-LT CRE infection.

Post-LT CRE Acquisition

During the post-LT period, 119 (30.8%) of the 386 patients acquired CRE after LT. In 7 (5.8%) cases, the patient tested positive for 1 CRE species in the pre-LT period and acquired a different species in the post-LT period (Figure 1). The most common species isolated in the post-LT period was *Klebsiella pneumoniae*, in 99 patients (83.2%). Of those 99 isolates, 26 (26.3%) were resistant to polymyxin. The SCs were positive in 111 (93.3%) of the 119 patients who acquired CRE after LT. In 106 (89.1%) patients, the first

FIGURE 1. Distribution of CRE infection according time of CRE acquisition.

CRE-positive culture was a SC, and 39 (36.8%) of those patients developed post-LT CRE infection.

Of the 119 CRE species identified in the post-LT period, 112 (92.6%) were tested for *bla*_{KPC}, of which 52 (46.4%) tested positive, among *bla*_{KPC} negative strains, 47 (78.3%) strains were positive by for CTX-M group 8 to 25 and 10 strains (16.7%) were positive for CTX-M group 1-2-9.

As can be seen in Figure 2, the proportion of CRE strains that were tested positive for *bla*_{KPC} increased over the study period ($P = 0.07$). No other carbapenemase was identified in study period.

Post-LT CRE Infection

In the post-LT period, 60 CRE infections were identified in 59 (15.7%) of the 386 patients. The most common type of infection was SSL, all classified as organ space, which was seen

in 28 (46.7%) of those 59 patients, followed by catheter-related bloodstream infection, in 11 (18.3%). The median time between LT and the first post-LT CRE infection was 11 days (range, 2-60 days), the median time between identified CRE acquisition and infection was 8 days (range, 0-219 days). Ten (16.9%) of the 59 patients developed a CRE infection despite showing no evidence of previous positive SC. Bacteremia was detected in 30 (50.0%) of the 60 post-LT CRE infections. Six patients evolved to death before starting the antibiotic therapy for CRE infection. Among those who received treatment, 15 (27.8%) received monotherapy and 72.2% (39) received combined therapy. The most common treatments used were carbapenem plus colistin (11-20.4%), and carbapenem + colistin + amikacin (11-20.4%) followed by carbapenem + colistin + tigecycline + amikacin (6-11.1%). Of the 59 patients with 60

FIGURE 2. Proportion of isolated strains that were *bla*_{KPC}-producing, as well as the proportion that showed an elevated MIC for polymyxin, among 99 patients found to be infected with carbapenem-resistant *Klebsiella pneumoniae* between January 2010 and December 2014.

TABLE 2.

Type of infection, concomitant bacteremia, time to infection after LT, and 60-day mortality in 59 patients with post-LT Carbapenem-resistant Enterobacteriaceae infection

Type of infection	n (%)	Bacteremia	Positive PCR for <i>bla_{KPC}</i>	Time from LT to infection (days)	Mortality ^a
		n (%)	n (%)	Median (range)	n (%)
SSI	28 (46.7)	13 (46.4)	22 (78.6)	11.0 (2-28)	19 (66.9)
Catheter-related bloodstream infection	11 (18.3)	11 (100.0)	8 (72.7)	17.0 (3-59)	3 (27.3)
Respiratory tract infection	8 (13.3)	2 (25.0)	8 (100.0)	7.5 (4-25)	5 (62.5)
Urinary tract infection	7 (11.7)	0	5 (71.4)	22.0 (10-58)	3 (42.9)
Primary bloodstream infection	4 (6.7)	4 (100.0)	1 (25.0)	33.5 (9-60)	0
Osteomyelitis	1 (1.7)	1 (100.0)	1 (100.0)	46	0
Balanoposthitis	1 (1.7)	0	0	5	0
Total	60 (100)	30 (50.0)	45 (75.0)	11 (2-60)	30 (50.8)

^a N = 59.

PCR, polymerase chain reaction.

post-LT CRE infection, 30 (50.8%) died within the first 60 days after LT (Table 2), no specific type of treatment was associated with a lower mortality.

Risk Factors

In the multivariate analysis, post-LT dialysis was identified as the only risk factor for post-LT CRE acquisition (Table 3).

Risk factors for developing a CRE infection were pre-LT CRE acquisition, MELD score greater than 32 at LT, combined transplant, reoperation, 3 or more post-LT dialysis sessions, and post-LT CRE acquisition (Table 4). In the CART analysis, the most important variable for discriminating between LT recipients who would and would not develop CRE infection was pre-LT CRE acquisition. The other major

TABLE 3.

Univariate and multivariate analyses of risk factors for post-liver transplantation carbapenem resistant Enterobacteriaceae acquisition in 325 LT recipients

Variable	Post-LT carbapenem resistant Enterobacteriaceae acquisition		Univariate analysis		Multivariate analysis	
	Yes	No	RR (95% CI)	P	OR (95% CI)	P
	(n = 119)	(n = 206)				
Pre-LT characteristics						
Male gender, n (%)	71 (59.7)	131 (63.6)	0.90 (0.68-1.20)	0.48		
Age: median (range), y	52 (14-71)	54 (16-52)	—	0.35		
Fulminant hepatitis, n (%)	12 (10.1)	20 (9.7)	1.00 (0.93-1.08)	0.91		
Hepatocellular carcinoma, n (%)	39 (32.8)	76 (36.9)	0.94 (0.80-1.11)	0.45		
Pre-LT hospital stay: median (range), d	1 (0-55)	1 (0-56)	—	0.01	1.03 (1.00-1.05)	0.09
Bacterial infection in the last 10 d before LT, n (%)	17 (14.3)	31 (15.0)	0.99 (0.90-1.09)	0.85		
Pre-LT serum creatinine	1.16 (0.47-10.54)	0.96 (0.32-10.9)	—	0.14		
MELD score at LT, median (range)	25 (5-57)	24 (6-56)	—	0.06		
Surgical characteristics						
Living-donor LT, n (%)	13 (10.9)	16 (7.8)	1.04 (0.96-1.12)	0.34		
Combined transplantation, n (%)	7 (5.9)	12 (5.8)	1.00 (0.95-1.06)	0.98		
Cold ischemia time: median (range), min	410 (81-740)	410 (15-910)	—	0.43		
ASA score > III, n (%)	67 (57.3)	95 (48.7)	1.20 (0.93-1.54)	0.14		
Senior surgeon, n (%)	26 (21.8)	60 (29.1)	0.91 (0.80-1.03)	0.15		
Roux anastomosis, n (%)	6 (5.0)	11 (5.3)	1.00 (0.95-1.05)	0.90		
Surgical time: median (range), min	420 (155-1150)	450 (250-1330)	—	0.09		
Blood transfusion during LT: median (range), units	2 (0-26)	1 (0-10)	—	0.31		
Post-LT characteristics						
Reoperation, ^a n (%)	32 (26.9)	41 (19.9)	1.10 (0.96-1.25)	0.15		
Retransplantation, ^a n (%)	14 (11.8)	26 (12.6)	0.99 (0.91-1.08)	0.82		
Biliary complication, n (%)	15 (12.6)	14 (6.8)	1.07 (0.99-1.15)	0.08		
Length of ICU stay: median (range), d	9 (1-73)	6 (2-71)	—	0.003		
Dialysis after LT, n (%)	62 (52.1)	78 (37.9)	1.30 (1.05-1.61)	0.13	1.67 (1.05-2.66)	0.03
Duration of drainage: median (range), d	8 (0-41)	8 (0-69)	—	0.60		

^a Performed in the first 60 days after LT.

ASA, American Society of Anesthesiologists (physical status classification); ICU, intensive care unit.

TABLE 4.
Univariate and multivariate analyses of risk factors for post-LT carbapenem-resistant enterobacteriaceae infection in 378 LT recipients

Variable	Patients with carbapenem-resistant enterobacteriaceae infection		Patients without carbapenem-resistant enterobacteriaceae infection		Univariate analysis		Multivariate analysis	
	(n = 54)		(n = 324)		RR (95% CI)	P	OR (95% CI)	P
Pre-LT characteristics								
Male gender, n (%)	30 (55.6)		206 (63.6)		0.82 (0.59-1.14)	0.26		
Age, in years, median (range)	52.0 (14-72)		54.0 (14-72)		—	>0.99		
Fulminant hepatitis, n (%)	5 (9.3)		31 (9.6)		1.00 (0.91-1.09)	>0.99		
Hepatocellular carcinoma, n (%)	12 (22.2)		113 (34.9)		0.84 (0.71-0.99)	0.07		
Pre-LT hospital stay, in days, median (range)	3 (0-46)		1 (0-96)		—	0.001		
Pre-LT CRE infection, n (%)	1 (1.9)		3 (0.9)		1.00 (0.97-1.05)	0.46		
Pre-LT CRE acquisition, n (%)	24 (44.4)		36 (11.1)		1.60 (1.26-2.04)	<0.001	12.75 (5.59-29.07)	<0.001
Pre-LT serum creatinine	1.59 (0.49-4.52)		1.03 (0.32-10.9)		—	0.003		
MELD score at LT > 32, n (%)	19 (35.2)		48 (14.8)		1.31 (1.07-1.61)	<0.001	2.06 (1.01-4.18)	0.05
Surgical characteristics								
Deceased-donor LT, n (%)	49 (90.7)		298 (92.0)		0.87 (0.35-2.16)	0.79		
Combined transplantation, n (%)	6 (11.1)		16 (4.9)		1.07 (0.97-1.18)	0.07	3.08 (1.00-9.84)	0.05
Cold ischemia time, in minutes, median (range)	416.0 (81-758)		410.0 (15-910)		—	0.43		
ASA score > III, n (%)	31 (57.4)		163 (50.3)		1.17 (0.84-1.62)	0.33		
Surgical prophylaxis with antibiotic active against CRE	1 (1.9)		9 (2.8)		0.51 (0.08-3.28)	0.78		
Senior surgeon, n (%)	21 (38.9)		97 (29.9)		1.15 (0.92-1.44)	0.19		
Roux anastomosis	5 (9.3)		17 (5.2)		1.04 (0.96-1.14)	0.24		
LT surgical time <365 min, n (%)	34 (63.0)		248 (76.5)		0.63 (0.43-0.94)	0.03		
Blood transfusion during LT, in units, median (range)	2.0 (0-26)		1.0 (0-44)		—	0.04		
Post-LT characteristics								
Reoperation, ^a n (%)	18 (33.3)		59 (18.2)		1.23 (1.01-1.49)	0.01	2.28 (1.04-5.03)	0.04
Retransplantation, ^a n (%)	8 (14.8)		37 (11.7)		1.04 (0.92-1.17)	0.48		
Biliary complications	7 (13.0)		25 (7.7)		1.06 (0.95-1.18)	0.20		
≥ 3 post-LT dialysis sessions, n (%)	33 (61.1)		111 (34.3)		1.69 (1.20-2.38)	<0.001	2.06 (1.04-4.32)	0.04
Colonization by <i>bla_{CEC}</i> -positive CRE, n (%)	38 (70.4)		39 (12.5)		2.95 (1.95-4.69)	<0.001		
CRAB colonization, n (%)	22 (40.7)		103 (31.8)		1.15 (0.91-1.45)	0.20		
Post-LT CRE acquisition, n (%)	25 (46.3)		84 (25.9)		1.38 (1.07-1.78)	0.002	4.14 (1.93-8.90)	<0.001
Post-LT polymyxin-resistant CRE acquisition, n (%)	21 (38.9)		15 (4.6)		1.56 (1.26-1.93)	<0.001		
Length of ICU stay, in days, median (range)	10.5 (1-46)		7.0 (1-102)		—	0.07		
Duration of drainage, in days, median (range)	8 (0-30)		8.0 (0-69)		—	0.67		

^a performed in the first 60 days after LT.

CRAB, carbapenem-resistant *Acinetobacter baumannii*.

FIGURE 3. CART analysis of risk factors for infection with CRE among 386 patients undergoing LT. In blue boxes the incidence of CRE infection in that subgroup, in node square the P value for the prediction of the outcome in this particular subgroup.

risk factor for post-LT CRE infection was requiring 3 or more post-LT dialysis sessions, which was found to increase that risk in regardless of the moment of CRE acquisition (Figure 3). When only CRE colonized patients were analyzed, the risk factors for developing CRE infection were length of time of abdominal drainage ($P = 0.02$; OR, 1.05 [1.01-1.09, 95%CI]), whether dialysis was performed after LT ($P = 0.01$; OR, 2.80 [1.28-6.15, 95%CI]), acquisition of CRE before LT ($P = 0.03$; OR, 2.20 [1.11-4.39, 95%CI]); combined transplant was almost statistically significant ($P = 0.06$; OR, 3.56 [0.94-13.53, 95%CI]).

The overall 60-day mortality rate was 27.4%. The most common cause of death was bacterial or fungal infection (50.9%), followed by primary graft dysfunction (13.2%),¹⁴ and surgical complications (8.5%).⁹ Among the patients who developed a CRE infection, 60-day mortality was 50.0%. Risk factors for 60-day mortality were fulminant hepatitis as the cause of LT, surgical time longer than 550 minutes, post-LT dialysis, and HAI with a *bla*_{KPC}-positive CRE species. The LT being performed by a senior surgeon was identified as a protective factor (Table 5).

PFGE

We analyzed 62 strains of carbapenem-resistant *K. pneumoniae* from 47 patients and identified 25 different PFGE clusters. A pulsotype A1 cluster was observed in the first quarter of 2010, and a pulsotype B1 cluster, in the second quarter of 2010. Of the 62 strains isolated, 26 were

KPC-producing. In 12 patients who developed post-LT CRE infection, paired strains were analyzed (from SCs— from clinical cultures related to infection). In 5 patients, pre-LT and post-LT paired strains were analyzed. Among those, 4 clusters were identified, and all paired strains belonged to the same cluster. In 7 patients, paired post-LT SCs and clinical cultures related to infection were analyzed. Seven PFGE clusters were identified, and all paired strains belonged to the same cluster (Figure S1, SDC, <http://links.lww.com/TP/B384>).

DISCUSSION

The incidence of post-LT CRE infection varies widely among transplant centers, ranging from 3% to 23%, and recent studies have reported higher rates of CRE colonization and infection.^{9,16,17} At our facility, the incidence of CRE colonization is high, and a progressive increase in the proportion of KPC-producing strains was noticed over the study period.¹⁸ Because of the high prevalence of CRE colonization, 15% of our patients developed post-LT CRE infection. However, the proportion of colonized patients who developed infection was lower than that reported in previous studies^{10,17}—36.8% versus 48.8% to 88.9%.

There have been few studies analyzing the risk of CRE acquisition and infection in LT patients in a context of weekly screening. Giannella et al¹⁷ analyzed 237 LT recipients who were screened weekly for CRE starting at LT and identified CRE acquisition at any time as the variable most strongly

TABLE 5.**Univariate and multivariate analyses of risk factors for 60-day mortality in 386 LT recipients**

Variable	Survival	Nonsurvival	Univariate analysis		Multivariate analysis	
	(n = 280)	(n = 106)	HR (95% CI)	P	HR (95% CI)	P
Pre-LT characteristics						
Male sex, n (%)	178 (63.8)	62 (58.5)	1.15 (0.80-1.69)	0.49		
Age: median (range), y	54 (14-71)	51 (16-52)	0.99 (0.98-1.00)	0.13		
Fulminant hepatitis, n (%)	16 (5.7)	21 (19.8)	3.00 (1.80-4.67)	<0.001	2.06 (1.18-3.59)	0.01
Hepatocellular carcinoma, n (%)	98 (35.0)	28 (26.4)	0.72 (0.47-1.11)	0.13		
Pre-LT hospital stay: median (range), d	1 (0-28)	2 (0-56)	1.01 (0.99-1.02)	0.24		
Pre-LT bacterial infection, n (%)	53 (19.0)	31 (29.2)	1.61 (1.06-2.45)	0.03		
Pre-LT CRE acquisition, n (%)	42 (15.0)	26 (24.5)	1.64 (1.05-2.55)	0.03		
MELD score at LT >32, n (%)	39 (13.9)	34 (32.1)	2.32 (1.54-3.49)	<0.001	1.42 (0.90-2.22)	0.13
Surgical characteristics						
Deceased-donor LT, n (%)	260 (92.9)	95 (89.6)	0.73 (0.39-1.36)	0.32		
Combined transplantation, n (%)	13 (4.6)	10 (9.4)	1.87 (0.97-3.58)	0.06		
Cold ischemia time: median (range), min	405 (15-786)	417 (15-910)	1.00 (0.99-1.00)	0.74		
ASA score > III, n (%)	135 (48.2)	66 (62.3)	1.58 (1.06-2.35)	0.03		
Senior surgeon, n (%)	78 (27.9)	44 (41.5)	1.67 (1.14-2.46)	0.009	1.60 (1.05-2.43)	0.03
LT surgical time < 550 min, n (%)	232 (82.9)	74 (69.8)	0.54 (0.36-0.82)	0.004	0.65 (0.41-0.86)	0.02
Blood transfusion during LT: median (range), units	1 (0-10)	2 (0-44)	1.08 (1.05-1.11)	<0.001		
Post-LT characteristics:						
Reoperation, ^a n (%)	66 (23.6)	45 (42.5)	1.88 (1.28-2.77)	<0.001	0.69 (0.45-1.05)	0.85
Retransplantation, ^a n (%)	24 (8.6)	25 (23.6)	3.38 (1.19-9.48)	<0.001	1.51 (0.95-2.40)	0.80
Post-LT dialysis, n (%)	103 (36.8)	90 (84.9)	6.96 (4.08-11.85)	<0.001	5.38 (3.02-9.57)	<0.001
Colonization by <i>bla</i> _{PC} -positive CRE, n (%)	55 (20.4)	28 (26.7)	0.80 (0.52-1.23)	0.31		
CRAB colonization, n (%)	80 (28.6)	48 (45.3)	1.76 (1.20-2.58)	0.004		
Post-LT CRE acquisition, n (%)	90 (32.1)	29 (27.4)	0.76 (0.50-1.17)	0.22		
Post-LT polymyxin-resistant CRE acquisition, n (%)	14 (5.0)	9 (8.5)	1.03 (0.57-1.88)	0.92		
HAI, n (%)	143 (51.1)	77 (72.6)	2.07 (1.35-3.17)	0.001		
HAI caused by MDR bacteria, n (%)	72 (25.7)	61 (57.5)	2.81 (1.91-4.13)	<0.001		
Post-LT CRE infection, n (%)	31 (11.1)	28 (26.4)	2.05 (1.33-3.15)	0.001		
Post-LT HAI with <i>bla</i> _{PC} -positive CRE, n (%)	18 (6.4)	26 (24.5)	2.69 (1.73-4.20)	<0.001	1.66 (1.02-2.69)	0.041

^a Performed in the first 60 days after LT.

correlated with post-LT CRE infection. In our study, previous colonization was also the most important risk factor for developing CRE infection, although we found pre-LT acquisition to be associated with a higher risk than was post-LT acquisition. One possible explanation for this finding is that pre-LT CRE acquisition could be a marker of poorer clinical conditions. Another is that the standard antibiotic prophylaxis might have been less effective in the patients colonized by CRE.

There are few data regarding outcomes in SOT recipients colonized by MDR-GNB before transplantation, and the impact of modifying the surgical prophylaxis has not been established.^{17,19,20} One study of infection with carbapenem-resistant *Acinetobacter baumannii* also reported that pre-LT acquisition is a risk factor for post-LT infection.²¹ However, the authors of that study found that adjusting the antibiotic prophylaxis was not preventive of infection. Our institutional protocol did not recommend adjusting prophylaxis regarding patient colonization. As a result, the number of patients in whom the antibiotic prophylaxis included an antimicrobial agent with proven activity against CRE was very small, which prevents us from drawing any conclusions regarding that at this time.

Although pre-LT CRE acquisition had a significant impact on the risk of developing CRE infection in our study, post-LT

acquisition was the second most important risk factor. This finding is similar to that reported by Giannella et al,¹⁷ supporting the recommendation to perform systematic post-LT SC for CRE at facilities where the prevalence of CRE infection is high. Early identification can increase the precision of empirical therapy in patients with infection and could reduce the mortality.²²

The need for dialysis was also identified as a risk factor for post-LT CRE infection in our study. Requiring dialysis is a well-documented risk factor for bacterial infection in LT and has previously been shown to increase the risk of CRE infection, as well as for infection with other MDR-GNB, in LT recipients.^{17,21,23} It is of note that, in the present study, dialysis increased the risk of CRE infection to a greater degree for the patients who had acquired CRE in the pre-LT period than for those who had not. The possible explanation for that finding is that patients requiring dialysis are more often subjected to the use of invasive devices and are more often admitted to the intensive care unit, both of which increase the risk of bacterial infection.^{1,6} Renal failure itself can be immunosuppressive and an indirect marker of graft malfunction. Acute kidney injury can also be seen in LT recipients with severe preoperative hepatic impairment. All of these factors can increase the of MDR-GNB invasive infection.

We found that the risk of CRE infection was also increased among the patients with severely impaired hepatic function at LT, especially among those requiring dialysis. A high MELD score at LT has been associated with a greater risk of bacterial infection, specifically post-LT bacteremia caused by Enterobacteriaceae.²⁴ One study evaluating the risk of developing infection with carbapenem-resistant or carbapenem-susceptible *K. pneumoniae* also found that a high pre-LT MELD score is a risk factor for post-LT infection.⁹

In the present study, the CART analysis identified a group at intermediate risk for post-LT CRE infection. That group comprised patients undergoing combined transplant or reoperation. It is reasonable to hypothesize that the risk of becoming infected with MDR bacteria is higher for patients undergoing surgical procedures that are more complex. Another study identified combined transplant as a risk factor for post-SOT infection with carbapenem-resistant *K. pneumoniae*.⁵ Reoperation has also been previously identified as a risk factor for post-LT SSI,²⁵ the most common type of CRE infection in the present study.

The 2 most common types of CRE infection in our sample were SSI and catheter-related bloodstream infection, similar to what has been reported in previous studies of CRE in LT.^{8–10,17} Although SSI was the most common site, CRE infection was not associated to biliary complication, probably because such infections occurred in the early post-LT period. We also identified a high rate of bacteremia associated with such infections. The 60-day mortality rate in patients with CRE infection was 50%, and mortality was highest among the patients with SSI or respiratory infection. In the literature, the mortality associated with CRE infection in LT recipients ranges from 41% to 72%.^{8,10,16,17,26} In our mortality analysis, we found that only infection with KPC-producing strains was associated with a worse outcome. Among the patients infected with KPC-producing CRE in our sample, the 60-day mortality rate after LT was 59.1%, compared with 13.3% among those infected with KPC-negative CRE and 23.9% among those not infected with CRE. Bogan et al²⁷ reported that mortality was higher among patients infected with KPC-producing *K. pneumoniae* than among those infected with extended-spectrum β -lactamase-producing *K. pneumoniae*, suggesting that the former is more virulent. That difference could be attributable to the fact that the initial antibiotic therapy was inappropriate in a high proportion of the patients infected with CRE.²⁷ Also, strains of KPC-negative CRE usually have lower MIC for carbapenem, which contributes to a better response to antibiotic therapy, specifically in the cases where carbapenem was used.²⁸

This study included a large number of patients in a scenario with high incidence of CRE, allowing statistical power. However, this was a single study center, and the results have to be extrapolated with caution, especially in institutions with low incidence of CRE. Another limitation was that only in half of the cases of CRE the carbapenem resistance was due to *bla*_{KPC} production, which is currently the most frequent mechanism of carbapenem resistance.

We found that the incidence of post-LT CRE infections was high among patients previously colonized by CRE, especially among those who had acquired CRE in the pre-LT period. Various combinations of risk factors have different effects on the risk of CRE infection, and knowledge of those interactions is crucial to designing strategies to prevent CRE infection and

to introduce empirical therapy for such infection. Patients who were colonized by CRE when they underwent LT were found to be at a high risk for developing infection, and measures for minimizing that risk, such as adjusting the antibiotic prophylaxis, should be investigated and implemented.

ACKNOWLEDGMENTS

The authors are grateful to laboratory assistant Juliana Januario Gautereto, for the technical support and assistance provided.

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Appendix C

ORIGINAL ARTICLE

FREIRE ET AL.

Carbapenem-Resistant *Acinetobacter baumannii* Acquired Before Liver Transplantation: Impact on Recipient Outcomes

Maristela Pinheiro Freire,¹ Ligia Câmara Pierrotti,⁴ Isabel Cristina Villela Soares Oshiro,¹ Patrícia Rodrigues Bonazzi,⁵ Larissa Marques de Oliveira,² Anna Silva Machado,¹ Inneke Marie Van Der Heijden,² Flavia Rossi,³ Silvia Figueiredo Costa,^{2,4} Luiz Augusto Carneiro D'Albuquerque,⁵ and Edson Abdala^{4,5}

¹Infection Control Team, ²Microbiology Research Laboratory, ³Microbiology Laboratory, Hospital das Clínicas, and ⁴Department of Infectious Diseases; and ⁵Liver and Intestinal Transplant Division, Department of Gastroenterology, School of Medicine, University of São Paulo, São Paulo, Brazil

Infection with carbapenem-resistant *Acinetobacter baumannii* (CRAB) after liver transplantation (LT) is associated with high mortality. This study aimed to identify risk factors for post-LT CRAB infection, as well as to evaluate the impact of pre-LT CRAB acquisition on the incidence of post-LT CRAB infection. This was a prospective cohort study of all patients undergoing LT at our facility between October 2009 and October 2011. Surveillance cultures (SCs) were collected immediately before LT and weekly thereafter, until discharge. We analyzed 196 patients who were submitted to 222 LTs. CRAB was identified in 105 (53.6%); 24 (22.9%) of these patients were found to have acquired CRAB before LT, and 85 (81.0%) tested positive on SCs. Post-LT CRAB infection occurred in 56 (28.6%), the most common site being the surgical wound. Multivariate analysis showed that the risk factors for developing CRAB infection were prolonged cold ischemia, post-LT dialysis, LT due to fulminant hepatitis, and pre-LT CRAB acquisition with pre-LT CRAB acquisition showing a considerable trend toward significance ($P = 0.06$). Among the recipients with CRAB infection, 60-day mortality was 46.4%, significantly higher than among those without ($P < 0.001$). Mortality risk factors were post-LT infection with multidrug-resistant bacteria, LT performed because of fulminant hepatitis, retransplantation, prolonged cold ischemia, longer LT surgical time, and pre-LT CRAB acquisition, the last showing a trend toward significance ($P = 0.08$). In conclusion, pre-LT CRAB acquisition appears to increase the risk of post-LT CRAB infection, which has a negative impact on recipient survival.

Liver Transplantation 22 615–626 2016 AASLD.

Received June 23, 2015; accepted November 9, 2015.

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Abbreviations: ASA, American Society of Anesthesiologists; CRAB, carbapenem-resistant *Acinetobacter baumannii*; CRE, carbapenem-resistant *Enterobacteriaceae*; CVC, central venous catheter; GNB, gram-negative bacteria; HAI, health care-associated infection; HBV, hepatitis B virus; HCV, hepatitis C virus; ICU, intensive care unit; LT, liver transplantation; MDR, multidrug-resistant; MELD, Model for End-Stage Liver Disease; MIC, minimum inhibitory concentration; PFGE, pulsed-field gel electrophoresis; RR, relative risk; SC, surveillance culture; SOT, solid organ transplantation; VRE, vancomycin-resistant enterococci.

Address reprint requests to Maristela Pinheiro Freire, M.Sc., Infection Control Team, Hospital das Clínicas, School of Medicine, University of São Paulo, Rua Dr Eneas de Carvalho Aguiar, 255, São Paulo, SP, 05403-900, Brazil. Telephone: +55-11-2661-6444; FAX: +55-11-2661-6444; E-mail: maristelapf@uol.com.br

The prevalence of multidrug-resistant (MDR) gram-negative bacteria (GNB) as agents of infection after solid organ transplantation (SOT) is increasing. It is estimated that 9.8–20.0% of SOT recipients become infected with MDR-GNB.^(1–3) These infections occur most often in the early posttransplant period, and the site of infection is usually related to the transplant process (at the surgical wound or in the transplanted organ). Various studies have reported decreased survival in SOT recipients infected with MDR-GNB.^(2–4) Certain risk factors have been associated with post-SOT infection with MDR-GNB, including previous antibiotic use, retransplantation, septic shock at

diagnosis of the infection, and prolonged mechanical ventilation. Specifically in liver transplantation (LT) recipients, additional risk factors include posttransplant renal failure and intra-abdominal infection.^(1,4,5)

Carbapenem-resistant *Acinetobacter baumannii* (CRAB) is an MDR-GNB that has been described in LT recipients. However, the reported proportion of CRAB infections among posttransplant bacterial infections varies widely among treatment facilities, ranging from less than 8.2% to 50.0%.^(1,5-7) Post-SOT infection with *A. baumannii* is associated with high mortality, reportedly as high as 80.0%, and carbapenem resistance appears to significantly increase the risk of death. Treatment of *A. baumannii* infection is a challenge because the bacterium often presents multiple mechanisms of resistance, thereby limiting the therapeutic options.⁽⁸⁾ Although the most often cited primary site of CRAB infection is the respiratory tract, some studies have shown that the abdominal cavity is the most common primary site of such infections among LT recipients.^(6,7,9,10)

Although there have been a number of case series investigating CRAB infection among LT recipients, the risk factors for CRAB colonization and infection after LT remain poorly defined in the literature. In addition, certain important aspects, such as the impact that pretransplant CRAB colonization or infection has on the incidence of posttransplant CRAB infection and survival among LT recipients, have not been evaluated. Therefore, the aim of this study was to identify risk factors for post-LT CRAB colonization and infection, as well as to evaluate the impact that pre-LT colonization has on the incidence of post-LT CRAB infection and on recipient survival.

Patients and Methods

STUDY DESIGN AND PATIENT SAMPLE

This was a prospective cohort study involving all patients who underwent LT at the Hospital das

Clínicas, School of Medicine, University of São Paulo, São Paulo, Brazil, between October 2009 and October 2011. Patients were followed from hospital admission until the end of the second month after transplantation. We excluded patients who died within the first 72 hours after transplantation.

Infections were identified through active surveillance in the LT ward and through review of outpatient records. The criteria used in order to identify and classify health care-associated infections (HAIs) were those outlined by the US National Healthcare Safety Network⁽¹¹⁾; the only exception that did not meet the criteria was the length of follow-up days which was reported up to 60 days after LT. Colonization was defined as CRAB being isolated in cultures of surveillance or clinical specimens with no evidence of infection. We defined CRAB acquisition as having a surveillance or clinical specimen that tested positive for CRAB in culture with no evidence of CRAB within the last 2 years. We defined the category of MDR bacteria as follows: carbapenem-resistant, nonfermentative gram-negative bacilli; extended-spectrum beta-lactamase-producing Enterobacteriaceae; Enterobacteriaceae resistant to at least 1 carbapenem; methicillin-resistant *Staphylococcus aureus*; and vancomycin-resistant *Enterococcus* spp. We defined effective treatment as having been treated for ≥ 48 hours with at least 1 antimicrobial agent that displayed in vitro activity against CRAB.

The standard surgical prophylaxis in use during the study period was a 48-hour course of ampicillin with cefotaxime. For cases in which patients underwent LT during treatment for an infection, as well as for those in which the donor had a suspected or confirmed infection, the prophylaxis protocol was modified. In such cases, the antibiotic used for prophylaxis was the same as that used for the treatment of the infection in question. The standard immunosuppression regimen was tacrolimus plus a corticosteroid, and immunosuppression was induced by administration of methylprednisolone.

During the follow-up period, clinical samples were collected for culture when any infection was suspected. Organisms were identified and antimicrobial susceptibility testing was performed with an automated system (VITEK 2; bioMérieux, Marcy l'Étoile, France). Minimum inhibitory concentrations (MICs) were interpreted according to the breakpoints established by the Clinical and Laboratory Standards Institute.⁽¹²⁾

SURVEILLANCE CULTURES (SCS)

SCs were collected immediately before LT and weekly thereafter, until the patients were discharged from the

This study received financial support from the Fundação de Amparo à Pesquisa do Estado de São Paulo (São Paulo Research Foundation; grant no. 2010/02267-6).

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View this article online at wileyonlinelibrary.com.

DOI 10.1002/lt.24389

Potential conflict of interest: Nothing to report.

hospital. Cultures were obtained from the rectum, axilla, and throat by swabbing with Stuart's transport medium. For patients on mechanical ventilation, we collected tracheal aspirate rather than swabbing the throat. Samples were directly inoculated into brain-heart infusion broth containing imipenem (10 $\mu\text{g/mL}$) and were cultured overnight, after which they were plated on MacConkey agar. Suspected colonies were characterized with a commercial microorganism identification kit (API 20NE; bioMérieux, Marcy-l'Étoile, France). Antimicrobial susceptibility testing was performed with the disc diffusion method, and MICs were interpreted according to the breakpoints established by the Clinical and Laboratory Standards Institute.⁽¹²⁾

Polymyxin resistance was confirmed by broth microdilution in clinical culture and SC for all strains with MIC ≥ 2 mg/L.⁽¹²⁾

PULSED-FIELD GEL ELECTROPHORESIS (PFGE) ANALYSIS

The chromosomal DNA of isolates was digested using the restriction enzyme SmaI (Amersham Pharmacia Biotech, Piscataway, NJ). PFGE was performed using the CHEF DR-II system (Bio-Rad Laboratories Inc., Hercules, CA) according to previously established protocols.⁽¹³⁾ The PFGE images were processed and analyzed with BioNumerics software, version 7.1 (Applied Maths, Sint-Martens-Latem, Belgium). The images were normalized through the use of standard molecular markers, and banding patterns were compared. A similarity analysis was performed using Dice coefficients, with a band position tolerance of 1.25% and an optimization of 0.5%. Isolates were separated into similarity clusters by the unweighted pair group method with arithmetic mean. Only bands larger than 45.5 kb were included in the analysis.

Statistical Analysis

In our analysis of risk factors for CRAB acquisition, infection, and mortality, we included each patient only once, regardless of the number of transplants they underwent, analyzing only the first LT performed during the study period. We evaluated the following variables related to the LT process: cold ischemia time; total ischemia time; number of units of blood transfused intraoperatively; reoperation; retransplantation in

the first 60 days after LT; level of experience of the lead surgeon (more than 8 years); American Society of Anesthesiologists (ASA) physical status classification; surgical time; type of biliary reconstruction; donor type (living or deceased); extensiveness of the procedure (liver-only or combined liver-kidney transplantation); and the type of surgical prophylaxis. We also evaluated variables related to the LT recipient: age; sex; presence or absence of fulminant hepatitis, hepatocellular carcinoma, or other underlying diseases; Model for End-Stage Liver Disease (MELD) score; and pretransplant serum creatinine level. In addition, we evaluated variables related to hospitalization: length of hospital stay before LT; post-LT need for dialysis; duration of abdominal drainage; acute cellular rejection; cytomegalovirus infection; infection in the last week before transplantation; and the type of immunosuppression therapy (standard versus tacrolimus, prednisone, and mycophenolate mofetil). For the patients developing CRAB acquisition or CRAB infection, the variables related to exposure time were recorded from hospital admission to the first CRAB-positive culture or from hospital admission to infection, respectively. The only exception was the use of antibiotic, which was recorded from 1 month after LT to CRAB acquisition or infection. Dichotomous variables were considered positive if the event occurred before CRAB infection or before the first CRAB-positive culture (for acquisition). For the remaining patients, those variables were recorded for the total time at risk during the first 60 days after transplantation.

The main outcome measure was CRAB acquisition during the first 60 days after transplantation (post-LT CRAB acquisition). A secondary outcome measure was infection with CRAB during that same period (post-LT CRAB infection). In our analysis of the risk of post-LT CRAB acquisition, we excluded patients in whom CRAB was detected before LT. In analyzing the risk of post-LT CRAB infection, we excluded patients who underwent LT with active CRAB infection, although we included patients in whom CRAB had previously been identified but who showed no evidence of infection.

For dichotomous variables, we performed univariate analysis using the chi-square test or Fisher's exact test. For continuous variables, we used the Mann-Whitney U test. Continuous variables were transformed into dichotomous variables through cluster analysis. Multivariate analysis was performed by stepwise binary logistic regression. The criterion for inclusion in the multivariate analysis was $P < 0.20$ in the univariate

analysis. Variables that then reduced the -2 log likelihood or showed $P < 0.05$ were retained in the model. For the survival analysis, the outcome measure used was death within the first 60 days after the first LT. We used Kaplan-Meier survival curves, and we calculated P values by log-rank test. Data were processed and analyzed with the SPSS Statistics software package, version 22.0 (IBM Corporation, Armonk, NY).

Results

CLINICAL CHARACTERISTICS AND EPIDEMIOLOGY ANALYSIS

During the study period, 202 patients underwent LT. Six (3.0%) patients died within the first 72 hours after transplantation. Therefore, we analyzed 196 LT recipients who were collectively submitted to 222 LTs, ie, 25 recipients underwent retransplantation (1 underwent retransplantation twice). The mean age was 53.0 years, and 69 (35.2%) patients were female. Of the 222 LTs, 25 (11.3%) were living donor transplants. In 12 (5.4%) transplantations, the procedure was a combined liver-kidney transplantation. As can be seen in Table 1, the most common reason for LT was cirrhosis due to hepatitis C virus (HCV) infection, which was present in 51 (26.0%) of the recipients. Nineteen (9.7%) of the recipients underwent LT for the treatment of hepatocellular carcinoma.

We identified CRAB in 105 (53.6%) of the 196 LT recipients evaluated, 24 (22.9%) of whom tested positive for CRAB before LT. Of those 24, 4 presented with pre-LT infection: 2 had primary bloodstream infections; 1 had pneumonia; and 1 had cholangitis. The median time from CRAB infection to LT was 19 days. Two of those recipients were submitted to LT during CRAB treatment. Among the remaining 22 recipients, the antibiotic prophylaxis was adjusted for CRAB colonization in only 4 (18.2%). In all 4 of those recipients, polymyxin was used in combination with standard antibiotic prophylaxis, and 2 of those patients developed CRAB infection after LT. For the strains of CRAB identified in both of those cases, polymyxin showed a high MIC (>16 mg/dL).

The SCs were positive in 85 (81.0%) of the 105 LT recipients in whom CRAB was identified; 20 patients had multiple negative SCs despite CRAB having been isolated in clinical cultures. In 59 (56.2%) patients, the first positive culture was a SC. A total of 73 recipients tested positive for CRAB in cultures of clinical

TABLE 1. Demographic Characteristics of Patients Submitted to LT

Characteristic	Values (n = 196)
Sex, male, n (%)	127 (64.8)
Age, median (range), years	53.0 (16-73)
Diagnosis, n (%)	
HCV infection	51 (26.0)
Alcoholic cirrhosis	26 (13.3)
Cryptogenic cirrhosis	21 (10.7)
Hepatocellular carcinoma	19 (9.7)
Fulminant hepatitis	17 (8.7)
HBV infection	14 (7.1)
HCV infection with alcoholic cirrhosis	13 (6.6)
Autoimmune hepatitis	6 (3.1)
Primary biliary cirrhosis	5 (2.6)
Hemochromatosis	5 (2.6)
Familial amyloid polyneuropathy	4 (2.0)
Budd-Chiari syndrome	4 (2.0)
Nonalcoholic steatohepatitis	4 (2.0)
Other	26 (13.3)
Living donor (first LT), n (%)	25 (12.8)
Liver-kidney transplantation, n (%)	12 (6.1)
Cold ischemia time, median (range), minutes	400 (20-960)
Early retransplantation, n (%)*	25 (12.8)
Immunosuppression therapy with 3 drugs, n (%)	88 (44.9)
Post-LT dialysis, n (%)	111 (56.6)

*Retransplant performed in the first 60 days after LT.

samples. Among those 73 recipients, SCs were positive in 54 (74.0%). Among the SC sites, the rectum showed the highest sensitivity (53.3%), followed by the throat and axilla (47.6% and 27.6%, respectively). For the recipients in whom CRAB was identified post-LT, the median time from LT to CRAB acquisition was 9.0 days (range, 1-58 days). The patients who acquired CRAB were also colonized by vancomycin-resistant enterococci (VRE) more often than were those who had not acquired CRAB (63.2% versus 39.4%; $P = 0.003$). However, there was no statistical difference between those 2 groups of patients, in terms of colonization by carbapenem-resistant Enterobacteriaceae (CRE).

A total of 56 patients developed CRAB infection after LT (Table 2). The most common site of infection, seen in 20 (35.7%) patients, was the surgical wound. Among those 20 patients, bacteremia was identified in 15 (75.0%), and reoperation (to treat the infection at its focus) was necessary in 7 (35.0%). All 15 of the primary bloodstream infections were attributed to the use of a central venous catheter (CVC), and 11 (57.9%) of the 19 patients had pneumonia that was attributed to the use of mechanical ventilation. Of the 56 LT recipients who developed CRAB infection after transplantation, 16 (28.6%) died within the first 24 hours of effective therapy; 32 (57.1%) received

TABLE 2. Source of Infection, Concomitant Bacteremia, Time to Infection After LT, and 60-Day Mortality in 56 LT Recipients With Post-LT CRAB Infection, by Infection Site

Site of Infection	Total Patients, n (%)	Bacteremia, n (%)	Time From LT to Infection, Median (Range), Days	Mortality, n (%)
Surgical wound/organ space	20 (35.7)	15 (75.0)	9.5 (5-43)	10 (50.0)
Respiratory tract	19 (33.9)	6 (31.6)	9.0 (2-37)	11 (57.9)
Bloodstream	15 (26.8)	15 (100.0)	9.0 (1-29)	10 (66.7)
Skin or soft tissue	1 (1.8)	1 (100.0)	4	1 (100.0)
Urinary tract	1 (1.8)	0 (0.0)	4	0 (0.0)
Total	56 (100.0)	37 (66.1)	9 (1-43)	40 (71.4)

polymyxin monotherapy; 5 (8.9%) received polymyxin plus aminoglycoside; 1 (1.8%) received polymyxin plus tigecycline; 1 (1.8%) received polymyxin, tigecycline, and gentamicin; and 1 (1.8%) received polymyxin plus sulbactam. Among the 40 patients who received treatment, the 60-day mortality rate for those who did and did not receive combination therapy was 50.0% and 37.5%, respectively ($P = 0.69$), corresponding to 4 of the 8 patients who received combination therapy and 12 of the 32 who did not.

The multivariate analysis revealed that the risk factors for post-LT CRAB acquisition were the previous use of carbapenem or piperacillin/tazobactam and retransplantation within the first 60 days after transplantation (Table 3). A primary diagnosis of hepatocellular carcinoma was identified as a protective factor against CRAB acquisition. The risk factors for developing an HAI with CRAB (CRAB-HAI) after LT were pre-LT detection of CRAB, LT performed because of fulminant hepatitis, post-LT need for dialysis, post-LT need for mechanical ventilation, a high MELD score at the time of LT, longer cold ischemia time, prolonged stay in the intensive care unit (ICU), and blood transfusion during LT. As can be seen in Table 4, the variables that remained significant in the final model of the multivariate analysis were longer cold ischemia time, post-LT need for dialysis, LT performed because of fulminant hepatitis, and pre-LT acquisition of CRAB, the last being marginally significant ($P = 0.08$).

Hospital stays were significantly longer among the patients with CRAB-HAI than among those without (31 versus 16 days; $P = 0.001$). In addition, 60-day mortality was significantly higher in the former group (46.4% versus 23.2%; $P < 0.001$; Fig. 1). In the multivariate analysis of 60-day mortality (Table 5), the following risk factors were identified: post-LT infection with MDR bacteria; LT performed because of fulminant hepatitis; retransplantation; prolonged cold

ischemia time; prolonged surgical time during LT; and pre-LT acquisition of CRAB, which showed a considerable trend toward significance ($P = 0.08$).

PFGE ANALYSIS

The PFGE analysis was performed in 85 strains isolated in specimens obtained from 57 LT patients who were colonized by or infected with CRAB. That analysis identified 7 separate types of strains. The most common strain (indistinguishable or closely related) was identified in 41 (71.9%) of those patients and had a homogeneous distribution throughout the study period. Among 3 patients, 2 different clones were identified.

We typed strains from 10 of the 22 patients in whom CRAB had been identified in the pre-LT period and who did not present active infection at the time of LT. Five different PFGE patterns were identified. In 8 patients, it was possible to analyze strains isolated in the pre-LT period and during an infectious episode in the post-LT period. Those paired (pre-LT and post-LT) strains were indistinguishable or closely related in 7 (87.5%) of the patients (Fig. 2).

Discussion

The reported prevalence of posttransplant CRAB infection among LT recipients varies widely across facilities, ranging from 2.8% to 16.8%.^(2,3,5) In the present study, the prevalence of CRAB infection was high—approximately 27.7%. At our facility, CRAB is the most common MDR-GNB pathogen of early post-LT infections. Other authors have shown that CRAB is the MDR-GNB that most often colonizes LT recipients before and after transplant.^(14,15) Although the prevalence of CRAB colonization was quite high in our patient sample, there are, to our knowledge, no

TABLE 3. Univariate and Multivariate Analyses of Risk Factors for Post-LT CRAB Acquisition in 172 LT Recipients

Variable	Post-LT CRAB Colonization		Univariate Analysis		Multivariate Analysis	
	Yes (n = 81)	No (n = 91)	RR (95% CI)	P Value	OR (95% CI)	P Value
Pre-LT characteristics						
Sex, male	51 (63.0)	61 (67.0)	0.89 (0.59-1.34)	0.58		
Age, years	50.0 (16-70)	53.5 (18-73)	—	0.29		
Fulminant hepatitis	12 (14.8)	2 (2.2)	1.15 (1.04-1.26)	0.004		
Hepatocellular carcinoma	17 (21.0)	36 (39.6)	0.77 (0.63-0.94)	0.008	0.46 (0.22-0.98)	0.05
Length of pre-LT hospital stay, days	1.0 (0-42)	1.0 (1-46)	—	0.22		
Bacterial infection in the last 10 days before LT	25 (30.9)	22 (24.2)	1.1 (0.91-1.32)	0.33		
MELD score at LT	26.0 (6-51)	19.0 (6-53)	—	0.01		
Surgical characteristics						
Living donor LT	11 (13.6)	13 (14.3)	0.99 (0.88-1.12)	0.89		
Combined transplantation	4 (4.9)	5 (5.5)	0.99 (0.93-1.07)	> 0.99		
Cold ischemia time, minutes	440.0 (20-960)	390.0 (28-754)	—	0.21		
ASA score > III	42 (51.9)	40 (44.0)	1.19 (0.86-1.65)	0.29		
Senior surgeon	46 (56.8)	44 (48.3)	1.20 (0.87-1.65)	0.27		
Surgical time, minutes	465.0 (210-920)	465.0 (245-1330)	—	0.42		
Blood transfusion during LT, units	2.0 (0-15)	0.5 (0-9)	—	0.04	1.17 (0.99-1.37)	0.06
Duct-to-duct biliary anastomosis	78 (96.3)	86 (94.5)	1.48 (0.36-6.01)	0.58		
Post-LT characteristics						
Reoperation*	17 (21.0)	19 (21.0)	1.01 (0.86-1.17)	0.99		
Replantation*	13 (16.0)	0 (0.0)	1.19 (1.08-1.31)	<0.001	10.09 (1.22-83.10)	0.01
Immunosuppression therapy with 3 drugs	41 (50.6)	39 (42.9)	1.16 (0.87-1.54)	0.31		
Length of ICU stay, days	7.0 (1-28)	5.0 (2-44)	—	0.02		
Dialysis after LT	47 (58.0)	33 (36.2)	1.52 (1.13-2.05)	0.004		
Mechanical ventilation use	49 (60.5)	38 (41.8)	1.47 (1.12-2.10)	0.01		
Duration of CVC use, days	8.0 (1-68)	4.0 (1-54)	—	0.002		
Duration of urinary catheter use, days	7.0 (1-28)	5.0 (1-47)	—	0.03		
Duration of abdominal drainage, days	7.0 (0-83)	6.0 (0-49)	—	0.43		
Previous carbapenem use	60 (74.1)	36 (39.6)	2.33 (1.56-3.49)	<0.001	3.31 (1.66-6.60)	0.001
Previous quinolone use	3 (3.7)	5 (5.5)	0.79 (0.32-1.96)	0.72		
Previous cephalosporin use	63 (77.8)	70 (76.9)	1.04 (0.60-1.81)	0.89		
Previous piperacillin/tazobactam use	47 (58.0)	30 (33.0)	1.60 (1.19-2.14)	0.001	2.19 (1.11-4.32)	0.02
Cytomegalovirus disease	6 (7.4)	14 (15.4)	0.91 (0.82-1.02)	0.10		
Acute cellular rejection	9 (11.1)	17 (18.7)	0.92 (0.81-1.04)	0.17		

NOTE: Data are given as n (%) or as median (range).

*During the first 60 days after LT.

TABLE 4. Univariate and Multivariate Analyses of Risk Factors for Posttransplant CRAB Infection in 194 LT Recipients

Variable	Post-LT CRAB-HAI		Univariate Analysis		Multivariate Analysis	
	Yes (n = 56)	No (n = 138)	RR (95% CI)	P Value	OR (95% CI)	P Value
Pre-LT characteristics						
Sex, male	39 (69.4)	87 (63.0)	1.16 (0.74-1.80)	0.51		
Age, years	51.5 (16-70)	53.0 (16-73)	—	0.83		
Fulminant hepatitis	10 (17.9)	8 (5.8)	1.14 (1.01-1.30)	0.01	3.41 (1.19-9.77)	0.02
Hepatocellular carcinoma	14 (25.0)	47 (34.1)	0.87 (0.72-1.05)	0.18		
Length of hospital stay before LT, days	2.0 (0-42)	1.0 (0-56)	—	0.25		
Bacterial infection in the last 10 days before LT	22 (39.3)	37 (26.8)	1.48 (0.95-2.30)	0.09		
Pre-LT CRAB acquisition	11 (19.6)	11 (8.0)	1.48 (0.96-2.26)	0.02	2.57 (0.94-7.05)	0.06
MELD score at LT	27.0 (6-52)	21.0 (6-53)	—	0.002		
Surgical characteristics						
Living donor LT	5 (8.9)	20 (14.5)	0.94 (0.84-1.04)	0.27		
Combined transplantation	3 (5.4)	8 (5.8)	0.99 (0.92-1.07)	>0.99		
Cold ischemia time, minutes	441.0 (50-960)	375.0 (20-754)	—	0.01	1.03 (1.01-1.06)	0.01
ASA score > III	34 (60.7)	67 (48.6)	1.42 (0.90-2.25)	0.13		
Senior surgeon	28 (50.0)	71 (51.4)	0.95 (0.70-1.29)	0.73		
Surgical time, minutes	467.5 (210-829)	460.0 (245-1330)	—	0.66		
Blood transfusion during LT, units	2.0 (0-15)	1.0 (0-9)	—	0.004		
Duct-to-duct biliary anastomosis	56 (100.0)	129 (93.4)	3.33 (0.43-26.00)	0.22		
Post-LT characteristics						
Reoperation*	16 (28.6)	35 (25.4)	1.03 (0.86-1.25)	0.72		
Re transplantation*	11 (19.6)	10 (7.2)	1.15 (1.00-1.32)	0.01		
Immunosuppression therapy with 3 drugs	29 (51.8)	57 (41.3)	1.19 (0.88-1.60)	0.24		
Length of ICU stay, days	9.5 (1-42)	6.0 (2-44)	—	0.02		
Post-LT dialysis	42 (75.0)	59 (42.8)	2.16 (1.37-3.42)	<0.001	3.08 (1.46-6.49)	0.003
Mechanical ventilation	39 (69.6)	61 (44.2)	1.76 (1.17-2.65)	0.002		
Duration of CVC use, days	10.0 (1-49)	6.0 (1-91)	—	0.008		
Duration of urinary catheter use, days	7.5 (3-35)	6.5 (3-56)	—	0.09		
Duration of abdominal drainage use, days	7.0 (1-35)	7.0 (0-49)	—	0.99		
Duration of previous carbapenem use, days	6.0 (0-40)	0.0 (0-72)	—	0.10		
Duration of previous quinolone use, days	0.0 (0-3)	0.0 (0-6)	—	0.77		
Duration of previous cephalosporin use, days	1.5 (0-15)	2.0 (0-21)	—	0.32		
Duration of previous piperacillin/tazobactam use, days	1.5 (0-19)	0.0 (0-33)	—	0.17		
Cytomegalovirus disease	6 (10.7)	26 (18.8)	0.91 (0.80-1.02)	0.15		
Acute cellular rejection	9 (16.1)	25 (18.1)	0.97 (0.85-1.11)	0.68		
Post-LT CRAB colonization	26 (46.4)	47 (34.1)	1.17 (0.96-1.43)	0.11	0.98 (0.96-1.01)	0.25

NOTE: Data are given as n (%) or as median (range).

*During the first 60 days after LT.

FIG. 1. Recipient survival in the first 60 days after LT, stratified by CRAB-HAI.

comparative data in the literature regarding the prevalence of CRAB colonization in the context of active, weekly surveillance after LT.

To control the spread of CRAB, a bundle of infection control measures was implemented on the LT ward. Such measures included weekly SCs for CRAB; contact precautions, each CRAB-colonized patient being isolated in a separate room; hand hygiene training; and systematic discussions about the cases in which CRAB acquisition was attributed to the LT ward. Those measures resulted in a reduction in the incidence of CRAB acquisition, from 12.2/1000 patient-days in 2009 to 5.5/1000 patient-days in 2014 (unpublished data).

One of the most important issues in CRAB control is the low sensitivity of SCs and the lack of standardization regarding the site from which such cultures should be collected. In the literature, the sensitivity of

SCs for CRAB ranges from 11.0% to 54.5%, and the best results are usually obtained when cultures are collected from multiple sites.⁽¹⁶⁻¹⁹⁾ Dalben et al.⁽¹⁸⁾ reported that the incidence of CRAB colonization was underestimated when cultures were collected from fewer than 3 sites with the proportion of cases missed ranging from 19.4% to 36.8%, depending on the site not sampled. The sensitivity observed in the present study (74.0%) was higher than that reported in the literature.⁽¹⁶⁻¹⁹⁾ When we analyzed only the SCs that were positive before CRAB had been identified in other cultures (ie, the capacity of SCs to identify asymptomatic patients), that sensitivity was 56.2%. We assume that the impressive results obtained in our study are attributable to the fact that the SCs were collected weekly and from 3 different sites. Another possibility is that immunocompromised patients have higher loads of MDR microorganisms colonizing the sites sampled.

TABLE 5. Univariate and Multivariate Analyses of Risk Factors for 60-Day Mortality in 196 LT Recipients

Variable	Survival (n = 128)	Non-Survival (n = 68)	Univariate Analysis		Multivariate Analysis	
			RR (95% CI)	P Value	OR (95% CI)	P Value
Pre-LT characteristics						
Sex, male	79 (61.7)	47 (69.1)	1.21 (0.79-1.84)	0.37		
Age, years	53.0 (16-73)	55.0 (16-70)	—	0.20		
Fulminant hepatitis	6 (4.7)	12 (17.6)	1.16 (1.03-1.30)	0.003	2.90 (1.01-8.64)	0.05
Hepatocellular carcinoma	41 (32.0)	20 (29.4)	0.96 (0.79-1.16)	0.65		
Length of hospital stay before LT, days	1.0 (0-46)	2.0 (0-56)	—	0.07		
Pre-LT bacterial infection	36 (28.1)	24 (35.3)	1.10 (0.90-1.36)	0.33		
Pre-LT CRAB acquisition	10 (7.8)	12 (17.6)	1.12 (0.99-1.26)	0.04		
MELD score at LT	21.0 (6-53)	27.0 (6-52)	—	0.003	2.52 (0.88-7.23)	0.08
Surgical characteristics						
Living donor LT	18 (14.1)	7 (10.3)	0.96 (0.86-1.06)	0.43		
Combined transplantation	6 (4.7)	5 (7.4)	1.03 (0.95-1.11)	0.52		
Cold ischemia time, minutes	390.0 (20-754)	417.5 (20-960)	—	0.09	1.02 (1.01-1.05)	0.04
ASA score > III	42 (32.8)	54 (79.4)	1.27 (1.02-1.58)	0.03		
Senior surgeon	63 (49.2)	36 (52.9)	1.06 (0.78-1.44)	0.70		
LT surgical time, minutes	446.0 (210-915)	490.0 (250-1330)	—	0.03	1.04 (1.01-1.06)	0.006
Blood transfusion during LT, units	1.0 (0-10)	2.0 (0-15)	—	0.002		
Duct-to-duct biliary anastomosis	121 (94.5)	64 (94.1)	0.68 (0.19-2.43)	0.55		
Post-LT characteristics						
Reoperation*	33 (25.8)	28 (41.2)	1.26 (1.01-1.57)	0.03		
Retransplantation*	8 (6.2)	18 (26.5)	1.27 (1.10-1.48)	<0.001	3.21 (1.19-8.62)	0.02
Immunosuppression therapy with 3 drugs	61 (47.7)	26 (38.2)	0.83 (0.65-1.08)	0.17		
Post-LT dialysis	50 (39.1)	60 (88.2)	5.13 (2.63-9.98)	<0.001		
Colonization by VRE	58 (45.3)	32 (47.1)	1.02 (0.77-1.34)	0.89		
CRAB colonization	60 (46.9)	45 (66.2)	1.02 (0.77-1.34)	0.01		
Colonization by CRE	53 (41.4)	34 (50.0)	1.70 (1.12-2.57)	0.01		
HAI	56 (43.8)	51 (75.0)	1.16 (0.88-1.53)	0.29		
HAI caused by MDR bacteria	37 (28.9)	42 (61.8)	2.22 (1.43-3.45)	<0.001		
Post-LT CRAB-HAI	24 (18.8)	32 (47.1)	1.85 (1.34-2.55)	<0.001	2.58 (1.30-5.14)	0.007
Surgical site infection	26 (20.3)	28 (41.2)	1.53 (1.20-1.94)	<0.001		
Pneumonia	14 (10.9)	19 (27.9)	1.35 (1.09-1.68)	0.002		
Cytomegalovirus disease	34 (26.6)	9 (13.2)	1.23 (1.05-1.45)	0.003		
Acute cellular rejection	26 (20.3)	26 (29.4)	0.84 (0.73-1.00)	0.03		
			1.12 (0.94-1.34)	0.17		

NOTE: Data are given as n (%) or as median (range).

*During the first 60 days after LT.

FIG. 2. Dendrogram of CRAB isolates from patients who acquired CRAB in pretransplant period.

To our knowledge, no single SC site has been defined as having the best sensitivity. Although some authors have reported that the throat has the best sensitivity (22.7%-46.6%), there is no evidence of a statistical difference between the sensitivity of the throat and that of other sites.⁽¹⁷⁻¹⁹⁾ We found the rate of positivity to be highest for the rectum, possibly because our patients were submitted to abdominal surgery with the disturbance of the gastrointestinal flora predisposing them to colonization by MDR bacteria.

There is little knowledge regarding outcomes in SOT recipients colonized by MDR-GNB before transplant, and the impact of modifying the surgical prophylaxis has not been established.^(20,21) In the present study, modifying the surgical prophylaxis did not prevent the occurrence of post-LT CRAB infection. However, because of the small number of patients, it was not possible to draw a conclusion about the efficacy of that measure. At our facility, surgical prophylaxis is not systematically modified on the basis of colonization of the recipient by MDR bacteria, but

rather only on the basis of preoperative infection. It is therefore likely that the patients in whom the surgical prophylaxis was modified were those who were the sickest. In addition, the total number of patients colonized before LT was too small to demonstrate the benefit of this strategy.

In our patient sample, pre-LT CRAB acquisition was associated with the risk of CRAB infection and with 60-day mortality. In addition, the PFGE analysis showed that most of the patients developed post-LT infection with the same strain that they harbored in the pre-LT period. That was also true for the patients who harbored strains other than the predominant clone. The impact that pre-LT colonization by MDR-GNB has on infectious outcomes after LT has not been well defined. Kim et al.⁽²⁰⁾ reported that, in a small number of LT recipients, colonization by *A. baumannii*, identified in sputum on the day of LT, was associated with the risk of *A. baumannii*-associated pneumonia in the posttransplant period. In another study, involving 475 LT recipients, microbiologically

proven pre-LT infection was found to be a risk factor for post-LT death from MDR-GNB infection.⁽⁵⁾

In our analysis, we identified other factors associated with an increased risk of post-LT CRAB infection: post-LT need for dialysis, prolonged cold ischemia time, and LT having been performed because of fulminant hepatitis. Cold ischemia time of >400 minutes has previously been identified as a risk factor for surgical site infection after LT.⁽¹⁴⁾ In general, it can be assumed that longer cold ischemia time is associated with slower post-LT recovery of the systemic inflammatory response, as well as, indirectly, with longer ICU stays, increased use of invasive devices, and delayed recovery of renal function.^(22,23)

The need for dialysis after LT is a well-known risk factor for early postoperative bacterial infections.⁽²⁴⁾ Patients requiring dialysis are more often subjected to the use of invasive devices and are more often admitted to the ICU, therefore having a higher incidence of bacterial infection.^(1,5) Renal failure itself plays an immunosuppressive role and can be an indirect marker of graft malfunction. Acute kidney injury can also be seen in LT recipients with severe preoperative hepatic impairment. In 2012, de Gouvêa et al.⁽¹⁰⁾ reported that hemodialysis after SOT is more common among recipients who evolve to infection with CRAB than among those who develop infection with carbapenem-susceptible *A. baumannii*.

Patients undergoing LT because of fulminant hepatitis typically show high MELD scores at the time of transplantation; such patients also often experienced longer ICU stays and required dialysis before LT.⁽¹⁴⁾ A high MELD score at LT has been associated with a greater risk of bacterial infection, specifically post-LT bacteremia caused by Enterobacteriaceae.⁽²⁵⁾ In a univariate analysis, Hsieh et al.⁽⁷⁾ demonstrated that a high MELD score is a risk factor for *A. baumannii* infection after LT.

In the present study, post-LT CRAB acquisition was also dependent on the degree to which hepatic function was impaired. Patients submitted to retransplantation recover hepatic function more slowly and more often develop postoperative complications such as biliary leaks and thrombosis.⁽²⁶⁾ In contrast, patients submitted to LT for the treatment of hepatocellular carcinoma typically present with better preserved hepatic function before LT, lower MELD score at LT, and fewer postoperative complications.⁽²⁷⁾

Another major finding of the present study was that the use of broad-spectrum antibiotics, such as carbapenem and piperacillin/tazobactam, increased the risk of

CRAB acquisition. The use of an antibiotic is frequently related to the emergence of MDR bacteria and has previously been correlated with CRAB colonization in immunocompetent individuals.^(28,29) In addition, the use of such antibiotics before or after SOT has been associated with a greater risk of MDR-GNB infection in the recipients.^(1,5,10)

Among our LT recipients with CRAB infection, post-LT survival was significantly lower and CRAB-HAI was strongly associated with an increased risk of death in the univariate analysis. However, in the final model of 60-day survival after LT, only infection with MDR bacteria remained statistically significant. Because SOT recipients infected with MDR bacteria are more likely to receive inappropriate initial antibiotic therapy, to have longer ICU stays, and to require mechanical ventilation, mortality rates are higher among such patients than among SOT recipients infected with bacteria that are drug-susceptible and among those without infection.⁽²⁻⁴⁾

In the present study, pre-LT CRAB acquisition was associated with higher 60-day mortality. Therefore, pre-LT CRAB acquisition might represent a marker of greater patient severity at the time of LT. Another possible explanation is that post-LT infection with CRAB is more invasive in patients who are colonized by CRAB before LT.

In conclusion, we found that patients who acquired CRAB before LT were at a greater risk for post-LT CRAB infection, which has a negative impact on recipient survival. Therefore, these patients should be carefully evaluated for the best moment for LT. Additional risk factors for CRAB acquisition and infection were mostly related to the degree of impairment of hepatic function at the time of LT and the intensity of broad-spectrum antibiotic use.

Acknowledgment: The authors thank laboratory assistant Juliana Januario Gautereto for technical support and assistance.

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Appendix D

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Transplant Infectious Disease, ISSN 1398-2273

Polymyxin use as a risk factor for colonization or infection with polymyxin-resistant *Acinetobacter baumannii* after liver transplantation

M.P. Freire, I.M. Van Der Heijden, G.V.B. do Prado, L.S. Cavalcante, I. Boszczowski, P.R. Bonazzi, F. Rossi, T. Guimarães, L.A.C. D'Albuquerque, S.F. Costa, E. Abdala. Polymyxin use as a risk factor for colonization or infection with polymyxin-resistant *Acinetobacter baumannii* after liver transplantation.

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Abstract: *Introduction.* *Acinetobacter baumannii* is a leading agent of healthcare-associated infection. The objective of this study was to evaluate cases of colonization or infection with polymyxin-resistant *A. baumannii* (PRAB) in liver transplant recipients and to identify the risk factors for the acquisition of PRAB.

Methods. We evaluated all patients undergoing liver transplantation (LT) between January and November of 2011. The exclusion criterion was death within the first 72 h after transplant. Patients were screened for PRAB through weekly rectal and inguinal swabs during their stay in the intensive care unit (ICU) and at ICU discharge. Patients who came from other hospitals or had been treated in the emergency room for >72 h were screened at ICU admission. The minimum inhibitory concentrations (MICs) for polymyxins were determined by broth microdilution, and clonality was determined by pulsed-field gel electrophoresis. The stepwise logistic regression was used to identify risk factors related to acquisition of PRAB, and Cox forward regression used to identify risk factors for 60-day mortality.

Results. We evaluated 65 patients submitted to LT, among whom PRAB was isolated in 7, 4 of whom developed infection. The MICs for polymyxin E ranged from 16 to 128 mg/mL. All patients with PRAB required dialysis. The median time of polymyxin use before PRAB isolation was 21 days. These 4 included 1 case of primary bloodstream infection (BSI), which was treated with the carbapenem-polymyxin combination; 1 case of surgical site infection, which was treated with gentamicin, polymyxin, ampicillin-sulbactam, and tigecycline; and 2 cases of pneumonia, treated with the combination of carbapenem-polymyxin. In the case of BSI and in 1 of the cases of pneumonia, the treatment was considered successful. Mortality was 71% among the cases, compared with 33% among the non-cases.

M.P. Freire¹, I.M. Van Der Heijden², G.V.B. do Prado¹, L.S. Cavalcante², I. Boszczowski¹, P.R. Bonazzi³, F. Rossi⁴, T. Guimarães¹, L.A.C. D'Albuquerque³, S.F. Costa^{2,5}, E. Abdala^{3,5}

¹Infection Control Service, Hospital das Clínicas, University of São Paulo School of Medicine, São Paulo, Brazil, ²Microbiology Research Laboratory, Hospital das Clínicas, University of São Paulo School of Medicine, São Paulo, Brazil, ³Liver and Gastrointestinal Transplant Division, Hospital das Clínicas, University of São Paulo School of Medicine, São Paulo, Brazil, ⁴Microbiology Laboratory, Hospital das Clínicas, University of São Paulo School of Medicine, São Paulo, Brazil, ⁵Department of Infectious Diseases, University of São Paulo School of Medicine, São Paulo, Brazil

Key words: multidrug resistance; early infection; polymyxin; *Acinetobacter baumannii*; liver transplantation; CRAB; PRAB

Correspondence to: Maristela P. Freire, MSc, Infection Control Service, Hospital das Clínicas, da Faculdade de Medicina, da Universidade de São Paulo, Rua Dr Enéas Aguiar, 255, 05403-000 São Paulo, Brazil
Tel/Fax: +55 11 2661 6444
E-mail: maristelapf@uol.com.br

Received 28 February 2013, revised 22 May 2013, 29 October 2013, accepted for publication 24 November 2013

DOI: 10.1111/tid.12210
Transpl Infect Dis 2014; 16: 369–378

Abbreviations: BSI, bloodstream infection; CPIS, Clinical Pulmonary Infection Score; CRAB, carbapenem-resistant *Acinetobacter baumannii*; GNB, gram-negative bacteria; HAI, healthcare-associated infection; ICU, intensive care unit; LPS, lipopolysaccharide; LT, liver transplantation; MDR, multidrug-resistant; MELD, model for end-stage liver disease; MIC, minimum inhibitory concentration; PFGE, pulsed-field gel electrophoresis; PRAB, polymyxin-resistant *Acinetobacter baumannii*; SSI, surgical site infection; VAP, ventilator-associated pneumonia

Conclusion. In the final model of the survival analysis, PRAB colonization or infection after LT was independently associated with mortality. One predominant clone was identified. The only risk factor identified in the multivariate analysis was polymyxin use. PRAB was an agent with high mortality, and the most important risk factor associated with colonization or infection for such bacterium was polymyxin use.

The incidence of bacterial infection is higher among patients undergoing liver transplantation (LT) than among those undergoing transplantation of other solid organs, as is the risk of septic shock and death due to such infection (1, 2). In addition, the incidence of post-LT healthcare-associated infections (HAIs) caused by multidrug-resistant (MDR) gram-negative bacilli (GNB) is on the rise. Few studies have examined the treatments and outcomes related to such infections in the setting of LT (3–5).

Among GNB, *Acinetobacter baumannii* is of growing importance. Although the incidence of post-LT infection with *A. baumannii* varies among transplant centers, it is always associated with high mortality (3, 4, 6–9). Treatment of *A. baumannii* infection is a challenge, because the bacterium often presents multiple resistance mechanisms, thereby limiting the therapeutic options. Strains of *A. baumannii* that are carbapenem resistant are usually sensitive only to tigecycline and polymyxin, so carbapenem is considered a good marker for an MDR phenotype (10). Infection with carbapenem-resistant *A. baumannii* (CRAB) is most often treated with polymyxin.

Polymyxin is a potent bactericidal drug against GNB. Its mechanism of action consists of binding to lipid membrane lipopolysaccharide (LPS), causing disruption of LPS and cell death (11). Little is known about the mechanisms of polymyxin resistance. The main mechanisms described for acquired resistance of GNB are changes in the lipid A component membrane. The addition of a phosphoethanolamine to lipid A causes a reduction in the negative charge and reduces the affinity of lipid A to polymyxins. These changes usually occur through point mutations in the genes of the *pmrAB* 2-component regulatory system (12, 13). Another mechanism of resistance described is the complete loss of LPS. When that occurs, sensitivity is regained to certain antibiotics, including cephalosporins and even drugs that are not normally active against GNB, such as vancomycin (14, 15).

Resistance to polymyxins is usually described as low, although cases of heteroresistance and the development of resistance during therapy have been reported

(16). Cases of high-level resistance to polymyxins have also been reported in recent years (17, 18). However, to our knowledge, polymyxin-resistant *A. baumannii* (PRAB) infections have not been reported in the LT population. The main objectives of this study were to describe cases of such infection in liver transplant recipients and to identify risk factors for colonization or infection with PRAB.

Patients and methods

Study design and sample

We conducted a descriptive study of patients with PRAB infection, together with a retrospective cohort study to identify risk factors for colonization or infection with PRAB. At our facility, an outbreak of PRAB infection occurred in 2011, with the first case being diagnosed in July and the last being diagnosed in October. Before and after this period, sporadic cases of PRAB were identified. Therefore, we evaluated all patients undergoing LT between January 2011 and November 2011. We excluded patients who died within the first 72 h post transplant. Follow-up started at hospital admission and continued until the end of post-transplant month 2. Cases were defined as post-LT colonization or infection with PRAB.

Surveillance cultures

Screening for CRAB patients was already done in transplant units as part of control measures for MDR agents, when the first case of PRAB was identified. Surveillance cultures were collected for all patients admitted to the intensive care unit (ICU) if they came from another hospital or had spent >72 h in the emergency room. Surveillance cultures were also collected weekly during the ICU stay and at ICU discharge. On the LT ward, surveillance cultures were collected weekly until 3 weeks after the last patient with a PRAB infection had been discharged. Cultures

were obtained through rectal and inguinal swab. The specimens were placed on screening medium directly in broth containing 0.5 mg/L of ertapenem.

Microbiological analysis

During all periods of study, surveillance cultures were performed using selective media supplemented with 6 µg/mL of imipenem. The strains of *A. baumannii* identified were tested to detect meropenem, imipenem, and polymyxin resistance using an automated system.

To determine minimum inhibitory concentrations (MICs), we used an automated system (VITEK 2; bioMérieux Clinical Diagnostics, Marcy l'Etoile, France). Cases in which *A. baumannii* infection was initially identified as polymyxin-resistant (MIC ≥2 mg/L) were subjected to screening by disk diffusion with polymyxin E. The clinical and surveillance samples were sent to a microbiology research laboratory to have their clonality assessed by pulsed-field gel electrophoresis (PFGE) and to confirm polymyxin resistance. The MICs for polymyxin were determined by broth microdilution and were interpreted using the Clinical Laboratory Standards Institute breakpoints (19). Endonuclease *Sma*I-digested genomic DNA samples were separated by PFGE (CHEF-DR III system; Bio-Rad, Hercules, California, USA). Restriction patterns were interpreted according to Tenover et al. (20). The criteria used to identify and classify HAIs were those outlined by the (United States) National Healthcare Safety Network (21).

Statistical analysis

We evaluated the following variables related to the transplantation procedure: surgical time, cold ischemia time, number of blood units transfused, American Society of Anesthesiologists physical status classification, type of biliary anastomosis, donor type (living or cadaveric), extensiveness of the procedure (liver-only or combined liver-kidney transplantation), level of experience of the lead surgeon, retransplantation within the first 2 months, and reoperation. We also evaluated variables related to the recipient: age, gender, fulminant hepatitis, hepatocellular carcinoma, model for end-stage liver disease (MELD) score, and infection within the last 7 days before transplant. In addition, we evaluated variables related to the post-transplant hospitalization period: length of hospital stay; need for and duration of dialysis; duration of the use of invasive devices (e.g.,

urinary catheter, abdominal drain, central venous catheter, and mechanical ventilation); immunosuppression therapy at 30 days post LT; duration of antimicrobial use, by drug class; corticosteroid pulse therapy for acute cellular rejection; cytomegalovirus infection; post-transplant HAI; colonization or infection with CRAB; and length of ICU stay. For cases, the variables related to exposure time were recorded from admission to the first PRAB-positive culture. For the remaining patients, those variables were recorded for the total time at risk during the first 2 months post transplant.

The endpoint of the risk analysis was post-LT infection or colonization with PRAB. For survival analysis, the endpoint used was death in 60 days after LT. In the univariate analysis, categorical variables were evaluated by the chi-square test or Fisher's exact test, as appropriate, whereas continuous variables were tested by the Mann-Whitney test. Multivariate analysis was performed by stepwise logistic binary regression, variables presenting $P < 0.3$ being included in the multivariate analysis; variables that reduced the $-2 \times \log$ likelihood error for the included variables or had $P < 0.1$ were retained in the model.

For survival analysis, the endpoint used was death in 60 days after LT. We evaluated 60-day mortality using Cox proportional-hazard regression for multivariate analysis. Infection or colonization with PRAB was treated as a time-dependent variable. Data were processed and analyzed using SPSS (version 16.0; SPSS Inc, Chicago, Illinois, USA).

Results

Over the course of the study period, we evaluated 65 patients submitted to a total of 75 LTs. At least 1 surveillance culture for PRAB was collected from each patient during the hospital stay (median, 2 cultures). The culture was positive for PRAB in 7 cases, and PRAB infection developed in 4 of those cases. All 7 cases occurred after the first LT. The median time between LT and PRAB isolation was 26 days (range, 12–99), and the median time between admission and the first PRAB-positive culture was 40 days (range, 22–119). In all cases, at least 1 surveillance culture was negative before PRAB identification.

Among the patients with PRAB infection was 1 case of surgical site infection (SSI), 2 cases of ventilator-associated pneumonia (VAP), and 1 case of catheter-related bloodstream infection (BSI). Cases of VAP and BSI were treated with polymyxin plus carbapenem. One of the patients with VAP due to PRAB died during therapy and was classified as a case of treatment

failure; her initial Clinical Pulmonary Infection Score (CPIS) was 9. The other patient with VAP was treated successfully. He was recovering from sepsis from *Pseudomonas aeruginosa*, using meropenem and polymyxin for 10 days. This patient began to show signs of sepsis, requiring vasopressor agent, had worsening of respiratory parameters, and a new infiltrate on a chest x-ray. Initial CPIS was 7 and increase to 9 in the next 72 h. At that time, PRAB was isolated in tracheal aspirate. The only modification made to the antibiotic therapy regimen was an increase in the dose of meropenem. The tracheal aspirate was still positive for PRAB at 20 days after symptom onset. The patient recovered slowly and was discharged after 30 days on antibiotic therapy. Although the case of BSI was considered successfully treated, the patient died 1 week after the end of treatment and CRAB was isolated in a blood culture performed at that time.

In the SSI case, the patient had biliary complication in the early post-LT period and evolved to PRAB infection-related cholangitis. The infection could not be controlled and the patient was submitted to retransplantation. Despite combined treatment with multiple antibiotics, the patient continued to test positive for

PRAB in peritoneal fluid and died from septic shock (Table 1).

The susceptibility profile of PRAB is shown in Table 2. All strains tested were found to be resistant to carbapenems and cephalosporin. The drug with the best sensitivity was gentamicin. The polymyxin MICs determined by broth dilution was 128 mg/mL for 4 patients, 64 mg/mL for 1, 32 mg/mL for 1, and 16 mg/mL for 1 patient. The MICs at which 50% and 90% of the tested isolates were inhibited were 24 and 128 mg/mL respectively.

All cases of PRAB infection had previously been identified as CRAB infection. The median time between CRAB isolation and PRAB isolation was 33 days (range, 3–100 days). In only 1 case, CRAB was isolated before LT. In 6 cases, CRAB was found to be the causative agent of an HAI (2 cases of pneumonia, 2 cases of SSI, 1 case of urinary infection, and 1 case of spontaneous bacterial peritonitis). In another 15 patients (23%), CRAB was isolated although PRAB was not. In 8 of those patients, CRAB was linked to an HAI (3 cases of pneumonia, 3 cases of BSI, and 2 cases of SSI). One case of colonization and 1 case of infection by CRAB occurred before LT. In only 1 case, the patient was not

Cases of infection with polymyxin-resistant *Acinetobacter baumannii* (PRAB): type of infection, treatment, and outcome

Case	Colonization date	Sample	Infection date	Type of infection	Bacteremia	Treatment	Duration of treatment, days	Outcome	Time between PRAB to outcome, days
1		Blood	July 2011	Bloodstream infection	Yes	Colistin; imipenem	28	Death	23
2		Tracheal aspirate	July 2011	Ventilator-associated pneumonia	No	Colistin; meropenem	50	Discharge	63
3	August 2011	Tracheal aspirate			No			Death	30
4		Tracheal aspirate	August 2011	Ventilator-associated pneumonia	No	Colistin; imipenem	14	Death	2
5	September 2011	Central venous catheter			No			Death	5
6	September 2011	Rectal screening			No			Discharge	24
7	October 2011	Rectal screening	October 2011 ¹	Surgical site infection	Yes	Colistin; gentamicin; ampicillin/sulbactam; tigecycline	90	Death	101

¹Time between colonization to infection was 11 days.

Table 1

Susceptibility profile of 7 strains of polymyxin-resistant *Acinetobacter baumannii*

Drug	MIC range (mg/mL)	Sensitivity (%)
Gentamicin	<1 >16	86
Amikacin	16 >64	14
Cefepime	32 >64	0
Imipenem	>16	0
Meropenem	>16	0
Tigecycline	2 4	14 ¹
Polymyxin	8 128	0

¹All others were classified as intermediate.
MIC, minimum inhibitory concentration.

Table 2

receiving carbapenem when PRAB was isolated. However, that patient had been under treatment with carbapenem for 30 days, ending the treatment 10 days before the PRAB infection. Among all patients submitted to LT, 20 (30%) used polymyxin in the first 60 days after LT; among patients that acquired PRAB, 7 (100%) used polymyxin before PRAB was identified.

In the univariate analysis, the following risk factors for PRAB colonization or infection were identified: dialysis; use of carbapenems, polymyxins, cephalosporins, or ampicillin; mechanical ventilation; and a high MELD score. In the multivariate analysis, only polymyxin use remained significant (Table 3). Colonization or infection with CRAB was not included in risk factor analysis, as all cases had CRAB previously isolated.

Mortality was 47% among patients colonized or infected with CRAB, compared with 71% for those colonized or infected with PRAB. Post-LT mortality was 27% among patients in whom *A. baumannii* had not been identified. The 60-day mortality rate for PRAB, CRAB, and patients without CRAB was 57%, 27%, and 27% respectively (Fig. 1). Despite these differences in mortality rate, PRAB infection was not independently associated with mortality in the final model of the survival analysis (Table 4).

The PFGE analysis was performed in 20 isolates of PRAB and in 3 isolates of CRAB (Fig. 2). Among the strains of PRAB, 5 patients had strains with the same PFGE profile. Another patient was infected with a strain classified as closely related, and 1 patient was infected with 2 strains – one with the predominant PFGE pattern and another with a distinct pattern. Among the 3 CRAB isolates analyzed by PFGE, the first, from Case 4, differed by only 1 band from the PRAB isolated from the same patient. The second CRAB isolate, from Case 5, was classified as possibly related to the predominant

strain of the outbreak. The third CRAB isolate, from Case 3, although differing from the outbreak strain, was closely related to the PRAB strain isolated from the same patient.

Discussion

LT patients are at risk for infection with MDR bacteria. This risk is attributable to the complexity of the surgery, the prolonged hospital stay, the need for multiple invasive procedures, the intensity of antimicrobial use, and the use of immunosuppression therapy (1). The incidence of MDR among GNB isolated from patients with post-LT bacteremia ranges from 5% to 22%. The reported proportion of *A. baumannii* among infections by MDR-GNB after LT ranges from 20% to 49% (2, 5, 22).

Although the incidence of CRAB infection varies among healthcare facilities, it is increasing worldwide, and liver transplant recipients are particularly affected by this epidemiologic change. The reported incidence of post-LT CRAB infection is 6–10%, and the associated mortality rate has been reported to be as high as 80% (3, 4, 7, 23).

To our knowledge, no studies have evaluated the risk factors for *A. baumannii* infection in liver transplant recipients. The risk factors described for CRAB in the general population are severe disease, antimicrobial use (especially the use of carbapenems and third-generation cephalosporins), prolonged ICU stay, the use of invasive devices, and a high incidence of colonization by CRAB in the ward (24, 25).

Our LT ward has a high incidence of infection with MDR bacteria, and the incidence of post-LT colonization and infection with GNB is highest for CRAB (31%), which accounts for 18% of all post-LT HAIs. Therefore, liver transplant recipients are often not only infected or colonized by CRAB but are also frequently subject to empirical therapy with polymyxin.

Although the incidence of polymyxin resistance among *A. baumannii* strains is described as low, hospital outbreaks of infection with *A. baumannii* showing high levels of polymyxin resistance have been reported in Korea and Greece. Nevertheless, few studies have described the clinical outcomes of such infections, and no treatment of choice has yet been established (26, 27).

We have described 4 cases of PRAB infection, all of which were considered to have a high inoculum: 2 cases of VAP, 1 case of BSI, and 1 case of SSI with cholangitis. We classified 1 case of VAP as having been successfully treated. The case of BSI was considered

Freire et al: *A. baumannii* in liver transplantationVariables related to infection with polymyxin-resistant *Acinetobacter baumannii*: univariate and multivariate analysis

Variable	Cases	Non-cases	Univariate analysis		Multivariate analysis		
			P-value	RR	P-value	OR	CI
Polymyxin use, days (mean)	24	6	<0.001		0.03	1.1	1.02 1.2
Mechanical ventilation, days (mean)	27	6	0.002				
Carbapenem use, days (mean)	22	11	0.002				
Time on dialysis, days (mean)	24	12	0.009				
Intensive care stay, days (mean)	44	15	0.02				
Penicillin use, days (mean)	1	3	0.03				
Dialysis (%)	7 (100)	34 (58)	0.04	1.21			
MELD score (mean)	33	25	0.05				
Cephalosporin use, days (mean)	1	3	0.05				
Acute cellular rejection (%)	3 (43)	8 (14)	0.09	1.27			
Hospital stay before LT, days (mean)	12	6	0.12				
Piperacillin-tazobactam use, days (mean)	5	2	0.26				
Fulminant hepatitis (%)	2 (29)	8 (14)	0.29	1.13			
Retransplantation (%)	2 (29)	8 (14)	0.29	1.14			
Combined liver-kidney transplant (%)	1 (14)	2 (3)	0.29	1.35			
Surgical time, min (mean)	438	492	0.36				
Vancomycin-resistant enterococci ¹ (%)	6 (85)	38 (65)	0.41	1.1			
Central venous catheter duration, days (mean)	40	20	0.43				
Urinary catheter duration, days (mean)	19	14	0.48				
Living-donor transplant (%)	0	8 (14)	0.58	0.88			
n of blood units transfused (mean)	3	3	0.59				
Hepatocellular carcinoma (%)	2 (29)	12 (21)	0.64	1.05			
Carbapenem-resistant enterobacteriaceae ¹ (%)	1 (14)	18 (31)	0.66	0.92			
Cytomegalovirus infection (%)	1 (14)	18 (31)	0.66	0.92			
Cold ischemia time, min (mean)	446	388	0.68				
ASA score = 4 (%)	5 (71)	32 (55)	0.69	1.57			
Female gender (%)	3 (43)	33 (43)	0.69	0.94			
Reoperation (%)	3 (43)	21 (36)	0.73	1.03			
Quinolone use, days (mean)	5	1	0.86				
Age, years (mean)	46	47	0.98				
Duration of abdominal drain, days (mean)	12	13	0.99				
Pre-transplant infection (%)	2 (29)	20 (34)	1	0.97			
Biliary reconstruction by CC (%)	7 (100)	56 (97)	1	1.12			
Immunosuppression therapy ² (%)	3 (43)	22 (38)	1	1.09			

¹Infection or colonization.
²Tacrolimus + corticosteroid vs. tacrolimus + corticosteroid + mycophenolate mofetil.
RR, relative risk; OR, odds ratio; CI, confidence interval; MELD, model for end-stage liver disease; LT, liver transplantation; ASA, American Society of Anesthesiologists (physical status classification); CC, choledochocholedochostomy.

Table 3

Fig. 1. Kaplan-Meier estimate of cumulative (Cum) probability of recipient survival after liver transplantation stratified by recipients who had colonization or infection by carbapenem-resistant *Acinetobacter baumannii*, colonization or infection by polymyxin-resistant *A. baumannii*, and liver transplant recipients with non-*A. baumannii* multidrug-resistant organisms isolated.

inconclusive, because the patient showed initial improvement of symptoms but deteriorated during therapy and died, with the blood culture performed on the day of death being positive for CRAB.

All of our cases of PRAB infection were related to the use of invasive devices or to the surgical procedure. The case of SSI was the only one with a deep focus (biloma), which was inaccessible for early infection control. Although that patient was later submitted to retransplantation in an attempt to eliminate the infection, the procedure was unsuccessful.

All of our patients were treated with combined antibiotic therapy, the most common combination being carbapenem and polymyxin. The effectiveness of combined antibiotic therapy in case of resistance to polymyxin is not well established. *In vitro*, the polymyxin-carbapenem combination has been reported to have a synergistic effect for non-fermenting polymyxin-resistant GNB strains (28, 29). The mechanism of action of carbapenem in GNB involves penicillin-binding protein located in the cytoplasmic membrane. Therefore, crossing over the outer membrane and reaching the periplasmic space is a critical point. Polymyxins can alter membrane permeability by increasing the concentration of carbapenems in the periplasmic space, which explains their bactericidal effectiveness. Despite the theoretical rationale for combined therapy, few studies have analyzed combined therapy for PRAB treatment, and clinical trials are needed to test this approach (30, 31).

Risk factors for 60-day mortality in 65 patients submitted to liver transplantation: univariate and multivariate analysis

Factors	Univariate analysis	Multivariate analysis		
	P-value	P-value	HR	CI
Central venous catheter duration	<0.001			
Surgical time	<0.001	0.001	1.02	1.01 1.08
MELD score	<0.001	0.003	1.08	1.03 1.13
Cold ischemia time	<0.001			
Age	<0.001			
Duration of dialysis	0.001			
ASA score	0.01			
Blood units transfused	0.01			
Dialysis	0.01			
Lead surgeon with <8 years' experience	0.04	0.006	5.1	1.60 16.4
PRAB	0.002	0.03	1.03	1.01 1.05
Fulminant hepatitis	0.13			
Duration of mechanical ventilation	0.17			
Retransplantation	0.24	0.12	2.34	0.80 6.87
Acute cellular rejection	0.28			

HR, hazard ratio; CI, confidence interval; MELD, model for end-stage liver disease; ASA, American Society of Anesthesiologists (physical status classification); PRAB, polymyxin-resistant *A. baumannii*.

Table 4

In the present study, we sought to identify risk factors related to the acquisition of PRAB infection. Among the 65 patients undergoing LT during the study period, PRAB was detected in 7 (11%). We consider this an alarming rate.

The risk factors identified in our univariate analysis can be broadly divided into factors related to the degree of patient immunosuppression, such as a high MELD score and renal failure; factors associated with increased risk of acquiring microorganisms by cross-transmission, such as prolonged ICU stay and mechanical ventilation; and factors related to resistance induction, such as prior isolation of CRAB and antibiotic use.

The use of polymyxin was the only independent risk factor for PRAB identified in our multivariate analysis. In the literature, the emergence of PRAB is typically related to polymyxin use. In a case-control study, Matthaiou et al. (32) analyzed 41 polymyxin-resistant GNB and identified the use of polymyxin as the only

Fig. 2. Pulse-field gel electrophoresis of *Acinetobacter baumannii* strains isolated from 7 patients. Polymyxin-resistant *A. baumannii* (PRAB) in lanes 1 and 2, Case 4; lanes 3–5, Case 3; lanes 6–8, Case 1; lane 9, Case 6; lanes 10–13, Case 7; lanes 14–19, Case 2; and lane 20, Case 5. Carbapenem-resistant *A. baumannii* is shown in lane 21, Case 4; lane 22, Case 5; and lane 23, Case 3.

risk factor for the emergence of this type of resistance. Other reported risk factors for the emergence of PRAB include the use of quinolones and glycopeptides, as well as prolonged ICU stay (33). In addition, cases of emergence of polymyxin resistance during polymyxin treatment of CRAB have been described, some of which were in patients submitted to solid organ transplantation (23, 34). Shields et al. (9) reported that colistin increased MICs in 40% of patients treated with regimens containing colistin, with high levels of resistance being observed in 2 patients. Polymyxin has been shown to have poor concentration at certain sites within the body, a pharmacodynamic feature that might lead to suboptimal dosage, thus contributing to the emergence of resistant strains (35). Park et al. (26) reported the concomitant emergence of different polymyxin-resistant strains during an outbreak. This report leads to the assumption that resistance induction, more than cross-transmission, plays the most important role in the emergence of PRAB.

In our present study, the epidemiologic assessment suggested the induction of resistance, and the molecular biology analysis demonstrated that this must have occurred in at least 2 patients. However, the presence of a single predominant clone allows us to infer that the main reason for the emergence of PRAB in nearly all cases observed was cross-transmission on the LT ward and in the ICU. Therefore, it is possible that the use of polymyxin had, in most cases, only favored colonization

of these patients by PRAB. This theory partially explains the statistical nonsignificance of previous CRAB isolation in the multivariate analysis.

The virulence of polymyxin-resistant strains has been much discussed. *In vitro* studies have demonstrated that changes in membrane LPS, especially its total loss, decrease the fitness of bacteria (36). An animal model study demonstrated that PRAB is less virulent than is *A. baumannii* strain ATCC 19606 (37). Rolain et al. (17) described the development of PRAB during colistin treatment of CRAB-induced VAP, with the PRAB being isolated in sputum and urine. In that case, the patient did not develop PRAB infection, leading the authors to infer that the PRAB strain was less virulent, as had previously been observed *in vitro* (17). In our study sample, this phenomenon did not occur. The cases of PRAB remained resistant to all beta-lactams, with susceptibility only to aminoglycosides. In addition, among the 7 colonized patients, 4 patients developed infection (57%), similar to the 64% observed for CRAB infection in our cohort. In the literature, the proportion of CRAB-colonized patients who evolve to infection ranges from 17% to 54% (24, 25).

Cases of PRAB in which polymyxin resistance does not result in a loss of virulence likely occur because the strains have acquired some compensatory mechanisms, as has previously been reported. Lopez-Rojas et al. (23) described a case of PRAB-related meningitis identified during treatment with colistin and reported

that it was necessary to change the antimicrobial regimen in order to achieve therapeutic success. Shields et al. (9) described high-level resistance to colistin during the treatment of *A. baumannii* infection in 2 patients undergoing solid organ transplantation (1 receiving a heart transplant and the other receiving a lung transplant); the outcomes in both cases were treatment failure and death.

It is possible that PRAB is a marker of severity, characterizing a population that is highly susceptible to all infections and other clinical complications. This theory may explain the fact that, although mortality rate was much higher among patients with PRAB, this variable was not associated with an increased risk of death in the final model of survival analysis.

Herein, we have shown that liver transplant recipients are particularly susceptible to acquiring infection with PRAB, which had a major impact on patient survival. Treatment with polymyxin was the most important risk factor for emergence of PRAB.

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