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 Câmera Pierrotti PhD [c], Larissa Marques de Oliveira MSc [d], Anna Silva Machado PhD [a], Inneke Marie Van Der Heijdenn 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.

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]

Colonization by MDR-GNB has been described as an important risk factor for infection by those same bacteria. Gianella et al[4] 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

* 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.

0196-6553/
http://dx.doi.org/10.1016/j.ajic.2016.12.010

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