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ORIGINAL ARTICLE

Predicting factors for infection or colonization by multidrug-resistant bacteria in a general hospital: a case-control study

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SUMMARY

Nosocomial infections are frequently caused by multidrug-resistant microorganisms. In everyday clinical practices, physicians face a dilemma when treating serious infections: to prescribe broad-spectrum antibiotics and contribute to increasing antibiotic resistance or use a narrow spectrum of antimicrobials and put patient prognosis at risk. The aim of this study was to identify potential predictors for the presence of multidrug-resistant bacteria and to build a clinical prediction model that can help physicians to recognize patients with different risks for infection or colonization by these microorganisms. We carried out a case-control study with all patients that had at least one culture performed. Cases were defined as patients that had a culture demonstrating a multi-resistant agent. Controls were all other patients that had at least one negative culture. The consensus definition from the Centers for Disease Control and the European Centre for Disease Prevention and Control was used to describe antibiotic multi-resistance. A backward logistic regression identified that a hospitalization history of 180 days, tube feeding, length of hospital stay before the culture, Charlson comorbidity index, central venous catheter, and tracheostomy were all independent predictors for patients that carried multidrug-resistant microorganisms. The bootstrap procedure was employed to assess internal validity. The shrinkage method was used to correct for optimism and the model was calibrated. The regression formula is described and the final model accuracy was evaluated by a receiver operating characteristic curve analysis. The area under the curve was 0.78, showing that the discriminative predictive capacity of the model was good.

INTRODUCTION

Nosocomial infections are a major threat to patient safety .[1] These infections are responsible for many losses and an increase in health-care related costs worldwide.[2] Among these infections, those caused by multidrug-resistant (MDR) bacteria are of special concern.[3] They impose additional costs, morbidity, and mortality.[4] One cause known to have these unfavorable outcomes is the inadequacy of the antibiotics first prescribed in life-threatening infections, such as pneumonia and sepsis.[5] The delay in administering a proper antibiotic regimen has been considered responsible for increases in deaths and prolonged hospital stays.[6] For these reasons, some medical specialty societies have stated guidelines to manage such clinical entities.[7] They focus on two aspects: first, the identification of patients with risk factors for MDR infections; second, if risk factors are present, the initial therapy should include broad-spectrum antibiotics, until the results of microbiological tests are available. However, broad-spectrum antimicrobial treatments can increase bacterial resistance and cause adverse reactions.[8] Thus, when dealing with such severe infections, physicians are challenged by this dilemma. As stated by the American Thoracic Society (ATS) and the Infectious Diseases Society of America (IDSA), institutional therapy protocols should reflect local characteristics, including the identification of potential risk factors for harboring MDR bacteria.[7] Frequently, studies have identified variables such as prior hospitalization, prior antibiotic use, long hospital stays, comorbidities, home treatments, nursing home residency, indwelling catheters, mechanical ventilation, and high colonization pressure as risk factors associated with bacterial resistance.[9, 10] Most research in this field focuses on ICU patients. Few studies have been published on the incidence and risk factors for nosocomial infections with MDR bacteria among overall hospitalized patients.[11] The present study seeks to recognize clinical and epidemiological variables that can be used as predictors and to develop a prediction model capable of identifying patients with different risks for MDR infection or colonization.

MATERIAL AND METHODOS

A case-control study was carried out at Hospital Santos Dumont, in the city of São José dos Campos, state of São Paulo, Brazil, between June 1, 2009 and June 30, 2011. The facility is a 90-bed tertiary hospital with two intensive care units (ICUs) with 10 beds each. There are no pediatric or obstetric wards. The study included all patients that had undergone at least one culture of any material during their hospital stay. Case was defined as a patient who had at least one culture that tested positive for MDR microorganisms as defined by the Centers for Disease Control and Prevention (CDC) and the European Centre for Disease Prevention and Control consensus.[3] Controls were defined as patients who had had at least one culture and that did not test positive for any MDR bacteria. Data were retrospectively extracted from electronic medical records. Surveillance cultures were excluded. Each patient was registered as to age, gender, culture date, specimens submitted to culture, microorganism isolated, microorganism group, Charlson comorbidity index, whether the patient was being transferred from other facility, history of admission in any health-care facility within the last 180 days – including home care and long-term care – and whether the patient had been admitted for a surgical-versus-medical service. The previous antibiotic use variable was defined among four groups: no use, prophylactic, prophylactic and therapeutic, and therapeutic use. Prophylactic use was the chosen group if the patient had received antibiotics at anesthetic induction and post-surgery for no longer than 48 hours; Therapeutic if the patient received any course of antibiotic therapy not characterized as prophylactic; and Prophylactic and therapeutic if both courses of antibiotics were present. The use of an antibiotic was considered only if it was administered before the collection of a specimen for culture. Indwelling catheters were studied: Central venous catheter, tube feeding, and tracheostomy were considered if present at any time before culture. The urinary catheter variable was considered present if a urinary catheter was left in place for 24 hours or more. Similarly, mechanical ventilation was considered if the patient received invasive respiratory assistance for at least 24 hours. Length of stay until culture was defined as the time between admission and culture collection. For the cases, the first culture with demonstration of MDR was considered, whereas for controls, it was the last culture collected. In many studies, the length of stay (LOS) predictor is usually log-transformed,[12] which is to say this variable was transformed into a natural logarithm. Cases were only considered at the first admission.

We estimated the number of patients required using the number of events per variable. Peduzzi at al. demonstrated that, for logistic regression models, a number of ten events per variable studied made it possible to obtain stable estimates.[13] As we studied 14 variables, the number of cases necessary was thereby 140.

Microbiological data were collected separately from clinical data to prevent bias. Microbiological identification and susceptibility tests were processed in an automated system, Vitek–2 compact (bioMérieux Inc. - France). Clinical and Laboratory Standards Institute (CLSI) recommendations were adopted.

The Mann-Whitney U test was used to compare continuous variables and the Pearson χ^2 or Fisher exact test compared categorical variables. In order to create a clinical prediction model of MDR infection or colonization, a backward logistic regression was used with all the studied variables. Odds ratios (OR) and their 95% confidence intervals (CIs) were calculated. All *p* values were two-tailed, and $p < 0.05$ was considered statistically significant. Internal validation was evaluated by the Bootstrap resampling method, considering all the variables studied and backward elimination.[14] Shrunken coefficients calculated by bootstrapping were used to construct an ROC curve to evaluate the final model accuracy. A bootstrap estimate of calibration accuracy assessed how well the model had been calibrated, and a calibration plot was constructed.

The statistical analysis was performed using Epi Info 3.5.1 and R software[15] v.2.15.0 with rms package[16] for all analyses.

This project was approved by the Research Ethics Committee of Faculdade de Ciencias Médicas, Universidade Estadual de Campinas, with written informed consent exemption, under number 15014/2012.

RESULTS

Within the study period, 753 patients were submitted to clinical cultures. MDR bacteria were identified in 146. The prevalence was 19.4% with a 95% confidence interval (CI 95%), ranging between 16.7 and 22.4%. Table 1 shows the distribution of clinical specimens submitted to culture according to MDR status.

Eight-four (11.2%) patients underwent blood cultures only. The median and the mean number of cultures per patient were 2.0 and 3.0, respectively. The rate of positive cultures was 24.6% and the percentage of cultures with MDR bacteria was 9.1%. The percentage of patients with a positive culture was 40.2%. Table 2 lists the frequency of bacterial groups and Table 3 shows the bacteria isolated from culture and MDR status. Clinical and demographic characteristics of patients and MDR status are listed in Table 4.

Table 1 – Distribution of cultivated samples according to MDR status.

Specimen	MDR n (%)	No-MDR n (%)	*p valor*
Blood	29 (15,4)	1109 (53,7)	< 0,0001
Urine	78 (41,5)	599 (29,0)	0,0003
Secretions	26 (13,8)	104 (5,0)	< 0,0001
Tracheal aspirate	28 (14,9)	78 (3,8)	< 0,0001
Catheter tip	16 (8,5)	53 (2,6)	< 0,0001
Liquor	2 (1,1)	46 (2,2)	0,43 (NS)
Bone fragment	3 (1,6)	24 (1,2)	0,49 (NS)
Pleural fluid	2 (1,1)	17 (0,8)	0,40 (NS)
Other specimens	4 (2,2)	37 (1,8)	0,77 (NS)
Total	188	2067	-

MDR – multidrug-resistant microorganism, NS – non-significant

Table 2 – Frequency of microorganism groups isolated from cultures.

Microorganism group	Frequency	Percentage (95% Confidence interval)
Negative	1701	75,4% (73,6-77,2)
Enterobacteria	227	10,1% (8,9-11,4)
Gram positives	212	9,4% (8,2-10,7)
Nonfermentative	91	4,0% (3,3-5,0)
Proteeae tribe	22	1,0% (0,6-1,5)
Neisseria spp.	1	0,0% (0,0-0,3)
Aeromonas genus	1	0,0% (0,0-0,3)
Total	2255	100,0%

Table 3 – Frequency of microorganism species isolated from cultures according to MDR status.

Microorganisms	MDR n (%)	No-MDR n (%)	*p* value
Klebsiella pneumoniae	47 (25,0)	16 (4,4)	<0,0001
Staphylococcus aureus	32 (17,0)	27 (7,4)	0,0008
Escherichia coli	29 (15,4)	52 (14,2)	0,79 (NS)
Acinetobacter baumannii	23 (12,2)	6 (1,6)	<0,0001
Enterobacter cloacae	18 (9,6)	13 (3,6)	0,0064
Pseudomonas aeruginosa	17 (9,0)	29 (7,9)	0,80 (NS)
Serratia marcescens	6 (3,2)	13 (3,6)	0,82 (NS)
Morganella morganii	4 (2,1)	9 (2,5)	1,00 (NS)
Proteus mirabilis	2 (1,1)	7 (1,9)	0,27 (NS)
Staphylococcus coagulase negativa	-	119 (32,5)	<0,0001
Enterococcus faecalis	-	20 (5,5)	0,0004
Enterobacter aerogenes	-	13 (3,5)	0,006
Others	10 (5,4)	58 (15,8)	0,0006
Total	188 (100,0)	366 (100,0)	–

MDR – multidrug-resistant microorganism, NS – non-significant

Table 4 – Clinical and demographic characteristics of patients according to MDR status.

Predicting factor	MDR		No-MDR		*p* valor
Male gender (%)	77	(52,7)	308	(50,7)	0,73
Age, years, median (IQR)	68	(25,0)	65	(34,0)	0,20
Admission for 180 days (%)	89	(61,0)	229	(37,7)	< 0,0001
Patient transferred from another facility (%)	45	(30,3)	104	(17,1)	0,0003
Clinical versus surgical (%)	83	(56,8)	376	(61,9)	0,30
Prior antibiotic use					
No	41	(28,1)	338	(55,7)	< 0,0001
Preventive	1	(0,7)	4	(0,7)	1,00
Prophylactic	12	(8,2)	63	(10,4)	0,52
Prophylactic and therapeutic	9	(6,2)	16	(2,6)	0,05
Therapeutic	82	(56,8)	186	(30,6)	< 0,0001
Length of stay until culture, days, median (IQR)	10	(20,0)	1	(6,0)	< 0,0001
Tracheostomy (%)	24	(16,4)	13	(2,1)	< 0,0001
Mechanical ventilation (%)	55	(37,7)	65	(10,7)	< 0,0001
Urinary catheter (%)	94	(64,4)	190	(31,3)	< 0,0001
Central venous catheter (%)	80	(54,8)	122	(20,1)	< 0,0001
Tube feeding (%)	72	(49,3)	85	(14,0)	< 0,0001
Number of cultures, median (IQR)	2	(2,0)	1	(0,0)	< 0,0001
Charlson comorbidity index, median (IQR)	4	(6,0)	3	(5,0)	0,0002

IQR – Interquartile range, MDR – multidrug-resistant microorganism

To deal with extreme values, the variable for length of stay until culture was encoded with a natural logarithm and truncated at a maximum value of 90 days. The logistic regression with backward elimination indicated that admission for 180 days, tube feeding, natural logarithmic transformation of length of stay until culture, Charlson comorbidity index, central venous catheter, and tracheostomy were all predicting factors for patients to be infected or colonized by MDR as summarized in Table 5.

Table 5 – Clinical and demographic characteristics that were selected as predictors for identifying patients colonized or infected with MDR bacteria by logistic regression with backward elimination method.

Predicting factor	Odds Ratio (95% Confidence interval)	*p* valor
Admission for 180 days	2,69 (1,78-4,07)	<0,001
Tube feeding	2,33 (1,42-3,83)	0,001
ln* (Length of stay until culture)	1,50 (1,22-1,84)	<0,001
Charlson comorbidity index	1,06 (0,99-1,13)	0,104
Central venous catheter	1,45 (0,87-2,43)	0,154
Tracheostomy	2,45(1,09-5,51)	0,029

Nagelkerke R2=0,27 C=0,80
Model Likelihood Ratio Test (p-valor <0,0001)
**ln – natural logarithm*
MDR - multidrug-resistant microorganism

In the validation phase, three hundred repetitions of bootstrap were performed and a logistic regression backward model, containing all the variables shown in table 4, was constructed for each bootstrap sample. The Nagelkerke's R2 for the original model was 27.3% and in the 300 bootstrap samples the mean apparent performance was 29.1%. When we tested the models from each bootstrap sample in the original sample, the R2 was 25.3%. The optimism was calculated (29.1% - 25.3% = 3.8%). Hence, the optimism-corrected R2 was 27.3 – 3.8 = 23.5%. The estimated Somers' D correlation coefficients for the original and the bootstrap models were 0.593 and 0.607, respectively. When the models from each bootstrap sample were tested in the original sample, the Somers' D correlation coefficient was 0.574. Hence, optimism was 0.034 and the optimism-corrected Somers's D coefficient was 0.559. The slope shrinkage factor was 0.91. The C statistics of the original and optimism-corrected model were similar, 0.797 and 0.780.

The new regression coefficients calculated from the bootstrap procedure using the shrinkage factor (0.91) are shown in Table 6. Box 1 shows the model formula for predicting MDR infection or colonization.

Tabla 6 - Original and shrunken logistic regression coefficients calculated from the bootstrapping procedure for the prediction model to identify patients colonized or infected with MDR bacteria.

Predicting factor	Original Regression Coefficient	Shrunken Regression Coefficient*
Admission for 180 days	1,001	0,911
Tube feeding	0,861	0,782
ln** (Length of stay until culture)	0,401	0,368
Charlson comorbidity index	0,063	0,048
Central venous catheter	0,401	0,343
Tracheostomy	0,896	0,792
Intercept	-3,271	-2,907

**Regression coefficient after multiplication by the shrinkage factor (0.91) from bootstrapping procedure*
***ln – natural logarithm*
MDR – multidrug-resistant microorganism

Box 1 – Logistic regression formula for MDR infection or colonization prediction model.

Probability of MDR = 1/1+exp-(-2.907
+ 0.368 × natural logarithm of length of stay until culture (in days)
+ 0.048× Charlson comorbidity index (points in the scale)
+ 0.911 × admission for 180 days (yes=1, no=0)
+ 0.782 × tube feeding (yes=1, no=0)
+ 0.343 × central venous catheter (yes=1, no=0)
+ 0.792 × tracheostomy (yes=1, no=0))
The logistic regression intercept is −2.907; the other numbers represent the shrunken regression coefficients (weight) of each predictor.

Figure 1 displays the receiver operating characteristic (ROC) curve when applying these shrunken coefficients to the original sample. The area under the curve was 79.6% with a 95% confidence interval of 76.6 to 82.5%.

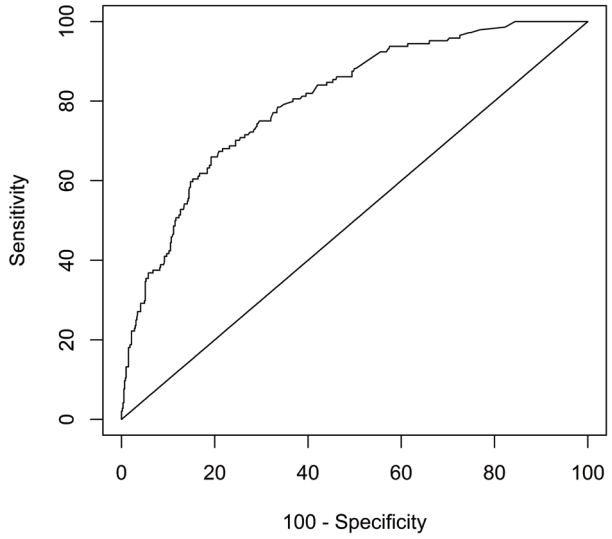

Figure 1 - Receiver operating characteristic (ROC) curve displaying sensitivity versus specificity for logistic regression with shrunken coefficients derived from bootstrapping procedure, comparing patients with and without MDR colonization or infection. The area under the ROC curve is 79.6%.

Figure 2 shows the bootstrap estimate of calibration accuracy for MDR infection or colonization from the final model. The figure displays the ideal, apparent, and bias-corrected curves from the bootstrap procedure with 300 samples.

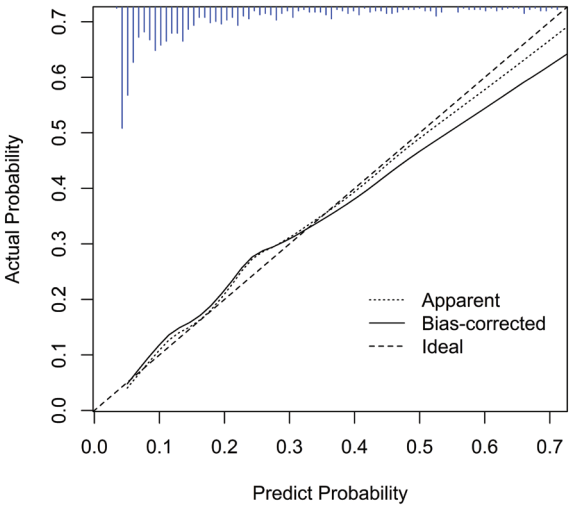

Figure 2 - Bootstrap estimate of calibration accuracy for MDR infection or colonization from the final model. The figure displays the ideal, apparent, and bias-corrected curves from the bootstrap procedure with 300 samples. The top shows the frequency of patients in each predicted probability.

The 0.9 quantile of absolute error in predicted probabilities between apparent and bias-corrected models is 0.035. The mean absolute error was 0.016.

DISCUSSION

The primary objective of this study was to assist physicians with some previous information about the results of a clinical culture. An appropriate initial antibiotic therapy is of paramount importance in clinical practice, as it is associated with a better prognosis. In this study, we described a predicting model that provides a potential clinical tool that may help physicians this initial choice. If the physician knows the probability of a patient harboring MDR bacteria and also the profile of antibiotic resistance in the facility, he can make a better-educated choice.

Most studies that have evaluated antibiotic resistance have focused on a single multi-resistant organism. We were intent on more accurately defining those patients most likely to harbor an MDR microorganism. This makes sense when faced with the fact that most risk factors studied are shared by several bacterial species. Other authors agree with this view.[17, 18]

There is much debate over the question of which group represents the best control group in case-control studies of antimicrobial resistance.[19] Some groups such as general hospital populations, patients with the susceptible forms of the microorganism, and patients who had a culture performed have been chosen as controls. According to Harris,[20] this choice must be based not only on the question being asked, but also on the generalization that is intended. A central concern about selecting as control patients those with the susceptible form of the microorganism is that it may cause overestimation of the association between prior antimicrobial use and the onset of resistance.[21] Our study design is in accordance with the recommendations made by Harris et al. for the case-control method applied to MDR microorganisms. The choice of control group was appropriate in order to answer the central question of this study: What is the probability that a culture required for a patient may indicate an MDR? Hence, our control group consisted of those patients with at least one culture ordered with no MDR microorganism isolation.[20] Thus, it enrolled all patients with any clinical cultures ordered during the 25-month study period. Furthermore, from the methodological point of view, two other recommendations addressed by Harris et al. were satisfied.[21] The first was to include the time at risk variable in the analysis. It is important to stress that this period of time needs to be measured before the outcome is observed. The second was the adjustment for comorbidity illness. The Charlson comorbidity index was used, since all hospital patients were involved. Other disease severity scores such as APACHE are difficult to obtain outside intensive care units.

Most studies on risk factors for MDR have been performed in Intensive Care Units (ICU), but few studies were carried out in an entire hospital.[11] Although critical-care patients are particularly at risk, frequent transfers between units and the extended length of stay in medical wards make a hospital-based study an attractive approach. This study included patients in these settings and this probably allowed it to identify predictors that sound more reliable in everyday clinical practices.

Our study evaluated traditional risk factors as predictors. Many studies were able to identify invasive procedures as central venous catheter, tube feeding, tracheostomy, mechanical ventilation, and urinary catheter as risk factors.[22-29] We were able to recognize associations with these invasive procedures and MDR status. However, in an attempt to construct an "easy-to-use" prediction model, we had to choose between several variables that undergo intense collinearity. In this sense, we used backward elimination in logistic regression. As a consequence, we had to deal with overfitting. Internal validation using bootstrapping and calibration shrinkage techniques are currently used for this purpose. Thus, a logistic regression model was built and validated using a bootstrap procedure considering all studied variables, as recommended by Steyerberg.[30]

Another feature that is frequently recognized as a risk factor

for harboring MDR bacteria is previous contact with health services.[31-34] We tried to identify this issue with two variables: patient transfer between facilities and previous admission within 180 days. These variables show intense collinearity. The second one showed a better performance in our model.

We found a significant association between tube feeding and MDR status. Even though identifying true risk factors was not the aim of the study, there are many hypotheses about this possible causal association. Schneider et al. observed that enteral artificial nutrition is associated with major qualitative and quantitative changes in the fecal microflora.[35] These changes are characterized by the preferential growth of aerobic bacteria to the detriment of anaerobes that would be responsible for colonization resistance. Moreover, tube feeding may be responsible for a weakening of the mucous membranes in the digestive system.

The area under the ROC curve for the original model was 0.80 and after internal validation performed by bootstrapping, the performance of the optimism-corrected model was 0.78. In the final model, with shrunken coefficients, the area was 0.80 (95% CI 0.77 to 0.83), showing that the discriminative capacity of the prediction model was good.

In the calibration plot, the bias-corrected estimate is slightly non-linear, but only slightly worse than the apparent calibration. The 0.9 quantile of absolute error in predicted probabilities between apparent and bias-corrected model is 0.035, demonstrating a small degree of overfitting in the original model.

This study has several potential limitations. Although selection bias is of concern in case-control studies, we included all patients that were submitted to cultures in the hospital. In this case-cohort design, the likelihood of selection bias tends to be diminished in comparison with traditional case-control study. Bias can also be introduced because of missing information. A special concern was the information quality concerning antibiotic use before hospital admission. As the definition did not specify a time gap to consider "prior antibiotic use," we believed that only antibiotic courses being used immediately before admission may have been mentioned in medical records. Thus, it is possible that a stronger association between antibiotics and MDR status might exist. Some attempts were made to include antibiotic use in the model. The dichotomization of the variable into any antibiotic use *versus* no-use, or any therapeutic use *versus* non-therapeutic-use plus no-use was attempted, but even then none of these changes allowed "prior antibiotic use" a place in the model. It was observed that the "length of stay until culture" variable showed intense collinearity with "prior antibiotic use". Probably for this reason antibiotic use was not in the final prediction model.

Finally, even though our prediction model appears to perform reasonably well in identifying patients harboring MDR bacteria, we should recognize the absence of a validation of the model in an independent group of patients. External validation of a prediction model is essential to support general applicability.

CONCLUSION

The history of admission within 180 days, tube feeding, length of hospital stay until culture and tracheostomy variables were independent predictors for MDR status. The logistic regression model was able to identify patients with different risks for MDR infection or colonization in the studied population.

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