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Brief Communication

NDM-producing *Enterobacterales* prevalence associated to COVID-19 in a tertiary hospital

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ABSTRACT

Colonizations/Infections caused by carbapenem-resistant *Enterobacterales* are of great clinical and epidemiological importance due to their rapid dissemination and high mortality rates. In this scenario, the use of antibiotics intensified by the COVID-19 pandemic has brought about a great warning on the real impact that this pandemic could have on antimicrobial management programs and long-term antimicrobial resistance rates. The objective of this study was to evaluate the increase of New Delhi Metallo β -Lactamase (NDM)-producing *Enterobacterales* cases in COVID-19 units of a complex Brazilian tertiary hospital. This retrospective observational study included all patients admitted to the hospital identified as colonized or infected by NDM-producing Gram negative bacilli (GNB), from January 2017 to April 2021. Forty-two NDM-producing *Enterobacterales* were identified in 39 patients. The rate of NDM cases per total surveillance cultures increased progressively between 2017 and 2021 (chi-2 for trend, $p < 0.0001$) and was associated with a higher occurrence specifically in COVID units (Fisher exact, $p < 0.0001$). The molecular investigation of the NDM-producing *Klebsiella pneumoniae* strains revealed the emergence of diverse clones during the COVID-19 period, also with possible evidence of horizontal transmission among patients within COVID units. NDM-producing *Enterobacterales* with multiple and different clonalities in the COVID-19 units also raised questions about the importance of other factors besides horizontal clonal transfer, including the increase of antimicrobial consumption by these patients.

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Introduction

New Delhi Metallo β -lactamases (NDM) production has been progressively identified in carbapenem-resistant Gram-

negative bacteria on all continents, representing a significant challenge for clinical management and public health worldwide.^{1,2} Since 2013, the increase of NDM-positive strains in Brazil has been described in different species of Gram-negative bacilli (GNB) recovered from human, animal, and environmental sources.²⁻⁵ The transmission of NDM-positive strains in health care settings is related to close contact, with hospitalized patients being the most vulnerable for

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colonization and infection. Although, in general the same infection control measures should be applied to any carbapenem-resistant organisms regardless of the resistance mechanisms,⁶ NDM- and *Klebsiella pneumoniae* carbapenemase (KPC)-producing isolates apparently present relevant epidemiological differences.^{1,2} While KPC-producing isolates have been widely described internationally belonging to a major well-adapted *K. pneumoniae* clonal complex (258),⁷ NDM-producing isolates are commonly described in multiple GNB⁸ with apparently no sustained global spread of specific high-risk clades.¹

Recently, the increased use of antibiotics may have been intensified by the COVID-19 pandemic worldwide^{9,10} raising concerns about the real impact of the pandemic on antimicrobial management programs and long-term antimicrobial resistance (AMR) rates,⁹ particularly strategies directed to COVID-19. The impact of the COVID-19 pandemic in Brazil and in São Paulo has posed significant threats to all, and in particular to the public hospital system¹⁰ in terms of allocated resources and quality of care. Most public hospitals have dedicated specific units (wards and intensive care units - ICU) for accommodating suspected and confirmed COVID-19 patients. In the study institution, the first case of carbapenemase detection (KPC) occurred in 2009 and spread in the postoperative ICU followed by some outbreaks of an endemic cluster of *Klebsiella pneumoniae* clonal complex 258 in the hospital environment.^{11,12} In this report, we describe the increase of another type of carbapenemase designated as NDM-1 producing *Enterobacterales* in COVID-19 units of a Brazilian high complexity tertiary hospital.

Material and methods

Population samples

This was a retrospective observational study based on surveillance cultures and target medical units conducted at Dante Pazzanese Institute of Cardiology (IDPC), a tertiary 350-bed hospital specialized in cardiovascular surgery. IDPC was included in the public COVID-19 efforts established in March 2020. As part of the efforts to manage the pandemic, IDPC infection control service and hospital administration established specific units and flows devoted to COVID-19 patients, while maintaining other units for their regular cardiothoracic patients. In the present study, we categorized these COVID-19 units (ICU or specific wards) and General units (ICU and Wards for non-COVID-19 patients). Since 2013, the infection control team performs weekly routine inguinal and anal surveillance cultures at the ICU, emergency room, and wards searching for carbapenem-resistant *Enterobacterales* (CRE), carbapenem-resistant non-glucose-fermentative Gram-negative bacilli (BGN-NF) (*Acinetobacter baumannii* and *Pseudomonas aeruginosa*) and vancomycin-resistant *Enterococci* (VRE), with the objective of placing those colonized patients in contact precautions. All patients admitted to COVID and non-COVID-19 units from January 2017 to April 2021, and identified as colonized or infected by NDM-producing GNB, were included in this study.

Microbiological methods

All surveillance cultures growing GNB were submitted to bacterial identification by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF/MS) technology, using Vitek MS[®] system (bioMérieux, Marcy-l'Étoile, France). The minimum inhibitory concentration (MIC) of all antibiotics was determined using Vitek[®] 2 System (bioMérieux), except for polymyxin B which was done by broth microdilution (Probac, Brazil). All surveillance cultures with GNB identified during the study period were screened for carbapenemase presence using commercially available disks containing carbapenems with and without EDTA (0.1 M), cloxacillin (75 mg/mL) or phenylboronic acid (40 mg/mL), as recommended by the Brazilian National Health Surveillance Agency. If screened positive, a sample was submitted to confirmation by Real-Time PCR for the detection of the following carbapenemase genes: *bla*_{KPC}, *bla*_{NDM}, *bla*_{IMP}, *bla*_{VIM}, *bla*_{GES}, and *bla*_{OXA-48-like}.¹³

Strains of the most prevalent NDM-producing species were analysed by Pulsed-Field Gel Electrophoresis (PFGE) for genetic relatedness, using the restriction endonuclease *SpeI* (New England Biolabs Inc., Ipswich, MA). PFGE profiles were analyzed and compared using BioNumerics version 8 software (Applied Maths, Sint-Martens-Latem, Belgium) and cluster analysis was performed with the unweighted pair group method with arithmetic mean, Dice coefficient and pattern interpretation. Isolates that exhibited a PFGE profile with ≥ 80% similarity were considered closely related.

Statistical analysis

A single NDM-producing isolate per patient was considered for the descriptive and statistical analysis. Information on variables age and sex of patients, NDM identification unit type (COVID or non-COVID-19), patient length-of-stay until NDM detection, surveillance culture, colonization or infection by NDM-producing GNB, and outcome (discharge or death) was collected. GraphPad Prism version 7.05 (San Diego, CA) was used for the chi-squared test for trend on the rate of new NDM patient (colonization's or infections) per the total number of surveillance cultures per year, from 2017 to 2021, and the Fisher's exact test on the rate of new NDM patient (colonization's or infections) per the total number of surveillance cultures per unit, COVID or non-COVID-19.

Results

A total of 13,004 active surveillance cultures were performed between January 2017 to April 2021. Nine hundred twenty-nine (7.1%) were positive for multidrug-resistant (MDR) bacteria: 616 (66%) CRE, 176 (28.5%) VRE, and 137 (22%) BGN-NF. Among CRE samples, there were 42 (6.9%) NDM-producing *Enterobacterales* isolated in 39 patients: 36 *Klebsiella pneumoniae*, two *Enterobacter cloacae*, two *Proteus mirabilis*, one *Morganella morganii* and one *Providencia rettgeri*. Three patients carried more than one NDM-producing in different species of *Enterobacterales*. From the 39 patients identified with at least one NDM-producing GNB during the study period, all were

Table 1 – (A) Total New Delhi Metallo β -Lactamase (NDM)-producing GNB cases detected per year and patient characteristics, and outcome (discharge or death). (B) Distribution of 36 NDM-producing *Klebsiella pneumoniae* strains identified in the present study according to their PFGE pattern, hospital unit and year of detection.

(1A) Number of NDM patients/Year	N	%
2017	2	5%
2018	3	8%
2019	7	18%
2020	9	23%
2021	18	46%
Total	39	100%
Age		
Mean (in years)	69 (43-91)	
Sex		
Male	25	64%
NDM Identification Unit		
COVID-19 (ICU or ward)	17	44%
Non-COVID-19 (ICU or ward)	22	56%
Length-of-stay until NDM identification		
Mean (in days)	26 (0-83)	
Underlying disease		
COVID-19	18	46%
Other	21	54%
NDM Colonization	39	100%
NDM Infection		
CLABSI	4	10%
Outcome Total		
Discharge	28	72%
Death	11	28%
COVID-19	8	72%
Clinical/Surgical	3	28%

(1B) PFGE Pattern (number of isolates identified with the same PFGE pattern)	2017	2018	2019	2020	2021
COVID-19 Ward					N (2), F (2)
COVID-19 ICU				H (3), J (1)	N (7), P (1)
Clinical Ward		C (1), D (1)	E (1), F (1), N (2)	K (1), J (1), M (1)	
Emergency room	A (1)		N (1)		
Surgical ICU			G (1)		O (1)
Surgical Ward				I (1)	
UCO				L (1)	L (2)
Clinical ICU		B (1)			F (1), Q (1)

considered colonized (rectal or inguinal sites) during the hospital stay and 4/39 patients presented central line-associated bloodstream infection (CLABSI). The time between colonization and infection ranged from four to seven days. Table 1A shows patient characteristics.

All 42 NDM-producing *Enterobacteriales* had no remaining carbapenemase genes evaluated and showed high-level of resistance to broad-spectrum cephalosporins, carbapenems, ciprofloxacin, gentamicin and piperacillin-tazobactam, according to CLSI and BrCAST guidelines.^{14,15} All *K. pneumoniae* samples (n = 36) were susceptible to polymyxin B (MIC \leq 0.5 μ g/mL) according to BrCAST or EUCAST guidelines.^{15,16}

Table 1B shows the PFGE method performed only on the *Klebsiella pneumoniae* isolates (n = 36) (one sample per patient). The dendrogram analysis revealed 17 PFGE patterns ("A" to "Q"). Five clusters (F, H, J, L and N) were detected with more than 80% of similarity by the Dice coefficient (Table 1B). The most prevalent cluster "N" was detected in 12 patients with 87.6% of similarity. Three of 12 *K. pneumoniae* strains cluster "N" was detected before the COVID pandemic period (year 2019), and nine strains were detected during the COVID

pandemic period (after March 2020) and within COVID units. Cluster "H" was found in three patients in COVID units at the same period with 100% similarity. Cluster "L" was detected in three patients in non-COVID units.

Fig. 1A shows the rate of NDM cases (colonizations or infections) per total surveillance cultures from 2017 to 2021, with the respective total amounts and chi-squared test for trend ($p < 0.0001$), and 1B the rate of NDM cases (colonizations or infections) per total surveillance cultures per COVID or Non-COVID units, with the respective total amounts and Fisher's exact test ($p < 0.0001$).

Discussion and conclusion

The COVID-19 pandemic posed a significant threat, not yet overcome, on health systems worldwide, and despite relevant public responses, public hospitals have faced important secondary impacts on their structure, particularly on increased density of inpatients, extenuated health care workers, and increased antimicrobial consumption.^{10,17,18} In the pandemic scenario

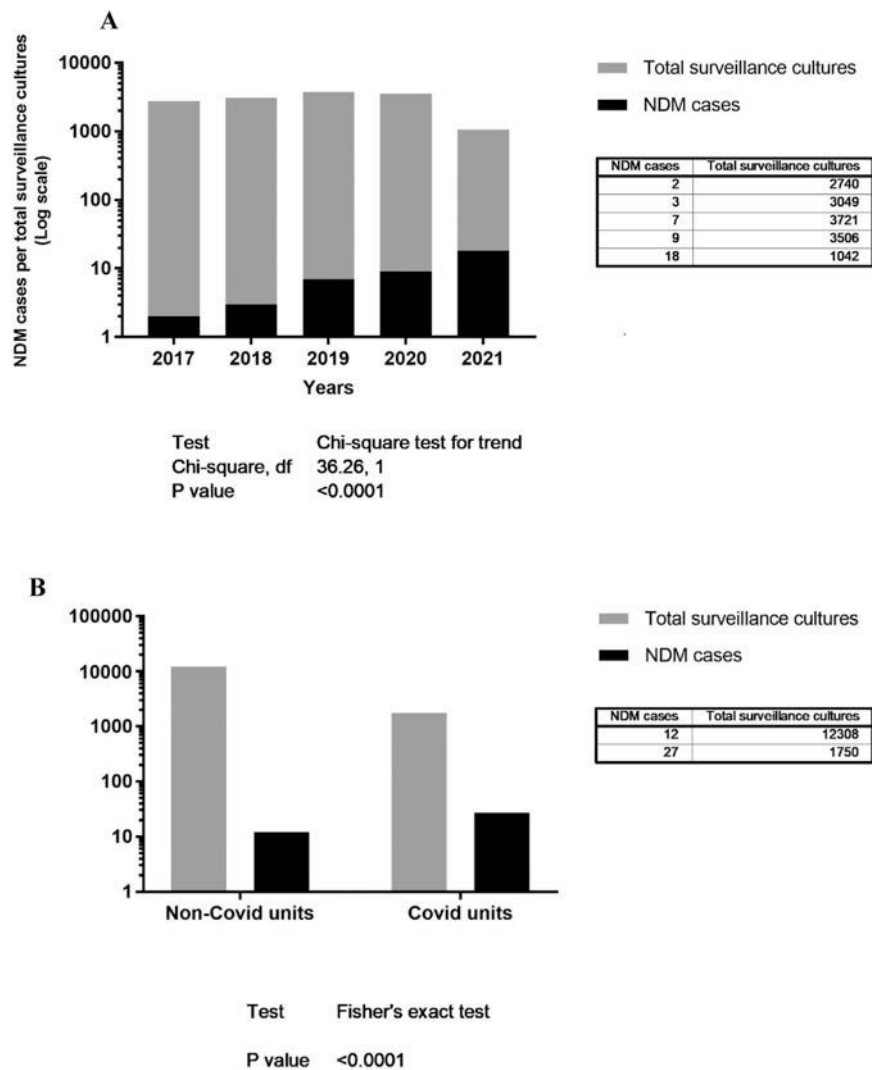


Fig. 1 – Ratio of NDM cases (colonizations or infections) per total surveillance cultures (in Log scale). (A) Per year, from 2017 to 2021, with total amounts and the chi2 test for trend. (B) Per unit, COVID-19 or non-COVID-19, with total amounts and the Fisher exact test.

(March 2020 to December 2021), to enable hospitalization of COVID-19 cases, the IDPC reduced the number of elective cardiovascular surgeries, setting up 30 ICU beds and 40 designated beds for COVID-19 patients. There was a 20% reduction in hospitalizations compared to previous years. Elective surgeries were suspended during part of this period, hence leading to a 30% reduction in the number of procedures. However, even with the reduction of hospitalizations, there was still an increase in the consumption of antimicrobials, according to hospital pharmacy information. In the period of January to December 2020, ceftriaxone consumption increased by 22%, piperacillin-tazobactam by 14%, and azithromycin by 70%. In a cohort of 52 critically ill patients with SARS CoV-2 pneumonia in hospital care in Wuhan city, the rate of broad-spectrum antibiotics use was 94%, although the reported incidence of secondary infections was lower than 15%.¹⁹

In the present study, we report five years of surveillance to multidrug resistant GNB in a complex hospital, with a significant increase in NDM-producing bacteria over time. There was a significant increase detected by chi-squared test for

trend on NDM-producing GNB ($p < 0.0001$) between 2017 and April 2021, specially in COVID-19 units ($p < 0.0001$) (Fig. 1). All isolates were highly resistant to the antimicrobial agents tested, and unfortunately, polymyxin B was considered the last resort to treat these patients. The emergence of such antimicrobial resistance pattern (including readily transmissible mobile genetic elements) in most of the cases is caused by suboptimal and/or excessive use of antimicrobials inside and outside hospital settings. Suboptimal antimicrobial usage often stems from the absence of or inappropriate interpretation of microbiological diagnosis.²⁰

The PFGE analysis showed the emergence of different clusters after March 2020, which coincides with the beginning of COVID-19 pandemic. Different clusters were identified during these periods, one of them (Cluster H) exclusively in COVID-19 units and another (Cluster L) exclusively in non-COVID-19 units (Table 1B). Among 17 patients admitted in COVID-19 units, nine were colonized by the same NDM-producing *K. pneumoniae* cluster “N” and three were colonized by cluster H. These findings are reasonable evidence of horizontal NDM-

producing *K. pneumoniae* transfer occurring between patients admitted in COVID-19 units, which may be attributed to the pandemic pressures of higher patient density, new and young healthcare employees at COVID-19 units, work overload even though the infection control team have performed several trainings to all professionals, including HAI infection prevention and isolation precautions.

The diversity of NDM-producing *Enterobacterales* species and *K. pneumoniae* clones observed in the present study brings up an interesting question. As previously shown, NDM-producing isolates are commonly described in multiple GNB⁸ with apparently no sustained spread of specific high-risk clades.¹ In addition, *bla*_{NDM} genes have also been detected in hospital sewage of several countries, which may reflect intestinal carriage of NDM-producing strains among the population of health care settings.¹ Thus, it seems reasonable to hypothesize that the cluster diversity and the major differences in clones detected pre- and post-March 2020 may have been associated to other factors, not only related to patient density and horizontal transmission. In this scenario, antimicrobial consumption might have provided the necessary selective pressure for the emergence of colonization by multi-drug-resistant pathogens carrying extended-spectrum beta-lactamase genes, including carbapenemase genes. Further studies should be carried out to clarify these findings.

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Ethical approval

The study was approved by the Ethical Committee of Instituto Dante Pazzanese de Cardiologia (São Paulo, Brazil) [n° 5162].

Conflicts of interest

The authors declare no conflicts of interest.

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REFERENCES

1. Wu W, Feng Y, Tang G, Qiao F, McNally A, Zong Z. NDM metallo- β -lactamases and their bacterial producers in health care settings. *Clin Microbiol Rev.* 2019;32:e00115–8.
2. Safavi M, Bostanshirin N, Hajikhani B, Yaslianifard S, van Belkum A, Goudarzi M, et al. Global genotype distribution of human clinical isolates of New Delhi metallo- β -lactamase-producing *Klebsiella pneumoniae*; a systematic review. *J Glob Antimicrob Resist.* 2020;23:420–9.
3. Carvalho-Assef AP, Pereira PS, Albano RM, Berião GC, Chagas TP, Timm LN, et al. Isolation of NDM-producing *Providencia rettgeri* in Brazil. *J Antimicrob Chemother.* 2013;68:2956–7.
4. Campana EH, Montezzi LF, Paschoal RP, Picão RC. NDM-producing *Klebsiella pneumoniae* ST11 goes to the beach. *Int J Antimicrob Agents.* 2017;49:119–21.
5. Monteiro J, Inoue FM, Lobo APT, Ibanes AS, Tufik S, Kiffer CRV. A major monoclonal hospital outbreak of NDM-1-producing *Klebsiella pneumoniae* ST340 and the first report of ST2570 in Brazil. *Infect Control Hosp Epidemiol.* 2019;40:492–4.
6. World Health Organization. Guidelines for the prevention and control of carbapenem-resistant *Enterobacteriaceae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* in health care facilities. Geneva, Switzerland: World Health Organization; 2017.
7. Lee CR, Lee JH, Park KS, Kim YB, Jeong BC, Lee SH. Global dissemination of carbapenemase-producing *Klebsiella pneumoniae*: epidemiology, genetic context, treatment options, and detection methods. *Front Microbiol.* 2016;7:895.
8. Hammoudi Halat D, Ayoub Moubareck C. The current burden of carbapenemases: review of significant properties and dissemination among gram-negative bacteria. *Antibiotics (Basel).* 2020;9:186.
9. Lai CC, Chen SY, Ko WC, Hsueh PR. Increased antimicrobial resistance during the COVID-19 pandemic. *Int J Antimicrob Agents.* 2021;57:106324.
10. Rossato L, Negrão FJ, Simionatto S. Could the COVID-19 pandemic aggravate antimicrobial resistance? *Am J Infect Control.* 2020;48:1129–30.
11. Abboud CS, Bergamasco MD, Doi AM, et al. First report of investigation into an outbreak due to carbapenemase-producing *Klebsiella pneumoniae* in a tertiary Brazilian hospital, with extension to a patient in the community. *J Infection Prevention.* 2011;12:150–3.
12. Abboud CS, Monteiro J, França JJ, Pignatari AC, De Souza EE, Camargo EC, et al. A space-time model for carbapenemase-producing *Klebsiella pneumoniae* (KPC) cluster quantification in a high-complexity hospital. *Epidemiol Infect.* 2015;143:2648–52.
13. Monteiro J, Widen RH, Pignatari AC, Kubasek C, Silbert S. Rapid detection of carbapenemase genes by multiplex real-time PCR. *J Antimicrob Chemother.* 2012;67:906–9.
14. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. 30st ed. USA: CLSI supplement M100. Clinical and Laboratory Standards Institute; 2020.
15. Brazilian Committee on Antimicrobial Susceptibility Testing – BrCAST. <http://brcast.org.br/documentos/>.
16. EUCAST. Breakpoint tables for interpretation of MICs and zone diameters. Version 10.0. 2020. <http://EUCAST.org>
17. Rawson TM, Moore LSP, Castro-Sanchez E, Charani E, Davies F, Satta G, Ellington MJ, Holmes AH. COVID-19 and the potential long-term impact on antimicrobial resistance. *J Antimicrob Chemother.* 2020;75:1681–4.
18. Gomez-Simmonds A, Annavajhala MK, McConville TH, Dietz DE, Shoucri SM, Laracy JC, et al. Carbapenemase-producing *Enterobacterales* causing secondary infections during the COVID-19 crisis at a New York City hospital. *J Antimicrob Chemother.* 2021;76:380–4.
19. Yang X, Yu Y, Xu J, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir Med.* 2020;8:475–81.
20. Morency-Potvin P, Schwartz DN, Weinstein RA. Antimicrobial stewardship: how the microbiology laboratory can right the ship. *Clin Microbiol Rev.* 2016;30:381–407.