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SUPPLEMENT ARTICLE

A 7-Year Brazilian National Perspective on Plasmid-Mediated Carbapenem Resistance in Enterobacterales, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* Complex and the Impact of the Coronavirus Disease 2019 Pandemic on Their Occurrence

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Background. Carbapenemase production is a global public health threat. Antimicrobial resistance (AMR) data analysis is critical to public health policy. Here we analyzed carbapenemase detection trends using the AMR Brazilian Surveillance Network.

Methods. Carbapenemase detection data from Brazilian hospitals included in the public laboratory information system dataset were evaluated. The detection rate (DR) was defined as carbapenemase detected by gene tested per isolate per year. The temporal trends were estimated using the Prais–Winsten regression model. The impact of COVID-19 on carbapenemase genes in Brazil was determined for the period 2015–2022. Detection pre- (October 2017 to March 2020) and post-pandemic onset (April 2020 to September 2022) was compared using the χ^2 test. Analyses were performed with Stata 17.0 (StataCorp, College Station, TX).

Results. 83 282 bla_{KPC} and 86 038 bla_{NDM} were tested for all microorganisms. Enterobacterales DR for bla_{KPC} and bla_{NDM} was 68.6% (41 301/60 205) and 14.4% (8377/58 172), respectively. *P. aeruginosa* DR for bla_{NDM} was 2.5% (313/12 528). An annual percent increase for bla_{NDM} of 41.1% was observed, and a decrease for bla_{KPC} of −4.0% in Enterobacterales, and an annual increase for bla_{NDM} of 71.6% and for bla_{KPC} of 22.2% in *P. aeruginosa*. From 2020 to 2022, overall increases of 65.2% for Enterobacterales, 77.7% for ABC, and 61.3% for *P. aeruginosa* were observed in the total isolates.

Conclusions. This study shows the strengths of the AMR Brazilian Surveillance Network with robust data related to carbapenemases in Brazil and the impact of COVID-19 with a change in carbapenemase profiles with bla_{NDM} rising over the years.

Keywords. carbapenemases; COVID-19; bla_{NDM}; bla_{KPC}; bla_{OXA-23}.

Antimicrobial resistance (AMR) has become a threat to public health due to the growing increase in multidrug-resistant microorganisms (MDROs) on a global scale. Carbapenems are among the last antimicrobials used for treating infections caused by Enterobacterales, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* complex (ABC). The broad spectrum of β-lactam hydrolysis by carbapenemases poses a significant threat to therapeutic options, including carbapenems [1]. Latin American countries reported a sustained increase in resistance in gram-negative bacteria from 2010 to 2019. Also, the Latin American Network for Antimicrobial Resistance Surveillance recently issued warnings about the emergence of previously uncommon carbapenemase-producing Enterobacterales in Latin America and the increase in the number of isolates expressing ≥1 carbapenemase [2, 3].

The coronavirus disease 2019 (COVID-19) pandemic posed an additional threat and placed pressure on the increase in AMR worldwide. The rapid increase in the number of COVID-19 cases overwhelmed health systems, creating a multifactorial problem. Hospitalization, especially in intensive care

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units (ICUs), increases the chances of healthcare-associated infections (HAIs). A significant increase in HAIs from 2019 to 2020 was reported, mainly associated with the rise in the use of invasive devices, such as mechanical ventilation and vascular catheters. Increased length of stay, human resource challenges, and other operational changes that limited the implementation and effectiveness of standard infection prevention practices also contributed to the increase in HAIs [2–4].

The use of antimicrobials for COVID-19 patients to treat potential bacterial pathogens has become a widely implemented empirical practice [5, 6]. In one study, antimicrobial prescribing was reported in 72% of patients admitted to hospitals and 94% of COVID-19 patients admitted to ICUs, despite the low incidence of superinfections (8% according to the Infectious Disease Society of America [7]) and secondary bacterial infections (10%–15%) [6–8]. In this context, improved AMR monitoring and data analyses are critical to evidence regional differences to allow public health policies and more efficient epidemiological measures against resistance and therapeutic planning [9]. In this study, our aim was to evaluate detection rates (DRs), temporal trends, and COVID-19 impact on the most common carbapenemase resistance genes in Enterobacterales, *P. aeruginosa*, and ABC recorded in public health databases in Brazil from 2015 to 2022.

METHODS

Bacterial Isolates

The State Public Health Laboratories Network–LACEN (SISLAB) receives clinical samples related to patient care, mainly carbapenem-resistant organisms (CROs), from state hospitals. The hospital size varies from small to quaternary. Surveillance isolates (eg, rectal swabs) were excluded from this database, and only 1 isolate per patient per year was included in the study. A CRO isolate is confirmed as a carbapenemase producer using molecular methods established at each state laboratory. If polymerase chain reaction (PCR) testing is not implemented locally, isolates are sent to a regional reference laboratory (RRL) or a national reference laboratory (NRL). The state public health laboratory in Paraná, Brazil (LACEN-PR), is an RRL and receives CRO isolates from hospitals in Paraná and 4 other states. LAPIH, located in Rio de Janeiro, Brazil, is an NRL that receives samples from state health laboratories from 18 additional states. All results are sent to the public laboratory information system for inclusion in the datasets.

Database

We used a unified database composed of 3 public laboratory information system datasets (see Supplementary Material). The 3 datasets were merged into the final database after an anonymization procedure with unique numeric identifications. The database was verified for duplicate samples. The main variables included in the database were date/year, microorganism (genus/species), resistance genes tested, and results. The database was composed of isolates received from January 2015 to September 2022.

Carbapenemase Molecular Detection

All CROs received by RRLs and NRLs were submitted for molecular analysis using conventional or quantitative PCR testing to detect multiple carbapenemases for the following genes: Enterobacterales: bla_{KPC}, bla_{NDM}, bla_{OXA-48}, bla_{IMP}, bla_{VIM}; *P. aeruginosa*: bla_{SPM}, bla_{KPC}, bla_{NDM}, bla_{IMP}, and bla_{VIM}; and ABC: bla_{KPC}, bla_{NDM}, bla_{OXA-23}, bla_{IMP}, bla_{VIM}, bla_{OXA-24}, bla_{OXA-58}, and $bla_{OXA-143}$.

Different PCR protocols are used at each state's public health laboratory, RRL, and NRL and have been validated independently.

Statistical Analyses

The DR was obtained by dividing the number of genes detected by gene tested per microorganism and per year. The DR time trend was analyzed using the Prais–Winsten model [10], which creates a line of best fit between the time series points by linear regression and establishes the quantitative trend of a rate. The trend considers the serial correlation of the model errors using the logarithm of the observed rate values to reduce the heterogeneity of the variance of the regression analysis residuals. The time trend of the rate is presented as an annual percentage change (APC) with 95% confidence intervals (95% CIs). The APC is classified as stationary, increasing, or decreasing. The impact of the COVID-19 pandemic was evaluated by comparing the frequency of each antimicrobial resistance gene (ARG) detection pre- and post-pandemic onset, which was defined as 1 October 2017 to 31 March 2020 and 1 April 2020 to 22 September 2022, respectively. For the latter analysis, only LACEN-PR and NRL databases contained within this timeframe were used (see Supplementary Material). Frequency comparison was performed using the χ^2 test, and $P < .05$ was considered significant. All analyses were performed with Stata 17.0 (StataCorp, College Station, TX).

RESULTS

During the study period, 83 282 bla_{KPC} were tested in all 3 gram-negative groups. The Enterobacterales DR was 68.6% (41 282 of 60 205; Table 1). Considering all groups, 86 038 bla_{NDM} were tested, with an Enterobacterales DR of 14.4% (8391 of 58 172), 0.4% (63 of 15 338) for ABC, and 2.5% (309 of 12 528) for *P. aeruginosa*. The highest DR for ARGs was bla_{OXA-23} (92.2%, 15 218 of 16 505) in ABC, and the lowest DR was bla_{VIM} in ABC at 0.07% (2 of 2841).

Table 1. Distribution of Resistance Genes Detected/Tested Over the Study Period

Microorganism/ microorganism group	Carbaopenemase gene detected	Study Year								
		2015	2016	2017	2018	2019	2020	2021	2022	Total
Enterobacterales	bla_{KPC}	74.5% 6042/ 8116	76.3% 5278/ 6916	72.3% 4978/ 6882	71.0% 7068/ 9948	65.6% 6754/ 10,290	61.6% 7128/ 11 574	64.6% 3148/ 4871	55.1% 886/ 1608	68.6% 41 282/ 60 205
	bla_{NDM}	4.1% 312/7700	4.7% 322/6816	8.2% 542/6596	8.7% 830/9500	15.7% 1532/9750	25.9% 2930/ 11 330	26.5% 1289/ 4872	39.4% 634/ 1608	14.4% 8391/58 172
	bla_{IMP}	0% 0/0	40.0% 4/10	25.0% 6/24	0% 0/60	1.4% 4/288	1.4% 18/1272	5.7% 2/35	0% 0/16	2.0% 34/1705
	bla_{VIM}	0% 0/0	0% 0/2	25% 4/16	12.5% 6/48	0.7% 2/278	0.2% 2/1280	8.5% 4/47	11.1% 2/18	1.2% 20/1689
	bla_{OXA-48}	3.1% 124/3986	0.7% 16/2210	0.5% 8/1606	0.7% 32/4346	0.1% 4/4204	0.4% 18/5036	0.7% 8/1121	2.8% 4/141	0.9% 214/22 650
Pseudomonas aeruginosa	bla_{KPC}	2.5% 40/1576	5.0% 50/1008	9.6% 102/1064	7.1% 98/1386	6.7% 142/2136	10.0% 320/3188	14.1% 219/1554	13.2% 94/713	8.4% 1065/12 625
	bla_{NDM}	0.3% 4/1570	0% 0/1010	0% 0/1062	0.4% 6/1370	1.2% 24/2078	2.8% 90/3182	8.8% 136/1546	6.9% 49/710	2.5% 309/12 528
	bla_{SPM}	22.5% 402/1786	11.8% 146/1232	9.0% 122/1350	11.2% 164/1460	9.3% 194/2074	4.1% 126/3084	4.5% 70/1546	4.0% 28/708	9.5% 1252/13 240
	bla_{IMP}	0% 0/0	7.3% 16/218	0% 0/106	6.0% 14/234	12.0% 140/1212	11.0% 160/1450	4.5% 44/971	5.1% 33/648	8.4% 407/4839
	bla_{VIM}	0% 0/0	0% 0/74	3.8% 22/582	7.8% 68/870	6.3% 130/2052	11.9% 370/3098	8.7% 133/1523	13.4% 95/708	9.2% 818/8907
Acinetobacter baumannii	bla_{KPC}	0.7% 14/2082	0.5% 8/1572	0.2% 2/930	0.5% 6/1312	0.5% 8/1554	0.4% 8/2106	0.5% 4/831	3.1% 2/65	0.5% 52/10,452
	bla_{NDM}	0.8% 16/2078	0.4% 6/1570	0.2% 2/1232	0% 0/1804	0% 0/2304	0.3% 8/2814	1.0% 28/2804	0.4% 3/732	0.4% 63/15 338
	bla_{IMP}	0% 0/0	0% 0/0	0% 0/2	0% 0/10	33.3% 2/6	3.3% 2/60	0% 0/5	0% 0/4	4.6% 4/87
	bla_{VIM}	0% 0/0	0% 0/0	0% 0/0	0% 0/2	0% 0/2	0.3% 2/622	0% 0/1948	0% 0/267	0.1% 2/2841
	bla_{OXA-23}	96.5% 1950/ 2020	91.9% 1620/ 1762	86.5% 1254/ 1450	89.5% 1790/ 2000	89.5% 2302/2572	91.6% 2916/3182	97.1% 2710/ 2790	92.7% 676/ 729	92.2% 15 218/16 505
	bla_{OXA-24}	0% 0/0	100% 2/2	8.9% 36/406	6.4% 50/784	4.5% 32/706	6.8% 30/440	47.4% 9/19	33.3% 1/3	6.8% 160/2360
	bla_{OXA-58}	0% 0/0	0% 0/0	0% 0/406	0.5% 4/798	5.4% 40/742	16.4% 104/636	38.9% 7/18	0% 0/3	6.0% 155/2603
	$bla_{OXA-143}$	30% 24/80	5.1% 28/548	13.1% 70/536	10.7% 94/ 878	5.5% 82/1486	6.9% 154/2246	2.7% 22/820	8.6% 5/58	7.2% 479/6652

Detection Rate Temporal Trend

During the period considered for the temporal trend, the Enterobacterales DR of bla_{KPC} decreased from 74.5% in 2015 to 55.1% in 2022 (4.0% APC decline; 95% CI, −4.8% to −3.3%). Furthermore, the Enterobacterales DR of bla_{NDM} increased from 4.1% in 2015 to 39.4% in 2022 (41.1% APC increase; 95% CI, 35.8% to 46.6%), all driven by the Enterobacterales species tested. These increases became more evident starting in 2017 (Tables 1 and 2, Figure 1*B*), with a peak in 2022 for *Escherichia coli*, *Enterobacter* spp., and *Klebsiella pneumoniae* with annual increases of 75.7%, 47.1%, and 39.5%, respectively (Table 2, Figure 1*B*). The Enterobacterales DRs for bla_{VIM}, bla_{IMP}, and bla_{OXA-48} were stationary. However, specific Enterobacterales resistance trends could not be tested for all ARGs. In summary, the most relevant findings relate to the decreasing trend in the DR of bla_{KPC} for Enterobacterales and the increasing trend in the DR for bla_{NDM} for Enterobacterales over time.

ABC resistance remained stationary over time for most genes. The ABC DR temporal trend for bla_{IMP} and bla_{VIM} could not be tested. For *P. aeruginosa*, bla_{SPM} decreased from 22.5% in 2015 to 3.9% in 2022 (20.6% APC decline; 95% CI, −26.8% to −13.9%) and was the only gene with resistance reduction over time (Table 2). The *P. aeruginosa* DR temporal trend for bla_{NDM} had the highest APC observed in this species (71.6% increase; 95% CI, 29.8% to 126.8%). Figure 1 shows the most representative ARGs for the temporal trend of microorganisms. For *P. aeruginosa*, it is relevant to note the rise in bla_{NDM} and the decrease in bla_{SPM} over time.

Impact of the COVID-19 Pandemic on Bacterial Resistance Genes

Compared with pre-pandemic onset, the Enterobacterales DR of bla_{KPC} increased from 57.1% to 61.8% in the post-onset

Table 2. Number of Genes Tested, Detection Rate, and Annual Percent Change From 2015 to 2022 for Each Gene Detection Rate Among the Main Enterobacterales, *Acinetobacter baumannii* Complex, and *Pseudomonas aeruginosa*

Microorganism	Gene	Tested (n)	Detected (%)	Annual Percent Change	95% Confidence Interval	Pattern
Enterobacterales	bla_{KPC}	60 205	68.6	−4.0	**−4.8 to −3.3**	Decreasing
Klebsiella pneumoniae		41 224	74.7	−3.1	**−4.1 to −2.1**	Decreasing
Escherichia coli		2466	42.2	−13.9	**−19.7 to −7.8**	Decreasing
Citrobacter spp.		647	49.0	−11.1	**−16.3 to −5.5**	Decreasing
Enterobacter spp.		4573	49.2	−14.6	−27.5 to .6	Stationary
Serratia spp.		3240	64.4	−3.3	−9.9 to 3.7	Stationary
Enterobacterales	bla_{NDM}	58 172	14.4	41.1	**35.8 to 46.6**	Increasing
K. pneumoniae		40 407	13.1	39.5	**30.6 to 49**	Increasing
E. coli		2390	18.4	75.7	**48.2 to 108.2**	Increasing
Citrobacter spp.		635	43.1	27.7	**22.2 to 33.4**	Increasing
Enterobacter spp.		4479	15.1	47.1	**25.8 to 71.9**	Increasing
Serratia spp.		3203	5.9	75.1	**28.4 to 138.7**	Increasing
Enterobacterales	bla_{IMP}	1705	2.0	−42.4	−77.6 to 47.8	Stationary
Enterobacterales	bla_{VIM}	1689	1.2	−16.9	−83.2 to 310.3	Stationary
Enterobacterales	bla_{OXA-48}	22 650	0.9	−3.8	−42.7 to 61.6	Stationary
K. pneumoniae		14 536	1.3	−9.6	−42.5 to 42.2	Stationary
Acinetobacter baumannii	bla_{KPC}	10 452	0.5	15.1	−11.3 to 49.4	Stationary
	bla_{NDM}	15 338	0.4	5.3	−16.8 to 33.3	Stationary
	bla_{OXA-23}	16 505	92.2	0.0	−1.8 to 1.9	Stationary
	bla_{OXA-24}	2360	6.8	−4.3	−100 to 97.5	Stationary
	bla_{OXA-58}	2603	5.9	302.9	**71 to 849.5**	Increasing
	$bla_{OXA-143}$	6652	7.2	−15.7	**−23.8 to −6.8**	Decreasing
Pseudomonas aeruginosa	bla_{KPC}	12 625	8.4	22.2	**9.1 to 37**	Increasing
	bla_{NDM}	12 528	2.5	71.6	**29.8 to 126.8**	Increasing
	bla_{IMP}	4839	8.4	−5.8	−25.7 to 19.7	Stationary
	bla_{VIM}	8907	9.2	20.0	**11.8 to 28.7**	Increasing
	bla_{SPM}	1 324	9.5	−20.6	**−26.8 to −13.9**	Decreasing

Bold values are for increasing or decreasing numbers.

period. Within the same time frame, bla_{NDM} in Enterobacterales increased from 18.7% to 28.0% ($P < .001$) and increased in all main species studied, *E. coli*, *Enterobacter* spp., and *K. pneumoniae*, with the DR reaching 51.3%, 43.0%, and 21.9% ($P < .001$). The ABC showed an increase from 0.4% to 0.7% in bla_{NDM} and from 91.9% to 95.8% in bla_{OXA-23}. The *P. aeruginosa* DR increased from 8.8% to 11.8% for bla_{KPC} and from 1.1% to 6.8% for bla_{NDM} during the post-pandemic onset period. bla_{SPM} and bla_{IMP} for *P. aeruginosa* were the only genes with a reduced DR (Table 3). It is important to note that during the post-pandemic onset period, there was an increase in the detection of bla_{KPC} and bla_{NDM} in Enterobacterales, although the temporal trend of bla_{KPC} decreased over the 7-year period.

DISCUSSION

Since the start of the 21st century, gram-negative bacteria have become an increasing problem as they relate to MDRO in Brazil, especially carbapenemase-producing organisms [11]. This situation became critical during the COVID-19 pandemic [2, 3], which has been the most severe pandemic of this century, causing 6 588 769 deaths worldwide; of those, 687 962 (10.4%) were in Brazil [12]. The death rate in Brazil was 4 times higher than the global median (319.5 × 82.5 deaths/100 k habitants globally) [13, 14].

In cooperation with the National Health Surveillance Agency (ANVISA—Portuguese acronym) and the Pan America Health Organization, the Brazilian Ministry of Health has made efforts to detect and control AMR since 2005 by establishing the AMR Network [15]. In 2015 for AMR Net, ANVISA chose 4 RRLs in 4 Brazilian states (Paraná, São Paulo, Brasília, Piauí) and 1 NRL (LAPIH- Fundação Oswaldo Cruz Rio de Janeiro) to provide AMR referral testing in healthcare services [16]. In 2018, Brazil started participating in the World Health Organization–Global Antimicrobial Resistance Surveillance System (GLASS) and published its National Acting Plan on AMR, which established the need to create a national surveillance program on AMR (BR-GLASS) [15, 17–19]. By 2020, the BR-GLASS database contained more than 30 000 isolates. After an international call from the US Centers for Disease Control and Prevention as part of the Global Antimicrobial Resistance Laboratory and Response Network, the General Coordination of Public Health Laboratories (Minister of Health (MoH)) proposed a new

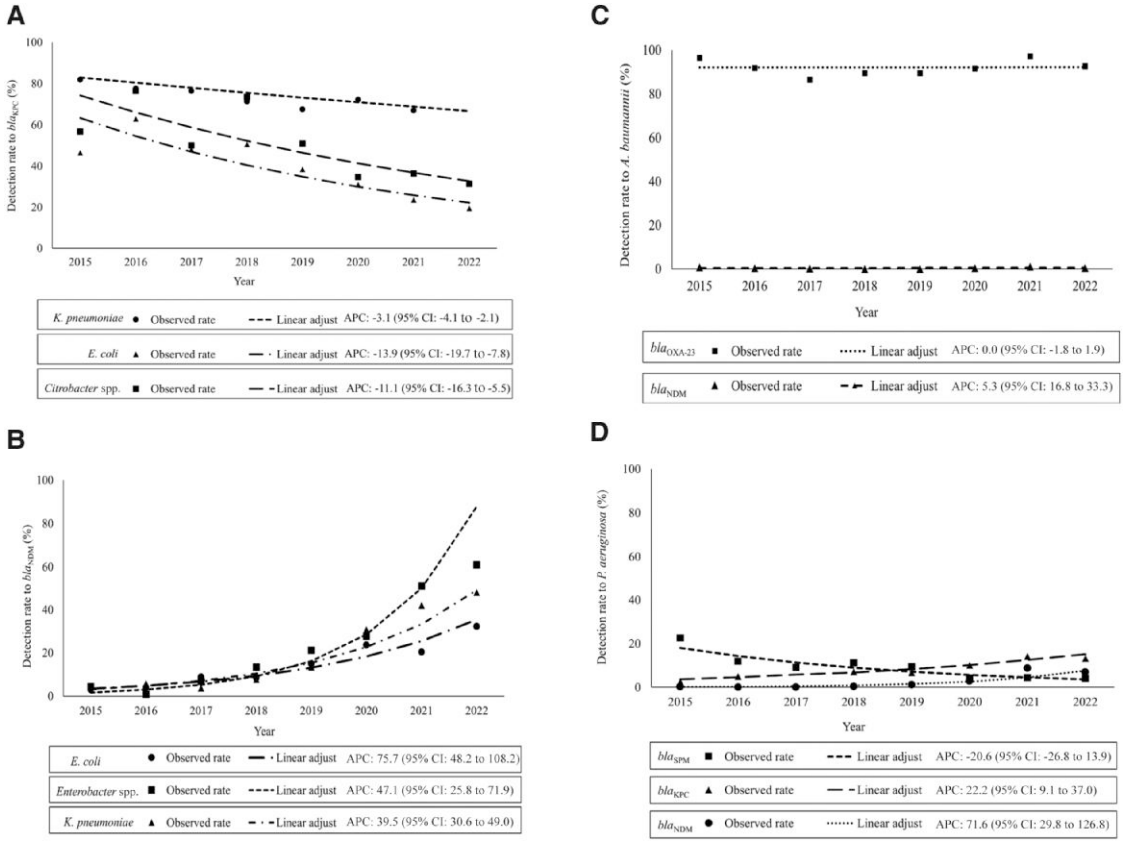

Figure 1. Temporal trend pattern for the detection rate of bla_{NDM} and bla_{KPC} in Enterobacterales, *Acinetobacter baumannii* complex, and *Pseudomonas aeruginosa*. Temporal trend pattern (APC) for *Klebsiella pneumoniae, Escherichia coli,* and *Citrobacter* spp. resistance to gene bla_{KPC} *(A), E. coli, Enterobacter* spp., and *K. pneumoniae* resistance to gene bla_{NDM} *(B); A. baumannii* resistance to bla_{OXA-23} and bla_{NDM} *(C);* and *P. aeruginosa* resistance to bla_{SPM}, bla_{KPC}, and bla_{NDM} *(D).* Abbreviations: APC, annual percentage change; CI, confidence interval.

program, the Strengthening of the Brazilian Surveillance System on AMR, supported by multiple national and state partners.

Because of these initiatives and better structuring of the Brazilian AMR Net, the analysis included here represents results from the molecular detection of carbapenemases at SISLAB from 2015 to 2022 in Brazil. Here, we demonstrate the substantial increase in the prevalence of carbapenemase genes during the post-pandemic onset period (2020–22) compared with the pre-onset period (2017–2020).

Although we observed an increase in carbapenemase production, the resistance rate of carbapenem from primary bloodstream infections that were reported to ANVISA through the national program for the prevention and control of HAIs was very similar when we compared pre- and post-pandemic onset periods, even though this report analyzed data up to 2021 [20].

Since it was first described in 2006 [21], bla_{KPC} has become one of the most worrisome resistance genes among Enterobacterales in Brazil. Many outbreaks have been described in Brazil. Since 2015, it has reached an endemic state, as shown in our study, as well as in many countries of Latin America, as described by PAHO at the CARBA-LA Project (Pillonetto et al, 2023, manuscript in preparation). Since 2015, our data have shown that the bla_{KPC} DR seems to be decreasing for Enterobacterales; it has also decreased for *K. pneumoniae*, *Citrobacter* spp., and *E. coli*. The same decrease in bla_{KPC} was found in a Brazilian study published by Wink et al [22]. Several factors may explain the declines observed in bla_{KPC}. First, some Brazilian hospitals are using methods for the detection of bla_{KPC} (phenotypically or genotypically) and no longer refer bla_{KPC}-positive isolates to the reference laboratories for confirmation because they consider bla_{KPC} to be endemic. Second, a larger number of isolates were tested for bla_{KPC}, including strains that were polymyxin-resistant but not necessarily carbapenem-resistant. Finally, the increase in bla_{NDM} detection, as demonstrated in the present analysis, could mean a possible replacement of carbapenemases in Brazil. This situation was also noted by Arend et al who showed that the increase in NDM-producing bacteria in southern Brazil was probably due to the presence of this gene in different plasmids [23]. Comparing the pre- and post-pandemic onset

Table 3. Detection Rate for Resistance Genes During the Pre-Onset and Post-Onset Periods of the Coronavirus Disease 2019 Pandemic

Microorganism/Gene	October 2017 to March 2020 % (n Tested / n Detected)	April 2020 to September 2022 % (n Tested / n Detected)	*P* Value
Increased detection on rate			
Enterobacterales *bla*$_{KPC}$	57.1% (3539/6201)	61.8% (5872/9506)	**<.001**
Klebsiella pneumoniae bla$_{KPC}$	66.7% (3097/4646)	70.9% (5246/7395)	**<.001**
Citrobacter spp. *bla*$_{KPC}$	19.1% (20/105)	33.0% (62/188)	**.011**
Enterobacterales *bla*$_{NDM}$	18.7% (1158/6203)	28.0% (2664/9507)	**<.001**
K. pneumoniae bla$_{NDM}$	14.4% (668/4646)	21.9% (1621/7393)	**<.001**
Escherichia coli bla$_{NDM}$	36.0% (151/420)	51.3% (192/374)	**<.001**
Enterobacter spp. *bla*$_{NDM}$	23.5% (158/672)	43.0% (299/696)	**<.001**
Serratia spp. *bla*$_{NDM}$	8.4% (13/155)	29.7% (89/300)	**<.001**
Acinetobacter baumannii bla$_{OXA-23}$	91.9% (2726/2965)	95.8% (4770/4979)	**<.001**
Pseudomonas aeruginosa bla$_{KPC}$	8.8% (176/2002)	11.8% (386/3265)	**<.001**
P. aeruginosa bla$_{VIM}$	7.8% (121/1546)	10.2% (331/3228)	**.007**
P. aeruginosa bla$_{NDM}$	1.1% (22/1997)	6.8% (222/3255)	**<.001**
Decreased detection on rate			
P. aeruginosa bla$_{SPM}$	7.5% (150/1998)	4.1% (134/3251)	**<.001**
P. aeruginosa bla$_{IMP}$	9.0% (83/926)	6.0% (116/2077)	**.001**
Did not change			
E. coli bla$_{KPC}$	21.0% (88/420)	19.5% (73/374)	.616
Enterobacter spp. *bla*$_{KPC}$	28.0% (188/671)	24.9% (173/694)	.196
Serratia spp. *bla*$_{KPC}$	64.9% (100/154)	68.0% (204/300)	.511
Citrobacter spp. *bla*$_{NDM}$	65.7% (69/105)	69.2% (130/188)	.546
A. baumannii bla$_{KPC}$	0.1% (1/862)	0.5% (7/1456)	.148
A. baumannii bla$_{NDM}$	0.4% (11/2896)	0.7% (35/4999)	.072
A. baumannii bla$_{OXA-24}$	58.3% (14/24)	48.5% (16/33)	.062
A. baumannii bla$_{OXA-58}$	0% (0/14)	22.6% (7/31)	.053

Bold values are for increasing or decreasing numbers.

periods and considering the database for LACEN-PR and NRL only, an increase in the KPC DR of 5% was observed within the Enterobacterales order. The same was observed for the main species that produce *bla*$_{KPC}$, *K. pneumoniae*. However, our analysis shows that not only was the *bla*$_{KPC}$ DR rising during the post-pandemic onset period but also the total amount of strains sent to the reference laboratories for Enterobacterales and for *K. pneumoniae*, where the total isolates tested increased by more than 3000 and the positive tests surpassed more than 2300 isolates (see Table 3).

In addition to the high endemicity for *bla*$_{KPC}$ in Brazil, *bla*$_{NDM}$ was first detected in Enterobacterales, in 2012 in *Enterobacter hormaechei* and in 2013 in *Providencia rettgeri* [24, 25]. Our study showed that the Enterobacterales DR for *bla*$_{NDM}$ increased consistently from 2015 to 2022 (from 4.2% to 23.8%), becoming more evident starting in 2017, with its peak in the pandemic years (2020–2021, mainly in 2022). Also, the total amount of *bla*$_{NDM}$ detected in Enterobacterales rose in more than 1500 isolates comparing pre- and post- pandemic onset (see Table 3).

Accordingly, da Silva et al [26] reported 81 *bla*$_{NDM}$ cases in 9 states, 4 in 2012–2013, 27 in 2014, and 50 in 2015. Also, Thomas et al [3] observed an increase in *bla*$_{NDM}$ in many Latin American countries, which is supported by the PAHO CARBA-LA project, which includes data from 12 countries from 2015 to 2020 (Pillonetto et al, 2023, personal communication). The rise in *bla*$_{NDM}$ cannot be explained by a selective pressure caused by the use of the newer β-lactam/β-lactamase inhibitor because this class of drugs has a very high cost for low- and middle-income countries such as Brazil and other countries in Latin America. Consequently, its use is very restricted. Also, most of the recently published studies, including ours, show a more evident increase from 2018 on, peaking during the pandemic years (2020–2022) [3, 22]. One hypothesis for the higher increase in the pandemic years compared with the pre-pandemic years is the clonal expansion related to overcrowded hospitals, hiring unprepared health professionals, and the indiscriminate use of antibiotics, as shown in some studies with up to 94% of COVID-19–infected patients receiving antimicrobials, especially broad-spectrum drugs [27].

The detection of the main carbapenemase gene in ABC (*bla*$_{OXA-23}$) started in Brazil during the first outbreak that was reported globally in 1999 [28]. However, a continuous increase over the last 2 decades was seen in Brazil, where the clonality and carbapenem resistance kept spreading [29, 30]. Although we did not observe a significant change in *bla*$_{OXA-23}$ detection over our study period, a 4% increase of the *bla*$_{OXA-23}$ DR was seen during the

post-pandemic onset period, with an additional 2000 bla_{OXA-23} isolates of ABC strains detected at the reference laboratories. The increase in carbapenem-resistant *A. baumannii* (CRAB) that we observed during the post-pandemic onset period has been reported in other studies [27]. At least 2 outbreaks of CRAB were reported in Brazil during the pandemic. Shinohara et al [31] reported 14 isolates in 1 ICU, and Camargo et al [32] found 224 patients colonized or infected by international clone 2, which is relatively uncommon in Brazil. Polly et al [33] showed a significant increase (+108.1%) in incidence density (ID) of MDR infections by CRAB in all hospitals when comparing pre-pandemic and pandemic periods and a 48% increase in ICUs ID for CRAB during the pandemic.

Although much less common than bla_{OXA-23}, the presence of bla_{NDM} in *Acinetobacter* spp. is another interesting finding, with the first Brazilian isolate detected in *Acinetobacter pittii* in September 2012 [34] followed by the detection in 2014 in *A. baumannii* [35], *Acinetobacter bereziniae* [36], and *Acinetobacter nosocomialis* [37].

Pseudomonas aeruginosa's primary mechanisms of resistance to carbapenems are overexpression of the efflux pump and overproduction of AmpC β-lactamase, which is associated with the inactivation of the OprD outer membrane protein. However, the production of carbapenemases has played an increasing role in this species [38]. Although many carbapenemase genes have been described globally in *P. aeruginosa*, the bla_{VIM} and bla_{IMP} genes are the most prevalent [38]. From 2015 to 2020 in 12 Latin American countries, bla_{VIM} and bla_{KPC} were detected at 52.2% and 22.4%, respectively (Pillonetto M et al, 2023, personal communication). Until recently, bla_{SPM} was the most prevalent ARG in Brazilian *P. aeruginosa* isolates. This gene was first isolated in 1997 in São Paulo, Brazil [39], and has since been detected in almost every region of Brazil [40, 41]. The peak of bla_{SPM} detection in Brazil occurred between 2000 and 2012 and was associated with a single clone, ST277. However, in the last 10 years, a decrease in the prevalence of bla_{SPM} and a higher frequency of isolation of other carbapenemases, mainly bla_{KPC}, bla_{NDM}, and bla_{VIM}, have been observed in some hospitals in Brazil [42]. Our present study corroborates these findings (APCs of bla_{SPM}: −20.6, bla_{VIM}: 19.9, bla_{KPC}: 22.2, and bla_{NDM}: 71.6). One possible explanation for this change in the profile of carbapenemase production in *P. aeruginosa* is the presence in Brazil of high-risk multidrug-resistant clones, such as ST233 and ST244, which carry carbapenemases such as bla_{KPC} and bla_{VIM} [43, 44]. An important rise in the total *P. aeruginosa* strains sent to the reference laboratories (from 12 404 to 19 013, 65.3%) and a significant increase in bla_{NDM} during the post-pandemic onset period were observed, with the DR rising from 1% in the pre-onset period to 7% in the post-onset period. Perez et al [45] also found NDM-producing *P. aeruginosa* in 27 of 156 (17.3%) patients during the COVID-19 pandemic.

To our knowledge, this is the first study in Brazil to compile data from 27 state and federal districts. A main strength of our study is the total number of strains studied (more than 80 000) over a long period of time (>7 years), including pre- and post-pandemic onset. No duplicates or surveillance swabs were included. The study did have limitations. There was no unique protocol for all states regarding referral of samples to the state reference laboratories. Also, there is no guarantee that all state reference laboratories used the same PCR protocol for ARG detection and that the protocols for receiving and investigating ARGs did not change during the study period. Some reference laboratories experienced an overload of isolates during the post-pandemic period, and stricter rules had to be implemented to limit the number of samples received. This action could have caused an underestimation of the overall increase in ARGs during the COVID-19 pandemic. Finally, the total number of hospitals that sent isolates to the reference laboratories could not be accessed.

This study shows the strengths of the Brazilian AMR Surveillance Network, with robust data related to carbapenemases in Brazil over time and the impact of COVID-19. The data clearly show an increase in total isolates sent to the reference laboratories, especially from 2020 to 2022. Additionally, we observed a tendency for the modification of carbapenemases profiles, mainly with an important annual rise in bla_{NDM} over the study period. It is unclear if ARGs will continue to increase steadily after the pandemic. However, this could have a direct impact on the use of new carbapenemase inhibitor drugs since they have no effect on metallo- β-lactamase. All of the AMR surveillance data from Brazil will be of great importance in enhancing projects that are already underway to improve the Brazilian health system and its AMR efforts and coordinate new and stronger actions for identifying risk factors for the spread of MDROs with adequate allocation of resources and policies intended to improve the diagnosis, prevention, and treatment in the country.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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