International Journal of Clinical Trials
Pereira HO et al. Int J Clin Trials. 2025 Aug;12(3):181-186
http://www.ijclinicaltrials.com
pISSN 2349-3240 | eISSN 2349-3259

DOI: https://dx.doi.org/10.18203/2349-3259.ijct20252195

Original Research Article

Impact of the COVID-19 pandemic on the distribution and resistance of multidrug-resistant genes in Minas Gerais, Brazil: challenges and implications

Hoberdan Oliveira Pereira[1]*, Marcelo Carneiro[1], Braulio Roberto Goncalves Marinho Couto[2], Dhian Renato Almeida Camargo[3], Carmem Dolores Faria[3], Amanda Aparecida Felizardo[4]

[1]Santa Cruz Hospital and Postgraduate Program in Health Promotion, University of Santa Cruz do Sul, Rio Grande do Sul-Brazil
[2]Biobyte Technology in Epidemiology, Brazil
[3]Ezequiel Dias Foundation, Bacterial and Fungal Diseases Service, Brazil
[4]Institute of Biological Sciences, Department of Morphology, Federal University of Minas Gerais, Brazil

Received: 17 May 2025
Revised: 14 July 2025
Accepted: 15 July 2025

***Correspondence:**
Dr. Hoberdan Oliveira Pereira
E-mail: hoberdanoliveira2013@gmail.com

ABSTRACT

Background: Following the COVID-19 pandemic, a significant rise in multidrug-resistant pathogens such as *Acinetobacter*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Providencia*, *Pseudomonas* and *Serratia spp*. was observed in Minas Gerais, Brazil. Diagnostic disparities and antimicrobial overuse for COVID-19-associated pneumonia likely contributed to resistance gene spread.

Methods: This retrospective, cross-sectional, descriptive study analysed 13,012 Gram-negative bacterial isolates collected from hospitals across Minas Gerais between 2016 and 2022. Samples, including urine, tracheal secretion, blood and surgical wound exudate, were referred to the state public health laboratory (LACEN-MG) based on resistance patterns detected via antibiograms. Genotypic analysis involved PCR screening for resistance genes (*bla*KPC, *bla*OXA, *bla*NDM, *bla*SPM, *bla*VIM, *bla*IMP), categorized by pandemic period (pre-, during and post-COVID-19). The presence of at least one gene indicated multidrug resistance.

Results: Among isolates, 70.8% were fermenting bacilli, mainly *Klebsiella spp*. (73.8%), 29.2% were non-fermenting, mostly *Acinetobacter spp*. (62.1%) and *Pseudomonas spp*. (37.9%). Post-pandemic resistance increased significantly ($p<0.05$). *bla*KPC declined in relative frequency (66.3% pre-pandemic to 44.4% post-pandemic) but absolute numbers rose (n=2,906). *bla*OXA and *bla*NDM increased to 41.1% and 13.5%, respectively, with *bla*NDM increasing over sixfold. Mcr-1 appeared exclusively post-pandemic in four isolates. *bla*SPM, *bla*VIM and *bla*IMP remained rare; *bla*IMP was detected only post-pandemic (0.24%, n=16).

Conclusions: Post-pandemic, resistance gene profiles diversified, with expansion of *bla*OXA, *bla*NDM and emergence of *mcr-1*. Strengthened molecular surveillance, equitable diagnostic access and antimicrobial stewardship are urgently needed in Brazil.

Keywords: Anti-microbial stewardship, *bla*KPC, Infection control, Molecular diagnostics, Multidrug-resistant, Resistance genes, Surveillance

INTRODUCTION

The COVID-19 pandemic has had a devastating impact on healthcare systems worldwide, disrupting even the most robust ones. Minas Gerais, a state in south-eastern Brazil with over 20 million inhabitants, was no exception, as the already fragile system for investigating hospital-acquired resistance genes came to a near standstill in

2020. This phenomenon occurred throughout Brazil, leading to underdiagnosis, along with the interruption of essential elective healthcare services, which contributed to the rise of antimicrobial resistance.[1] Globally, antimicrobial resistance (AMR) was already recognized as a critical health threat prior to the pandemic, associated with approximately 4.95 million deaths in 2019.[2] During the COVID-19 pandemic, several studies indicated that empirical and often inappropriate use of antimicrobials to treat suspected secondary infections accelerated the spread of resistance genes, particularly among Gram-negative bacteria.[3,4] In hospital settings, especially in intensive care units (ICUs), there was a marked increase in infections caused by multidrug resistant organisms (MDROs), a phenomenon attributed to both excessive antimicrobial use and breakdowns in infection prevention measures under the pressures of the pandemic.[5]

The spread of resistance mediated by plasmids, facilitated by environmental contamination and transmission by healthcare workers' hands (cross-contamination), reflects the vulnerability arising from inadequate hygiene and infection control practices.[6] These dynamic underscores the crucial role of robust infection prevention programs and rational antimicrobial use, whose disruption during the pandemic created opportunities for resistance genes to spread more rapidly.[7] Additionally, studies emphasize that low- and middle-income countries like Brazil face added challenges due to centralized laboratory networks and the uneven distribution of diagnostic capabilities, further hindering the timely identification and control of resistant pathogens.[8]

Recent systematic reviews reinforce global concerns regarding the increase in antimicrobial resistance during the COVID-19 pandemic. Langford et al identified a significant rise in resistance among Gram-negative pathogens in patients with COVID-19, especially in hospital settings. Similarly, Sulayyim et al reported high resistance rates of *Acinetobacter baumannii* and *Klebsiella pneumoniae* to various antibiotics, including colistin, during the pandemic period. These findings highlight the urgent need to strengthen surveillance systems and promote rational antimicrobial use.[9,10]

In Minas Gerais, the centralization of biological sample analysis (genotyping) in a single regional public laboratory highlights the concentrated technical capacity and limited infrastructure of advanced laboratories in other microregions. Consequently, the post-pandemic period represents a critical window to assess the long-term impacts of interrupted surveillance systems and the inappropriate use of antimicrobials on the genetic landscape of hospital-acquired bacterial resistance. Therefore, this study aimed to analyze and compare the genotypic profile of hospital bacterial resistance in Minas Gerais, Brazil, across the pre-, during and post-COVID-19 pandemic periods. By examining variations in the detection of resistance genes over time, the study seeks to highlight the potential consequences of disrupted diagnostic services and weakened infection control practices on the spread and evolution of antimicrobial resistance in a middle-income healthcare setting.

METHODS

This is a cross-sectional, retrospective, descriptive study conducted between 2016 and 2022 in the state of Minas Gerais, Brazil. A total of 13,012 Gram-negative bacterial isolates from hospitals were analyzed, extracted from the database of the Central Public Health Laboratory of Minas Gerais (LACEN-MG). The samples, collected from cases of infection and/or colonization, were sent by healthcare institutions mainly hospitals as recommended by Technical Note No. 01/2013 of the National Health Surveillance Agency (ANVISA).[11]

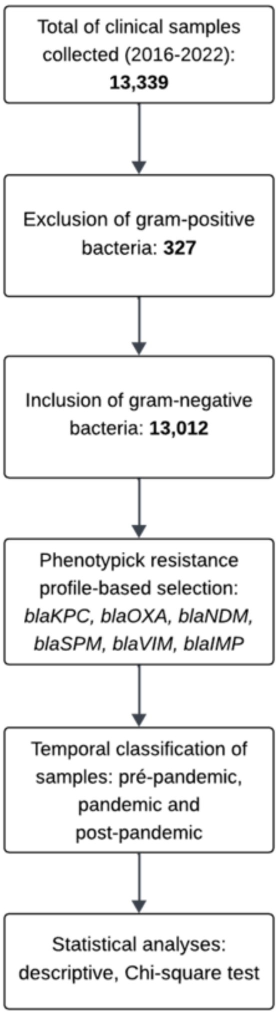

Figure 1: Methodological flowchart of the study on resistance genes in gram-negative bacteria isolated from hospitals in Minas Gerais, Brazil (2016–2022).

The samples were collected from 37 municipalities across all regions of the state, with a focus on the capital, Belo Horizonte, which accounted for 65% of the total biological samples. The materials mainly included urine, tracheal secretion, blood and surgical wound exudate.

The samples were sent to LACEN based on phenotypic resistance profiles identified by the microbiology laboratories of the participating units. All materials that showed resistance in the antibiogram to one or more classes of antimicrobials, such as carbapenems or other critical classes, were analyzed. Genotypic analysis was performed by detecting resistance genes *bla*KPC, *bla*OXA, *bla*NDM, *bla*SPM, *bla*VIM and *bla*IMP using polymerase chain reaction (PCR). The presence of one or more of these genes was considered indicative of multidrug resistance (MDR).

For temporal analysis, the data were categorized into three periods pre-pandemic (until March 2020), pandemic (April 2020 to December 2021) and post-pandemic (January 2022 to December 2023). Descriptive statistics and univariate analyses were applied to assess the distribution of microorganisms and resistance genes. The comparison of resistance rates between periods was conducted using the chi-square test, with a significance level set at $p<0.05$. As an inclusion criterion, all samples of biological materials sent by hospitals with carbapenem-resistant microorganisms were considered, according to Technical Note 01/2013. As an exclusion criterion, gram-positive bacterial isolates (n=327) were excluded from the analysis. This study was approved by the Research Ethics Committee of the Ezequiel Dias Foundation (FUNED), under protocol number CAAE: 65564322.0.0000.9507.

RESULTS

A total of 13,012 samples of gram-negative bacteria of hospital origin were analyzed from 37 municipalities (4.3%), covering all regions of Minas Gerais, located in the south-eastern region of Brazil, with 65% of the samples coming from the capital, Belo Horizonte. The biological urine samples were predominantly observed (37.6%), with Gram-negative fermenting bacilli found in 9,225 (70.8%) samples and *Klebsiella spp*. accounting for 73.8%. The most requested biological samples were tracheal secretions (46%), with non-fermenting bacilli found in 3,801 (29.2%) samples, comprising 62.1% *Acinetobacter spp*. and 37.9% *Pseudomonas spp*.

In the comparison of the pre- and post-pandemic periods, there was an increase in resistance ($p<0.05$) (Table 1). Demonstrates the most prevalent genes during the pre-pandemic, pandemic and post-pandemic periods of COVID-19. Four patients were found to have *mcr1* genes, including two *Klebsiella pneumoniae* (from surgical wound secretion and tracheal aspirate) and two *Escherichia coli* (from peritoneal fluid and surgical wound secretion), all of which were identified during the post-pandemic period.

Table 1: Distribution and univariate analysis of multidrug-resistant (MDR) microorganisms during pre-pandemic, pandemic and post-pandemic periods of COVID-19.

Micro organism	Period	Samples	MRD (n)	MRD risk (%)	P value
Acinetobacter sp.	Pre-pandemic	784	138	18	<0.001
	Covid-19 pandemic (Brazil)	231	4	2	
	Post-pandemic	1346	840	62	
Citrobacter sp.	Pre-pandemic	31	28	90	>0.100
	Covid-19 pandemic (Brazil)	6	6	100	
	Post-pandemic	35	35	100	
Enterobacter sp.	Pre-pandemic	356	283	79	<0.001
	Covid-19 pandemic (Brazil)	53	43	81	
	Post-pandemic	219	204	93	
Escherichia sp.	Pre-pandemic	124	105	85	<0.001
	Covid-19 pandemic (Brazil)	24	20	83	
	Post-pandemic	156	153	98	
Klebsiella sp.	Pre-pandemic	3145	2780	88	<0.001
	Covid-19 pandemic (Brazil)	757	712	94	
	Post-pandemic	156	153	98	
Morganella sp.	Pre-pandemic	26	18	69	0.080
	Covid-19 pandemic (Brazil)	4	3	75	
	Post-pandemic	21	20	95	
Proteus sp.	Pre-pandemic	141	122	87	0.070
	Covid-19 pandemic (Brazil)	40	36	90	
	Post-pandemic	121	115	95	
Providencia sp.	Pre-pandemic	114	75	66	<0.001
	Covid-19 pandemic (Brazil)	44	36	82	
	Post-pandemic	167	164	98	
Pseudomonas sp.	Pre-pandemic	424	31	7	<0.001
	Covid-19 pandemic (Brazil)	80	6	8	

Continued.

Micro organism	Period	Samples	MRD (n)	MRD risk (%)	P value
	Post-pandemic	937	762	81	
Serratia sp.	Pre-pandemic	435	161	37	<0.001
	Covid-19 pandemic (Brazil)	51	24	47	
	Post-pandemic	228	201	88	
Total	Pre-pandemic	5580	3741	67	<0.001
	Covid-19 pandemic (Brazil)	1290	890	69	
	Post-pandemic	6142	5335	87	

Distribution and univariate analysis of multidrug-resistant (MDR) microorganisms during pre-pandemic, pandemic and post-pandemic periods of COVID-19. The table presents the number of samples (n), the number of MDR isolates (MRD), the proportion of MDR risk (%) and the p value for each microorganism during the three study periods. Significant differences in MDR prevalence across periods are indicated by p-values <0.05.

Table 2: Percentage of resistance genes in gram-negative isolates across pre-pandemic, pandemic and post-pandemic periods.

Gene	Pre-pandemic (%)	Pandemic period in Brazil (%)	Post-pandemic (%)	Pre-pandemic (n)	Pandemic period in Brazil (n)	Post-pandemic (n)
***bla*KPC**	66.3	55.6	44.4	3538	784	2906
***bla*OXA**	30	32.9	41.1	1603	464	2691
***bla*NDM**	2.2	10.9	13.5	119	153	884
***bla*SPM**	1.3	0.3	0.5	67	4	31
***bla*VIM**	0.2	0.3	0.3	12	4	17
***bla*IMP**	0	0	0.2	0	0	16
Total	100	100	100	5339	1409	6545

Data represent the percentage and absolute number (n) of gram-negative isolates carrying each resistance gene during the three COVID-19 time periods: pre-pandemic (until March 2020), pandemic (April 2020 to December 2021) and post-pandemic (from January 2022 to December 2023).

The Table 2 reveals significant changes in the distribution of resistance genes between the periods analyzed, evidencing a shift in the genetic profile of bacterial resistance in the state of Minas Gerais during and after the COVID-19 pandemic. The *bla*KPC gene, associated with carbapenem resistance and considered highly prevalent in Brazil, showed a progressive reduction in its relative frequency: from 66.3% in the pre-pandemic period, it dropped to 55.6% during the pandemic and reached 44.4% in the post-pandemic period. Despite this percentage drop, the absolute number of samples with this gene increased again in the post-pandemic period (n=2,906), indicating that resistance remains widespread and due to the need to continue surveillance in the research of resistance genes.

On the other hand, the *bla*OXA and *bla*NDM genes showed an increase in their relative prevalence, especially in the post-pandemic period. The *bla*OXA gene increased from 30.0% to 41.1% and the *bla*NDM gene frequency increased more than sixfold, from 2.2% to 13.5%. These data suggest the possible dissemination of emerging bacterial clones with these genes or even a scenario of selective pressure resulting from the intensive use of antimicrobials during the pandemic to treat infections caused by viruses and secondarily by bacteria. Less prevalent genes, such as *bla*SPM, *bla*VIM and *bla*IMP, remained at low levels, although *bla*IMP was detected exclusively in the post-pandemic period (0.24%, n=16), which may indicate its recent emergence in the local context.

This dataset highlights a diversification of the genotypic resistance profile after the COVID-19 pandemic, with an increase in the participation of less frequent genes and a relatively substantial reduction in the dominance of *bla*KPC. These changes require continued surveillance and reinforcement of infection control policies and rational use of antimicrobials, especially in the antimicrobial stewardship program, since the genetic diversity of resistance mechanisms can compromise the effectiveness of current and future therapies.

DISCUSSION

The concentration of genotyping requests predominantly in the metropolitan region of Belo Horizonte suggests disparities in access to diagnostic services and laboratory resources across the state. These disparities may lead to underreporting and underdiagnosis in more remote regions, hindering preventive measures such as in-hospital contact isolation and exacerbating the problem of antimicrobial resistance.[12] AMR, intensified by the SARS-CoV-2 pandemic, is a global crisis that exacerbates infection control challenges, especially in low- and middle-income countries. Tackling this threat requires multisectoral and coordinated efforts with clinical, socioeconomic and policy approaches at both global and local levels.[13]

The COVID-19 pandemic negatively impacted the control of multidrug-resistant bacteria due to the diversion of resources towards viral testing, exacerbating pre-existing problems, particularly the underdiagnosis of microbial resistance and the lack of focus on the surveillance of colonization and hospital-acquired infection genes.[14] Reports from the World Health Organization had already highlighted concerns regarding these issues.[15]

The empirical use of antimicrobials in COVID-19 patients, justified by the severity of the disease, the use of corticosteroids, invasive devices (such as orotracheal tubes and central venous catheters) and prolonged stays in intensive care units, increased the risks.[16]

Lima et al may reflect a transient impact on the incidence of MDR *A. baumannii*, however, the effects of COVID-19 on hospital-acquired MDR infections can vary across healthcare services, a reality shaped by precautionary measures and rational antimicrobial use.[17]

Data analysis from the state of Minas Gerais revealed an increase in genotypic resistance profiles, primarily in descending order for *Klebsiella spp*., *Acinetobacter spp*., *Pseudomonas spp*. and *Serratia* spp. These endemic pathogens showed a worsening genotypic profile in the post-pandemic period. The predominance of resistance genes such as *bla*KPC, *bla*OXA, *bla*NDM, *bla*SPM, *bla*VIM and *bla*IMP reflects this concerning pattern of carbapenem resistance, complicating and limiting therapeutic options for severe infections.[18] However, there was a low prevalence of *mcr-1* detection, in total the four cases.

The management of MDR *Klebsiella pneumoniae* is a critical challenge, with high rates of hospital mortality. Infection control and rational antibiotic use are essential to slow down resistance, especially with new antimicrobials now available to treat this infection.[19]

The study highlights the urgent need to strengthen surveillance and infection control systems in Minas Gerais and other affected regions, particularly by improving access to point-of-care technology for early molecular diagnosis of highly pathogenic and epidemiologically significant microorganisms. Enhancing hospital hygiene practices, the effectiveness of antimicrobial stewardship programs and the training of healthcare professionals and family members are essential measures to minimize the impact of resistant bacterial dissemination and improve the response to future health crises.

Carbapenem-resistant *Klebsiella pneumoniae* is a major public health threat due to its multidrug resistance and high mortality rates. A Chinese study shows that *Klebsiella pneumoniae* is the second most isolated bacterium in Chinese clinics, with meropenem resistance increasing from 2.9% in 2005 to 30.0% in 2023, significantly limiting therapeutic options.[20] This study highlights a significant change in the genetic and epidemiological profile of antimicrobial resistance after the pandemic. A percentage reduction in the prevalence of the *bla*KPC gene was observed, although there was an increase in the absolute number of cases in the post-pandemic period. Simultaneously, there was a significant increase in the frequency of the *bla*OXA and *bla*NDM genes, indicating possible dissemination of emerging clones or horizontal acquisition of these genes by different bacterial species.[21]

The concentration of diagnostic resources in the metropolitan area of Belo Horizonte highlights the critical need to improve access to microbiological testing and infection control in smaller, underserved regions.

The detection of highly prevalent resistance genes such as *bla*KPC, *bla*OXA and *bla*NDM, along with the emergence of *mcr-1* in some cases, underscores the urgency of action. Strengthening surveillance systems, improving hospital hygiene practices and enhancing antimicrobial stewardship programs are vital to contain the spread of these resistant bacteria. Additionally, investments in point-of-care molecular diagnostic technologies, especially in resource-limited regions, are crucial to mitigating future public health threats.

As this is a retrospective epidemiological study, underreporting of the submission of biological specimens by healthcare facilities may occur, underestimating microbial resistance.

CONCLUSIONS

This study demonstrates a significant post-pandemic shift in the genotypic profile of hospital-acquired bacterial resistance in Minas Gerais, Brazil. Despite the proportional decline of the *bla*KPC gene, its absolute frequency remained high, while the increased detection of *bla*OXA, *bla*NDM and the emergence of *mcr-1* indicate a diversification in resistance mechanisms. These findings reflect not only the biological impact of the COVID-19 pandemic but also systemic weaknesses in diagnostic infrastructure and infection control measures, particularly in underserved regions. Strengthening molecular surveillance, ensuring equitable access to microbiological diagnostics and reinforcing antimicrobial stewardship are essential strategies to contain the spread of multidrug-resistant organisms. Targeted investments in laboratory capacity and infection prevention programs are urgently needed to improve response capacity and reduce morbidity and mortality from resistant infections in Brazil's public health system.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. World Health Organization. Antimicrobial stewardship programmes in health-care facilities in low- and middle-income countries: a WHO practical

toolkit. Geneva: WHO. 2019. Available at: https://iris.who.int/handle. Accessed in 21 February 2025.

2. Murray CJL, Ikuta KS, Sharara F, Swetschinski L, Aguilar GR, Gray A, et al. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet. 2022;399(10325):629–55.
3. Rawson TM, Moore LSP, Zhu N, Ranganathan N, Skolimowska K, Gilchrist M, et al. Bacterial and fungal coinfection in individuals with coronavirus: a rapid review to support COVID-19 antimicrobial prescribing. Clin Infect Dis. 2020;71(9):2459–68.
4. Hsu J. How COVID-19 is accelerating antimicrobial resistance. BMJ. 2020;369:1983.
5. Bonomo RA. The impact of COVID-19 on antimicrobial resistance in healthcare-associated infections in ICUs: a global perspective. Clin Infect Dis. 2022;75(1):29–31.
6. Partridge SR, Kwong SM, Firth N, Jensen SO. Mobile genetic elements associated with antimicrobial resistance. Clin Microbiol Rev. 2018;31(4):87–9.
7. Huttner BD, Catho G, Pano-Pardo JR, Pulcini C, Schouten J. COVID-19: don’t neglect antimicrobial stewardship principles! Clin Microbiol Infect. 2020;26(7):808–10.
8. CDC Global Health. Combating antimicrobial resistance globally during COVID-19. Atlanta: CDC. 2022.
9. Langford BJ, So M, Raybardhan S, Leung V, Soucy JPR, Westwood D, et al. The impact of COVID-19 on antimicrobial resistance: a systematic review. Lancet Microbe. 2023;4(3):179–91.
10. Al Sulayyim AS, Khan M, Aslam B, Almutairi F, Jabbar A, Aljarba NH, et al. Antimicrobial resistance trends during the COVID-19 pandemic: a systematic review. Int J Environ Res Public Health. 2022;19(21):13746.
11. Brasil. Agência Nacional de Vigilância Sanitária (ANVISA). Nota técnica nº 1/2013: medidas de prevenção e controle de infecções por enterobactérias multirresistentes. Brasília: ANVISA; 2013. Available at: http://antigo.anvisa.gov.br/documents. Accessed on 22 Febbruary 2025.
12. World Health Organization. Hand hygiene technical reference manual: to be used by health-care workers, trainers and observers of hand hygiene practices. Geneva: WHO. 2009. Available at: https://iris.who.int/bitstream. Accessed on 21 February 2025.
13. Corrêa JS, Zago LF, Silva-Brandão RR, Oliveira SM, Fracolli LA, Padoveze MC, et al. antimicrobial resistance in Brazil: an integrated research agenda. Rev Esc Enferm USP. 2022;56:10589.
14. Ribeiro EA, Machado GS, Tavares JVA, Rocha MJ, Rabelo PWL. Impacto da pandemia de COVID-19 na ocorrência de resistência bacteriana frente aos antimicrobianos: revisão integrativa. Braz J Dev. 2022;8(7):54080–99.
15. World Health Organization. Global antimicrobial resistance and use surveillance system (GLASS) report Geneva: WHO. 2022. Available from: https://iris.who.int/bitstream. Accessed on 22 February 2025.
16. World Health Organization. WHO report on antibiotic use in COVID-19 patients. Available at: https://www.who.int. Accessed on 21 January 2025.
17. Polly M, Almeida BL, Lennon RP, Cortês MF, Costa SF, Guimarães T. Impact of the COVID-19 pandemic on the incidence of multidrug-resistant bacterial infections in an acute care hospital in Brazil. Am J Infect Control. 2022;50(2):238–9.
18. Shams AM, Rose LJ, Edwards JR, Cali S, Harris AD, Jacob JT, et al. Assessment of the overall and multidrug-resistant organism bioburden on environmental surfaces in healthcare facilities. Infect Control Hosp Epidemiol. 2016;37(12):1426–32.
19. Soto-Giron MJ, Rodriguez-R LM, Luo C, Elk M, Ryu H, Hoelle J, et al. Biofilms on hospital shower hoses: characterization and implications for nosocomial infections. Appl Environ Microbiol. 2016;82(9):2872–83.
20. Russo A, Fusco P, Morrone HL, Trecarichi EM, Torti C. New advances in management and treatment of multidrug-resistant *Klebsiella pneumoniae*. Expert Rev Anti Infect Ther. 2022;21(1):41–55.
21. Xavier BB, Lammens C, Ruhal R, Malhotra-Kumar S, Butaye P, Goossens H. Identification of a novel plasmid-mediated carbapenem-hydrolyzing β-lactamase (*bla*NDM-5) in *Escherichia coli*. J Antimicrob Chemother. 2016;71(3):596–602.

Cite this article as: Pereira HO, Carneiro M, Couto BRGM, Camargo DRA, Faria CD, Felizardo AA. Impact of the COVID-19 pandemic on the distribution and resistance of multidrug-resistant genes in Minas Gerais, Brazil: challenges and implications. Int J Clin Trials 2025;12(3):181-6.