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MONITORING THE BACTERIAL RESISTANCE PROFILE OF HEALTH CARE-RELATED INFECTIONS (HAI) POST-TRAINING FOR THE PHENOTYPIC DETECTION OF KPC

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Abstract: Introduction: the Regional Laboratory Center of the Adolfo Lutz Institute in Ribeirão Preto - VI (CLR-IAL-RP-VI) is a Regional Reference Laboratory for studies and molecular detection of resistance genes in Multi-Drug-resistant Bacteria (BMR). Phenotypic screening with phenylboronic acid (AFB) in multidrug-resistant (MDR) strains increases the percentage of resistance gene confirmation *Klebsiella pneumoniae* carbapenemase (KPC) by PCR. From April 8 to 12, 2019, theoretical and practical training was offered to microbiologists from local laboratories that serve hospitals, in order to decentralize the screening technique with AFB. **Objective:** to evaluate the efficiency of previous screening with AFB on the resistance profile of Gram negative bacilli of the order *Enterobacterales* to carbapenem antibiotics, in the city of Ribeirão Preto, from January 2016 to August 2019, considering the training offered at CLR-IAL-RP-VI. **Methodology:** in the CLR-IAL-RP-VI MDR strains isolated from clinical samples of hospitalized patients are received. These are phenotypically identified in genus and species and subjected to sensitivity tests by the disk diffusion method according to the "Clinical and Laboratory Standards Institute" and Technical Note 01/2013 of the National Health Surveillance Agency and PCR to detect genes of resistance. **Results:** from 2016 to March 2019, 22 MDR strains were received. After training, we received 68 MDR strains, previously screened by AFB, being 39 (57%) KPC, two (3%) extended-spectrum beta lactamase (ESBL) by phenotypic detection, 25 (37%) KPC and ESBL and two (3%) strains not carrying resistance genes. **Conclusion:** after training and adherence of some participants, a larger number of strains were received for further molecular studies. Prior screening with AFB in the local laboratory is important to direct the effectiveness of PCR confirmation of resistance genes.

Keywords: Phenylboronic acid, Multi-resistance, *Klebsiella pneumoniae*, carbapenemase.

INTRODUCTION

Bacterial resistance is a serious global health problem, resulting in high rates of morbidity and mortality. The indiscriminate and incorrect use of antimicrobials in the community and in the hospital environment has favored the occurrence of resistance genes in bacterial species, especially those considered pathogenic for humans and animals (PRATES, et al., 2020).

The "Alexandre Vranjac" Epidemiological Surveillance Center coordinates the State Plan for the Prevention and Control of Multi-resistant Bacteria (PECBMR) in the state of São Paulo, which aims to monitor the incidence of Health-Related Infections - HAIs (infection and colonization) and disseminate data defining levels endemic and epidemic (SES-SP, 2016).

The Ribeirão Preto Regional Laboratory Center - Instituto Adolfo Lutz VI (CLR-IAL-RP-VI) is part of the network of regional reference laboratories for molecular detection and further studies of antimicrobial resistance genes. In this context, it receives multidrug-resistant (MDR) bacterial isolates from different sites of patients admitted to hospitals in the city of Ribeirão Preto.

One method proposed by Technical Note 01/2013 – ANVISA is phenotypic screening with phenylboronic acid (AFB), a carbapenemase enzyme blocker, with a specificity equal to or greater than 99% in MDR bacterial isolates. The CLR-IAL-RP-VI offered theoretical and practical training in the phenotypic detection of *Klebsiella pneumoniae* carbapenemase (KPC), in bacterial isolates that produce

carbapenemases, for microbiologists from local laboratories that serve hospitals, in order to decentralize the screening technique with AFB (BRASIL, 2013).

GOAL

To evaluate the adherence of participants to PECBMR and the efficiency of previous screening with AFB to carbapenem antibiotics in the resistance profile of Gram negative bacilli (GGN) fermenting bacteria of the *Enterobacteraceae* family in the city of Ribeirão Preto, considering the training offered in this CLR-IAL-RP- SAW.

MATERIAL AND METHODS

This is a retrospective study of analysis of laboratory data from January 2016 to August 2019. From April 8 to 12, 2019, theoretical and practical training on the phenotypic detection of KPC in bacterial isolates that produce carbapenemases was carried out for training of microbiologists from the local laboratories that serve hospitals, in order to decentralize the screening technique with AFB.

The bacterial isolates sent to the Bacteriology laboratory of the CLR-IAL-RP-VI were presumptively characterized, sown on Mc Conckey Agar and were confirmed as to identification in genus and species by biochemical tests according to microbiology manuals (BALOWS et al., 1991; HOLT et al., 1994; JORGENSEN et al., 1999; WAUTERS & VANEECHOUTTE, 2015). The resistance profile analyzed followed the Kirby Bauer disk diffusion method, with interpretation by the "Clinical and Laboratory Standards Institute" (CLSI 2015, 2017, 2018, 2019) and adapted by ANVISA Technical Note 01/2013 (BRASIL, 2013). Afterwards, real-time PCR was performed to detect resistance genes (RIBEIRO, 2016).

RESULTS

Figure 1 and table 1 show the results obtained after the training offered, where it can be noted the adherence of some participants who began to refer a larger quantity of bacterial isolates for additional molecular studies, duly notified and well isolated.

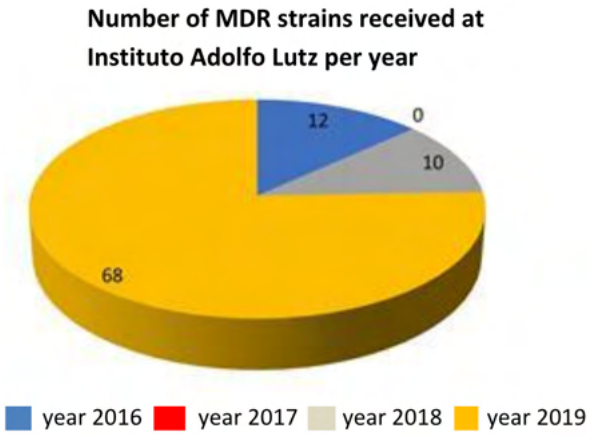

Figure 1. Number of MDR strains received, before and after training.

In the period studied, 90 bacterial isolates were analyzed. In 2016, 12 MDR bacterial isolates were received, resulting in three (25%) carriers of the KPC resistance gene, confirmed by PCR, and one (8%) extended-spectrum beta lactamase (ESBL) by phenotypic detection. In 2017, no MDR isolates were sent for further studies. In 2018, ten MDR isolates were received, three (30%) KPC, one (10%) ESBL and one strain was not investigated because it was unviable. In 2019, until the month of March, no MDR isolates were sent. After training and adherence of the participants, we received 68 MDR isolates, previously screened by the AFB, being 39 (57%) KPC, two ESBL (3%), 25 (37%) KPC and ESBL and two (3%) non-carrier isolates of resistance genes.

	KPC	ESBL	KPC+ESBL	Negative
2016 (12)	25%	8%	0	67%
2017 (0)	0	0	0	0
2018 (10)	30%	10%	0	60%
2019 (68)	57%	3%	37%	3%

Abbreviations: ESBL= Extended Spectrum Beta Lactamase, KPC=*Klebsiella pneumoniae* carbapenemase producer.

Table 1. Annual percentage of resistance genes found in suspected MDR strains submitted to CLR-RP-IAL-VI.

CONCLUSIONS

After the training, some participants joined, who began to refer a larger quantity of bacterial isolates for additional molecular studies, duly registered.

Prior screening with AFB in the local laboratory is important to direct the actions of the Center for Hospital Infection Control and Epidemiological Surveillance in order to prevent the spread of antimicrobial resistance in the hospital environment and for the effectiveness of the confirmation of resistance genes by PCR.

We also emphasized the need to send bacterial isolates that show unusual resistance to the CLR-IAL-RP-VI, in order to monitor the circulation of new profiles.

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