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https://doi.org/10.22239/2317-269x.01480

Epidemiologically relevant antimicrobial resistance phenotypes in surveillance cultures of a tertiary health service in Aracaju-SE

Fenótipos de resistência antimicrobiana epidemiologicamente importantes em culturas de vigilância de um serviço terciário de saúde em Aracaju-SE

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Received: Mar 05 2020
Approved: Jul 22 2020

ABSTRACT

Introduction: The constant emergence and spread of bacterial phenotypes of resistance to multiple drugs has become a public health problem worldwide. **Objective:** To investigate the occurrence of critical antimicrobial resistance phenotypes in bacteria isolated from surveillance cultures. **Method:** Microbiological data were collected from surveillance cultures performed between January and December 2015 at a tertiary health service. 1,590 surveillance cultures using the VITEK 2 system for phenotypic identification and susceptibility to antimicrobials of the isolates were analyzed. The detection of extended-spectrum ß-lactamases (ESBL) was performed using the approach disc and carbapenem resistance method and methicillin-resistant *Staphylococcus aureus* (MRSA) using diffusion disc methods. The detection of bacteria carrying the *bla*KPC, *bla*NDM and *bla*OXA-48 genes was carried out through the polymerase chain reaction in house. **Results:** The analysis identified 201 (12.64%) ESBL phenotypes, 173 (11.87%) resistant to carbapenems and 3 (0.18%) MRSA. 34 strains carrying genes encoding carbapenemases were detected, where 23 (67.64%) carried the *bla*KPC gene, 8 (23.53%) *bla*NDM and 3 (8.82%) both genes. **Conclusions:** It is a challenge to control the spread of critical resistance bacterial phenotypes. The predominance of ESBL in the referent hospital, as well as the occurrence of genes that have not been reported for carbapenemases until then, confirm the importance of epidemiological surveillance and the encouragement of subsequent research.

KEYWORDS: Carbapenemase-producing Enterobacteriaceae; Bacterial Genes; Nosocomial Infection; Epidemiological Surveillance

RESUMO

Introdução: O constante surgimento e a disseminação de fenótipos bacterianos de resistência a múltiplas drogas têm se tornado problemas de saúde pública em todo o mundo. **Objetivo:** Investigar a ocorrência de fenótipos críticos de resistência aos antimicrobianos em bactérias isoladas de culturas de vigilância. **Método:** Os dados microbiológicos foram coletados a partir de culturas de vigilância realizadas entre janeiro e dezembro de 2015 em um serviço terciário de saúde. Foram analisadas 1.590 culturas de vigilância utilizando o sistema VITEK 2 para identificação fenotípica e suscetibilidade aos antimicrobianos dos isolados. A detecção de ß-lactamases de espectro estendido (ESBL) foi realizada por método de disco aproximação e a resistência aos carbapenêmicos e *Staphylococcus aureus* resistente à meticilina (MRSA) por métodos de disco difusão. A detecção de bactérias portadoras dos genes *bla*KPC, *bla*NDM e *bla*OXA-48 foi realizada através da reação em cadeia da polimerase *in house*. **Resultados:** Foram identificadas 201 (12,64%) fenótipos de ESBL, 173 (11,87%) de resistência aos carbapenêmicos e 3 (0,18%) de MRSA. Foram detectadas 34 cepas portadoras de genes codificadores de carbapenemases, onde 23 (67,64%) carreavam o gene *bla*KPC, oito (23,53%) o *bla*NDM e três (8,82%) ambos os genes. **Conclusões:** É um desafio controlar a disseminação de fenótipos críticos de resistência bacterianos. O predomínio de ESBL no referente hospital, assim como a ocorrência de genes codificadores de carbapenemases até então não relatados, ratificam a importância da vigilância epidemiológica e do incentivo a pesquisas subsequentes.

PALAVRAS-CHAVE: Enterobacteriaceae Produtoras de Carbapenemase; Genes Bacterianos; Infecção Hospitalar; Vigilância Epidemiológica

INTRODUCTION

The emergence and spread of multidrug resistant (MDR) bacteria have become global public health problems as a consequence of the increase in the number of healthcare-associated infections (HAIs).[1] Bacteria are able to rapidly develop mechanisms that invalidate the administration of several drugs.[2,3]

Bacteria normally have antimicrobial resistance mechanisms, like modification of their cell membrane permeability through changes in protein channels (porins), as occurs with *Pseudomonas aeruginosa*, changes in the drug binding site in the case of methicillin-resistant *Staphylococcus aureus* (MRSA), efflux pumps that expel the drug from the interior of the bacterial cell, common in *Escherichia coli*'s resistance to tetracyclines, and the production and enzymatic inactivation of antimicrobial drugs applicable to *Klebsiella pneumoniae* Carbapenemase (KPC) and extended spectrum ß-lactamases (ESBL), which can occur in the Enterobacteriales order.[4,5,6,7]

MRSA, through the acquisition of the *mec*A gene, encodes an additional penicillin binding protein (PBP), PBP2a, which has low affinity for ß-lactams and, therefore, results in resistance to drugs of this class, with exception so far of cephalosporins ceftaroline and ceftobiprole.[8,9]

ESBL are a group of enzymes transmitted and encoded by plasmids in Gram-negative bacteria. They are capable of degrading third-generation cephalosporins and monobactams.[10] These enzymes can be inactivated by ß-lactamase inhibitors, like clavulanate, sulbactam and tazobactam.[10]

The KPC enzyme is a carbapenemase produced by Gram-negative bacteria carrying the *bla*KPC gene, capable of inactivating the entire class of beta-lactams, including carbapenems. The enzyme is detected by phenotypic methods and confirmed by molecular biology.[11,12] This carbapenemase was first described in 2001 in a strain of *K. pneumoniae* isolated in North Carolina.[10] The first reports of this mechanism of resistance in Brazil date back to 2009, from studies done with isolated strains in the cities of Recife and Rio de Janeiro between the years 2006 and 2008.[13,14] These were followed by many other reports in hospitals from several regions of the country.[15]

A critical resistance phenotype is defined as the ability of a microorganism to inactivate the main class of clinically effective antimicrobials (e.g., KPC and MRSA).[9,16] The expression of resistance mechanisms can induce resistance to multiple classes of first-line antimicrobial drugs in the treatment of severe infections. Therefore, usually the bacteria that express critical resistance phenotypes are also MDR.[9,16]

In this context, it is important to pay attention to the emergence of critical phenotypes through antimicrobial resistance mechanisms responsible for inhibiting the effects of widely used antimicrobials, since some of these are epidemiologically relevant for infection control and public health.[9]

In the meantime, based on the case record of patients infected with KPC in August 2015 in a tertiary health service in Aracaju, the occurrence of critical phenotypes of antimicrobial resistance in bacteria isolated from surveillance cultures was investigated.

METHOD

Type of study

This is a retrospective, descriptive, cross-sectional study, with a quantitative approach, with the purpose of observing the occurrence of critical bacterial phenotypes isolated from surveillance cultures. The study sample consisted of information about the microorganism and its resistance profile from hospital infection control reports from surveillance culture files of a tertiary health service, located in the city of Aracaju, in the Brazilian state of Sergipe, between January and December 2015. The data were obtained from printed forms signed by the person in charge of the microbiology laboratory of that institution.

Only cases of critical bacterial phenotypes were investigated. The variables analyzed were the type of sample of critical phenotypes of epidemiological interest: MRSA, ESBL and the KPC, New Delhi Metallo-ß-lactamase (NDM) and OXA-48 carbapenemases.

Samples

During the study period, a total of 1,590 surveillance cultures from colonization were analyzed. Data were acquired from the availability of results from surveillance cultures distributed evenly among rectal, nasal and axillary swabs of patients, collected in intensive care unit (ICU), clinical, surgical and emergency wards beds. The material was collected according to the institution's infection control protocols.

Sample analysis

After culture for isolation, the microorganisms were analyzed using the VITEK® 2 bioMérieux equipment, which enables phenotypic identification and performance of the antimicrobial susceptibility test (AST) using a semiquantitative method. The antimicrobial sensitivity profile of the identified species was analyzed for subsequent testing to detect critical phenotypes in accordance with the standard procedures of the institution's clinical microbiology laboratory.

Critical phenotype detection

After identification and analysis of the AST, the microorganisms were submitted to phenotypic tests for confirmation. To detect the ESBL phenotype in Gram-negative microorganisms resistant to cephalosporins, we used the combined disc test (CDT) with discs containing cephalosporin alone (cefotaxime, ceftazidime and cefepime) and in combination with clavulanic acid.[17,18] The phenotype present in MRSA was detected by the disk-diffusion method using cefoxitin in *S. aureus* isolates. The initial screening for detection of

carbapenemases was performed by selecting microorganisms resistant to carbapenems (meropenem, imipenem and ertapenem) and subsequent confirmation by molecular methods.[17,18]

Genotypic detection of carbapenemases

To confirm this phenotype, the Gram-negative bacterial strains resistant to carbapenems were sent by the service to a support laboratory of the Oswaldo Cruz Foundation (Fiocruz) in Rio de Janeiro, where, through in-house polymerase chain reaction (PCR) methodology, the *bla*KPC, *bla*NDM and *bla*OXA-48 carbapenemase-encoding genes were researched.[10,19,20] Carbapenemase-producing phenotypes were considered to be those with one or more encoding genes. Bacteria that did not present the studied genes were considered as resistant strains to carbapenems due to the presence of other resistance mechanisms.

Data analysis

The extracted data were stored in Excel© version 2019 spreadsheets. Through descriptive statistics, data were presented using relative and absolute frequency distribution. The figure with the frequency of categorized variables was obtained using GraphPad Prism© 8 and Origin© 2018 software.

Ethical considerations

This work was approved by the Research Ethics Committee (CEP) of Universidade Tiradentes under opinion n. 3.801.168, in accordance with the Resolution of the National Health Council n. 466, of December 12, 2012.

RESULTS

Of the 1,590 surveillance cultures analyzed, 377 had microorganisms with critical resistance phenotypes. The rectal swabs have shown the highest percentage of detected phenotypes, totaling 210 (55.70%) of the isolates, whereas 92 (24.40%) were found in the axillary swabs and 75 (19.90%) in the nasal swabs. It was not possible to discriminate the sites of the institution where the swabs were collected due to lack of information in the database.

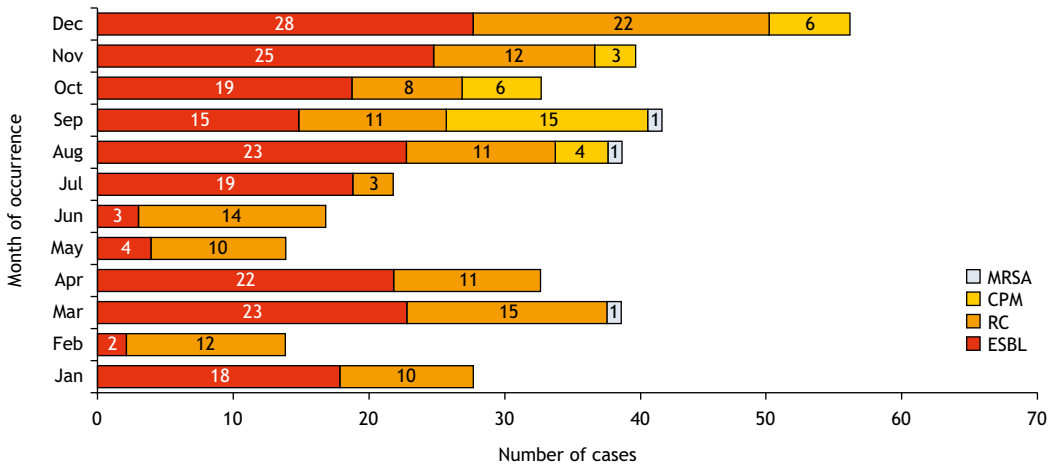
Regarding the total samples analyzed, we identified 201 (12.64%) ESBL phenotypes, 173 (11.87%) resistant to carbapenems and three (0.18%) MRSA.

When it comes to critical phenotypes, it is important to observe the presence of ESBL and resistance to carbapenems with variable frequency in all months of analysis. However, the month of December recorded a greater number of these phenotypes. Carbapenemases were detected from August to December, and September was the month with the highest number of cases. MRSA was the phenotype with the lowest number of records, appearing only in March, August and September (Figure).

Among the critical phenotypes identified in the present study, 120 (31.83%) isolates of *E. coli* and 81 (21.50%) of *K. pneumoniae* predominated as ESBL producers, and 139 (36.87%) isolates of *P. aeruginosa* were resistant to carbapenems. All 34 (9.01%) Enterobacteriaceae carrying genes for carbapenemase belonged to the *K. pneumoniae* species, and molecular tests have shown that 23 (67.64%) had the *bla*KPC gene, eight (23.53%) had *bla*NDM and three (8.82%) had both genes. The *bla*OXA-48 gene was not found in any of the bacterial isolates. Three (0.79%) MRSA were also identified in the study.

DISCUSSION

ESBL was initially documented in Europe, with later dissemination across the world. It is currently also present in the Brazilian territory with several variants already detected.[21,22] The present study evidenced the predominance of ESBL-producing phenotypes and corroborates previous research that revealed the prevalence of ESBL in surveillance cultures.[23,24,25]



Source: Prepared by the authors, 2020.
ESBL: extended spectrum ß-lactamase; RC: resistance to carbapenems; CPM: carbapenemases; MRSA: Methicillin-resistant Staphylococcus aureus.

Figure. Occurrence of critical bacterial phenotypes detected in surveillance cultures from January to December 2015 in a tertiary health service in Aracaju, Brazil.

The clinical relevance of ESBL is because it limits the therapeutic options to carbapenems as the class of choice for the treatment of infections caused by this phenotype.[26,27,28] This enzyme is encoded by genes present in plasmids, genetic segments that are easily transferable between bacteria, favoring the spread of resistance both in the hospital and in the community. The loading of genes conferring resistance to other antimicrobial classes is also common.[26,27]

The phenotype represented by MRSA was the least evident when compared to the others during the study period. This microorganism is reported as an important hospital pathogen. It presents high virulence and toxin production and causes infections on soft tissues and skin.[29] Patients with colonization of the nostrils by this agent are the main contributors to its spread.[30] MRSA strains are resistant to ß-lactam drugs, including carbapenems, and increase the probability of death up to five times when compared to methicillin-sensitive *S. aureus*.[31]

Regarding resistance to carbapenems and the production of carbapenemases, this class of enzymes has received attention due to its clinical impact, as it increases resistance to all ß-lactam agents, reducing therapeutic options and demanding greater efforts for its control and prevention, which, in turn, are hampered by the rapid transmission of plasmid genes encoding these enzymes. Greater emphasis is given to the KPC and NDM enzymes, disseminated worldwide.[14,29,32] In this study, 8.74% of the analyzed samples had carbapenem-resistant species and 3.13% had genes encoding carbapenemases. These are relevant data to help control spread and hospital infection rates.

Epidemiological studies aimed at researching genes encoding carbapenemases have shown predominance of the *bla*KPC gene[33,32,34] and, to a lesser extent, the *bla*NDM and *bla*OXA-4837 genes.[32,35] The present study is consistent with the aforementioned studies and also demonstrated the *bla*KPC gene as predominant and the *bla*NDM gene as secondary, with no evidence of the presence of *bla*OXA-48.[35]

The first finding of KPC in the tertiary service under this study occurred in August 2015 and was characterized as an outbreak, since there was no previous report of this phenotype there. According to Brazil's National Health Surveillance Agency,[34] an outbreak happens when there is an increase above the expected in the occurrence of cases of an event or disease in an area or among a specific group of people, in a given period.

To control and prevent the occurrence and spread of bacteria carrying resistance genes, control measures must be established and reinforced. In the community, sanitation and disease prevention practices are fundamental to reduce the emergence of infections. Hospital settings require educational policies and management programs about the use of antimicrobials and the control of hospital infection, with monitoring and reporting of hospital infections likely caused by critical resistance phenotypes.[36]

CONCLUSIONS

The targeted surveillance cultures enabled the identification of important and critical antimicrobial resistance phenotypes with a predominance of ESBL, frequent in tertiary health services.

Special attention should be given to the detection of genes producing carbapenemases in strains of *K. pneumoniae*, as this phenotype had not been reported in the service in question until then.

The application of this study model in other tertiary health services in the state of Sergipe is necessary to reveal the epidemiological reality regarding the KPC phenotype and confirm the importance of epidemiological surveillance.

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Authors' Contributions

Amâncio FLR, Carvalho IKNP – Conception, planning (study design), data analysis and interpretation, and writing of the manuscript. Menezes TA, Albuquerque Junior RLC – Data acquisition, analysis and interpretation. Pinheiro MS, Santos-Neto AG – Data analysis and interpretation, and writing of the manuscript. All authors approved the final draft of the manuscript.

Conflict of Interest

Authors have no potential conflict of interest to declare, related to this study's political or financial peers and institutions.