Original Article

REVISTA PREVENÇÃO DE INFECÇÃO E SAÚDE (REPIS)

Surveillance cultures: screening of carbapenemase producing microorganisms and patient safety

Culturas de Vigilância: Triagem de Microorganismos Produtores de Carbapenemase e Segurança do Paciente

Vigilancia de Culturas: Triagen de Microorganismos Productores de Carbapenase y Seguridad del Paciente

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ABSTRACT

Objective: to perform a carbapenemase screening in the main Gram negative microorganisms isolated from active cultures surveillance in patients of the Intensive Care Unit (ICU) of an emergency hospital in the city of Caruaru, Pernambuco, Brazil. **Method:** this is a descriptive, cross-sectional study where the samples obtained were collected following the principles described by the Ministry of Health for cultures surveillance. **Results:** the identification of patients colonized by carbapenemase through cultures surveillance showed a positivity of 88% of the total samples collected, with *Klebsiella pneumoniae* being the most frequent (31.81%). **Conclusion:** in addition to allowing the early identification of asymptomatic colonizers, cultures surveillance provide microbiological information on the epidemiological profile of the health unit. The high carbapenemase detection rate in this study shows the importance of performing this technique as a reinforcement measure aimed at patient safety.
Descriptors: Antimicrobials, Public Health Surveillance, Microbiology, Patient safety.

RESUMO

Objetivo: realizar triagem de carbapenemase nos principais microrganismos Gram negativos isolados de vigilância de culturas ativas em pacientes da Unidade de Terapia Intensiva (UTI) de um hospital de emergência da cidade de Caruaru, Pernambuco, Brasil. **Método**: trata-se de um estudo descritivo, transversal, no qual as amostras obtidas foram coletadas seguindo os princípios descritos pelo Ministério da Saúde para a vigilância de culturas. **Resultados**: a identificação dos pacientes colonizados por carbapenemases por meio de culturas de vigilância mostrou uma positividade de 88% do total de amostras coletadas, sendo *Klebsiella pneumoniae* a mais frequente (31,81%). **Conclusão**: além de permitir a identificação precoce de colonizadores assintomáticos, a vigilância de culturas fornece informações microbiológicas sobre o perfil epidemiológico da unidade de saúde. A alta taxa de detecção de carbapenemases neste estudo mostra a importância de realizar essa técnica como uma medida de reforço visando a segurança do paciente.
Descritores: Antimicrobianos, Vigilância em Saúde Pública, Microbiologia, Segurança do paciente.

RESUMÉN

Objetivo: realizar una detección de carbapenemasas en los principales microorganismos Gram negativos aislados de la vigilancia de cultivos activos en pacientes de la Unidad de Cuidados Intensivos (UCI) de un hospital de emergencia en la ciudad de Caruaru, Pernambuco, Brasil. **Método**: se trata de un estudio descriptivo, transversal, donde las muestras obtenidas fueron recolectadas siguiendo los principios descritos por el Ministerio de Salud para la vigilancia de las culturas. **Resultados**: la identificación de pacientes colonizados por carbapenemasas a través de la vigilancia de cultivos mostró una positividad del 88% del total de muestras recolectadas, siendo *Klebsiella pneumoniae* la más frecuente (31.81%). **Conclusión**: además de permitir la identificación temprana de colonizadores asintomáticos, la vigilancia de cultivos proporciona información microbiológica sobre el perfil epidemiológico de la unidad de salud. La alta tasa de detección de carbapenemasas en este estudio muestra la importancia de realizar esta técnica como una medida de refuerzo dirigida a la seguridad del paciente.
Descriptores: Antimicrobianos, Vigilancia de Salud Pública, Microbiología, Seguridad del paciente.

How to cite:
Almeida KRH, Silva NS, Rocha IV, Araújo AA, Silva LM, Oliveira SR. Surveillance cultures: screening of carbapenemase producing microorganisms and patient safety. Rev Pre Infec e Saúde[Internet]. 2018;4: 6977. Available from: http://www.ojs.ufpi.br/index.php/nupcis/article/view/6892 DOI: https://doi.org/10.26694/repis.v4i0.7028

INTRODUCTION

The spread of multidrug-resistant pathogens has become a public health problem worldwide, affecting both developed and developing countries[1,2]. One of the main ways involved in the process of acquisition of antimicrobial resistance includes the production of enzymes capable of inactivating the antimicrobial activity[3].

Klebsiella pneumoniae carbapenemase (KPC) is the main enzyme of the carbapenemases group produced by Gram negative bacteria, usually *Enterobacteriaceae*, predominantly by the genus *Klebsiella*, *Serratia*, *Citrobacter*, *Enterobacter*, *Escherichia*, *Samonella*, *Proteus* and *Morganella*[4], which confers resistance to carbapenem class of antimicrobials, largely used in the treatment of bacterial infections.

In addition to inactivating carbapenems, KPCs also confer resistance to various ß-lactam agents, such as cephalosporins and penicillins[1,3,5]. Often due to the lack of therapeutic options against infections of patients colonized by KPC-producers, antimicrobial agents such as polymyxin B, which had previously been disused because of its toxicity, were reintroduced in clinical practice[6].

The bla_{KPC} gene, which encodes the KPC enzyme, is usually located on a mobile plasmid that can be transferred between bacteria of the same species or between different species[7], however, it has also been identified in different regions of the bacterial chromosome.[8,9] Different KPC enzyme variants have already been identified, with KPC 2 and 3 being the most common variants found generally in *Enterobacteriaceae* and some Nonfermenting Gram-Negative Bacilli (NFGNB) species such as *Pseudomonas aeruginosa* and *Acinetobacter baumanii*[10]. The easy spread of this resistance mechanism makes the epidemics difficult to control, since the treatment options for infections caused by KPC-producing bacteria are extremely limited, favoring the increase of mortality rates[1].

One of the most significant ways of spread of hospital infections is the cross infection, which can occur by the transmission of microorganisms from one patient to another in the hospital units, specially by the health professionals during patient's care[11].

Cultures surveillance are defined as a screening technique performed in patients who are at risk of acquisition of multiresistant bacteria, being considered one of the most sensitive approach in the early detection of colonized patients[12]. Its periodic performance enables the implementation of prevention strategies such as contact precaution isolation, decolonization of patients, reinforcement of the policies of hand hygiene and cleanliness of the environment[13,14]. Prevention strategies become relevant because they limit the spread of multiresistant microorganisms and enable a decrease in the number of cases of cross-infection[13,15]. In order to develop such strategies, it is important the continuing education of the healthcare professionals and the reinforcement of orientations to the patient's visitors and companions[7,10].

Since multiresistant microorganisms are present more frequently in health institutions or hospitalization units, the increase in its transmission and infectious potential can be determined by the vulnerability of the patients,

the high number of patients colonized, the selective pressure exerted by the use of antimicrobials, and, more often, by the lack of compliance with the preventive measures[11].

Asymptomatic patients colonized with antimicrobial resistant bacteria are more susceptible to infectious diseases. Although the surveillance culture technique is recommended in the early detection of multiresistants in asymptomatic patients[16], the time dispend for its implementation and the additional costs of the procedure have made it difficult to be implemented in the most hospital routine, thus, the use of clinical criteria is the most frequently used option[8,14].

Considering that only a part of the colonized population is detected by the use of clinical cultures[7], the present study aimed to perform a screening of carbapenemase enzyme production in Gram negative microorganisms isolated from active surveillance cultures in patients of an Intensive Care Unit (ICU) of an tertiary hospital.

METHODS

This is a descriptive and cross-sectional study developed between February and November of 2016, in the Intensive Care Unit of a tertiary hospital located in Caruaru-PE, in the Northeast of Brazil. Ethical approval was obtained from the ethical committee of the Universitary Center Tabosa de Almeida Asces-Unita (code 1.256.462).

The samples were collected by convenience sampling, following the principles described by the Secretariat of Health Surveillance and Ministry of Health (Brazil)[14,17]. Bacterial screening was performed in patients hospitalized in the ICU, including all the 20 beds. The samples were obtained by the use of sterile swabs introduced into the nasal and rectal cavities, axillary region and the palms of the hands of the patients. The inclusion criterion was patients of any gender submitted to ICU stay during the study period. Considering the methodology of Landman[18], immediately after the collection, the swabs were stored in 10 mL of *Tryptic Soy Broth* (TSB) (Oxoid®) containing a meropenem disk (10µg) (Laborclin®) and incubated at 37 ± 2 ºC during 24 hours. After the growth in TSB, the samples were seeded in MacConkey agar and also incubated at 37 ± 2 ºC during 24 hours for colony isolation.

The identification of the isolates was performed according to macro and microscopic characteristics of the colonies and biochemical test results. For the identification of bacteria from the *Enterobacteriaceae* family, the carbohydrate fermentation test was used in Triple Sugar Iron (TSI) (Kasvi®), as well as biochemical tests using the Sulfide Indole Motility (SIM) (Kasvi®), Simmons' citrate (Kasvi®) and Christensen's Urea Agar (Kasvi®) growth mediums. Tests based on Oxidase (Laborclin®) were used for the identification of glucose-non-fermenting Gram-negative bacteria. As an additional step to confirm the identification of the bacterial species, MALDI-TOF MS (Matrix Associated Laser Desorption/Ionization - Time of Flight Mass Spectrometry) was performed. The mass spectra were manually obtained in duplicate for each

isolate and compared to the *Biotyper MALDI 2.0* software database.

The detection of carbapenemase production was performed by the CarbaNP[19] and the enzymatic inhibition by aminophenylboronic acid (ABPA) (Sigma Aldrich®) tests. As a negative control for carbapenemase production in CarbaNP test, *Escherichia coli* ATCC® 25922 strain was used and, as a positive control of carbapenemase production, *K. pneumoniae* Kp13[20] (imipenemase producer) and *K. pneumoniae* FL_C262[21] (metallo-ß-lactamase producer) strains were also included. ABPA test was performed and interpreted according to Borba[22] proposed methodology, in which tests considered positive were those that presented a bacterial growth inhibitory zone ≥ 5 millimeters in diameter around the disc containing the antimicrobial added with ABPA.

RESULTS

A total of 73 samples were collected. The bacterial growth was detected in 47 (64.38%) of the samples, three of them (6.38%) positive for the growth of more than one microorganism, totaling 50 bacterial isolates. It should be noted that at the time of collection, 7 patients did not have adequate physical conditions to obtain rectal samples, so this site was not evaluated in these patients.

Regarding the profile of the patients, 10 (55.5%) were men and the most prevalent age range was between 60 and 85 years.

As regard as collection regions, 17 (34%) of the bacterial isolates corresponded to the axillary region of the patients, 12 (24%) corresponded to the nasal region, 11 (22%) were from the rectal region and 10 (20%) from the palm of the hands (Table 1).

Table 1 - Bacterial species isolated from the evaluated body regions.

Isolated species	Axillary region n(%)	Nasal cavity n(%)	Rectal cavity n(%)	Palm of hands n(%)	Total n(%)
A. baumannii	4(23.52)	2(16.66)	5(45.45)	4(40)	15(30)
K. pneumoniae	9(52.94)	3(25.0)	2(18.18)	1(10)	15(30)
E. aerogenes	2(11.76)	2(16.66)	1(9.09)	3(30)	8(16)
P. mirabilis	2(11.76)	2(16.66)	1(9.09)	1(10)	6(12)
E. coli	0(0)	1(8.33)	2(18.18)	0(0)	3(6)
S. rubidaea	0(0)	1(8.33)	0(0)	1(10)	2(4)
P. aeruginosa	0(0)	1(8.33)	0(0)	0(0)	1(2)
Total	17(34)	12(24)	11(22)	10(20)	50(100)

Note*: n = number of isolates; % = percentual of isolates.

There was a higher occurrence of *Acinetobacter baumannii* and *Klebsiella pneumoniae*, representing 30% of the total isolates, followed by *Enterobacter aerogenes* (16%), *Proteus mirabilis* (12%), *Escherichia coli* (6%), *Serratia rubidaea* and P*seudomonas aeruginosa* (2%) (Table 1). Tests for carbapenemases detection

showed 44 (88%) positive strains for the production of the enzyme (Figure 1 and 2).

Among the carbapenemase-producing microorganisms, *K. pneumoniae* was the most frequent specie, representing 31.81% of the isolates, followed by *A. baumannii* (29.54%), *E. aerogenes* (15.9%), *P. mirabilis* (13.63%), *S. rubidaea* (4.54%) and *E. coli* and *P. aeruginosa* (2.27% of isolates each), as described in Figure 3.

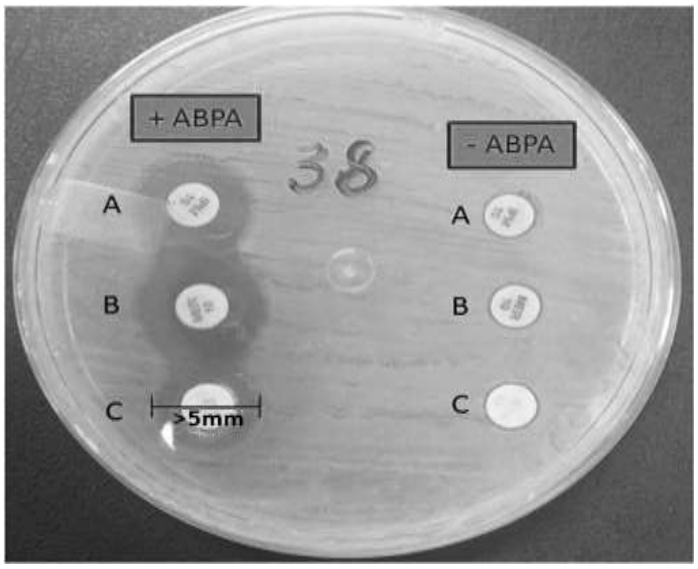

Figure 1 - Representation of the identification of carbapenemase production by CarbaNP test.

Note*: Column 1 = 0.5% phenol red solution (m/v) containing 0.1 mM ZnSO4; Column 2 = 0.5% phenol red solution (m/v) containing 0.1 mM ZnSO4 and 3 mg/mL of imipenem; Column C = 0.5% phenol red solution (m/v) containing 3 mg/mL of imipenem and 0.006 M ethylenediamine tetraacetic acid; A1/A2/A3 = control of solutions without bacterial lysate; B1/B2/B3 = positive control for carbapenemase production (strain Kp13 producing KPC-2); C1/C2/C3 = positive control for metallo-ß-lactamase production (strain FL_C262); D1/D2/D3 = negative control of bacterial lysate (E. coli ATCC® 25922); E1/E2/E3 = representation of the tested isolates. Photography taken after 2 hours of incubation at 37 °C.

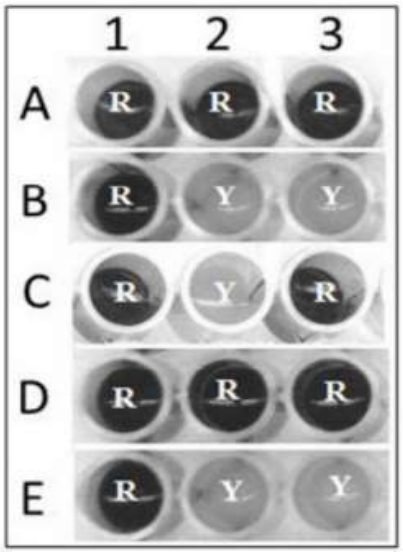

Figure 2 - Aminophenylboronic acid (ABPA) inhibition test for carbapenemase detection.

Note*: +ABPA = Test performed with addition of aminophenylboronic acid; -ABPA = Test performed without addition of aminophenylboronic acid; A = imipenem disk; B = meropenem disk; C = ertapenem disk.

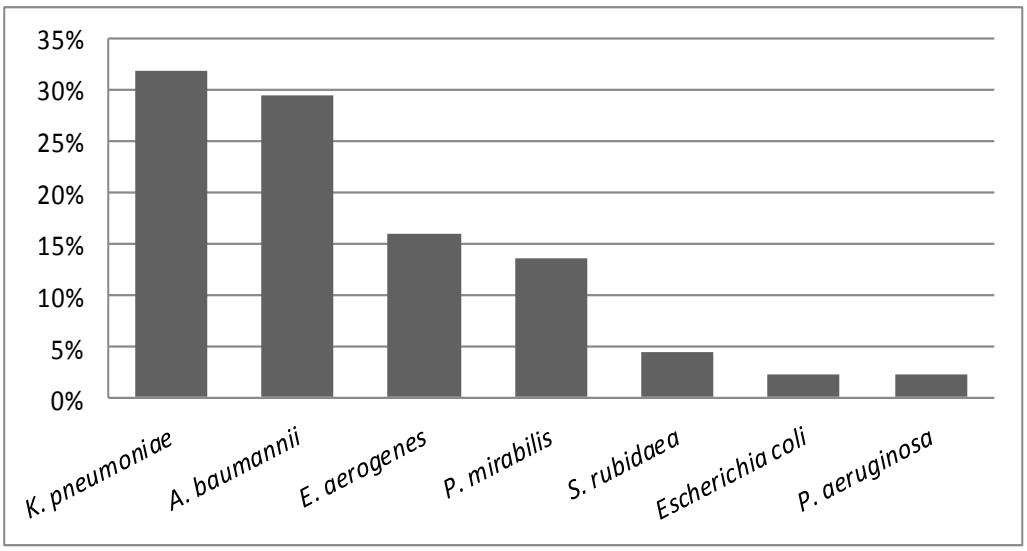

Figure 3 - Percentage of carbapenemase-producing isolates according to each bacterial species

DISCUSSION

The control of the dissemination of bacterial resistance mechanisms presents a great challenge for clinical practice, since the increase in cases of infections caused by multiresistants is an increasingly frequent public health problem[14].

Bacteria producing carbapenemase usually have high dissemination capacity, posing a threat to hospitalized patients and health institutions, especially due to difficulties in implementing early identification measures and the severe treatment restrictions, making the epidemics difficult to control and consequently raising mortality rates[1,16].

Surveillance cultures, when performed continuously, are an important component of infection control programs once it allows the early identification and isolation of microorganisms of asymptomatic patients, since they are considered important reservoirs for the transmission of resistant pathogens[7,17].

The high occurrence of carbapenemase-producing *Enterobacteriaceae* identified in the present study corroborate other studies[23-26], in which bacteria belonging to this family were predominantly associated with the production of carbapenemases, representing a serious clinical and epidemiological issue. Thus, the necessity of rapid detection of this resistance mechanism is essential to ensure patients safety, as well as the adoption of practices aimed to the prevention and control of its dissemination.

The high frequency of *K. pneumoniae* evidenced in this study has also been reported by Cotrim[27] and Rossi[8], that highlight the significant detection of the carbapenemase enzyme production in this species. Among the *Enterobacteriaceae*, *K. pneumoniae* represents an important opportunistic pathogen associated with a series of nosocomial infections, fact that has contributed to this species as a cause of several outbreaks of hospital infections[25,29].

Among the carbapenemase-producing NFGNB, *Acinetobacter baumannii* was the most prevalent species (29.54%). Similar data were

found by Mishra[30] in which a higher frequency of this microorganism was detected when compared to others NFGNB identified. These findings can be explained by the frequent presence of *A. baumannii* in the respiratory tract of intubated patients, especially those with clinical impairment.

The dissemination prevention of carbapenemase-producing bacteria depends, among other factors, on the early and correct detection of colonized patients[12]. Several orientations are recommended for the monitoring of patients during their admission, hospitalization or transference between hospitalization units, such as the screening of multiresistant microorganisms in patients at risk of colonization, especially those who are in ICUs, organ transplantation units and immunosuppressed patients. Once that strains of multiresistant bacteria are identified as colonizers of patients in these units, the dissemination prevention practices should be extended to those who share the same sector, and also to those transferred from other hospitals.

In addition to enabling the early identification of asymptomatic patients colonized by carbapenemase-producers, the cultures surveillance provides the epidemiological profile of the entire mapped unit. These information can contribute to the spread control of multiresistant microorganisms as well as the implementation of a targeted and more effective antimicrobial therapy[7].

CONCLUSION

The high detection rate of carbapenemase-producing bacteria in this study shows the importance of the cultures surveillance. Its application in the detection of patients colonized by multiresistant microorganisms, even if they do not present clinical symptoms of infection, may contribute to the adoption and/or reinforcement of practices aimed to patients safety.

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COLLABORATIONS

Almeida KRH, Silva NS and Rocha IV collaborated in the collection and processing of the samples, data analysis and article construction. Araújo AA and Silva LM collaborated in the critical analysis of the data

and final revision of the article. Oliveira SR collaborated in the supervision of the work, analysis of the data and final revision of the article.

INTEREST CONFLICTS

There are no conflicts of interest to report

CORRESPONDENCE

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