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EXTENDED-SPECTRUM β-LACTAMASES PRODUCING *KLEBSIELLA PNEUMONIAE* ISOLATED IN TWO HOSPITALS IN GOIÂNIA/BRAZIL: DETECTION, PREVALENCE, ANTIMICROBIAL SUSCEPTIBILITY AND MOLECULAR TYPING

Daniella Fabíola dos Santos[1]; Fabiana Cristina Pimenta[2]; Rodrigo Alves[1]; Edlaine Rodrigues Montalvão[1]; Daniela Braz dos Santos[1]; José Rodrigues do Carmo Filho[1]*

[1]Universidade Católica de Goiás, Goiânia, GO, Brasil; [2]Universidade Federal de Goiás, Goiânia, GO, Brasil

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ABSTRACT

This study was developed to evaluate the prevalence of extended-spectrum β-lactamases (ESBL) producing *Klebsiella pneumoniae* in two hospitals (A and B) in Goiânia, GO, Brazil. The antimicrobial susceptibility of the isolates was determined using the MicroScan WalkAway (Dade Behring, USA). Tests to evaluate the genetic correlation between the isolates were also performed. For the ESBL phenotypic test, the Double-disk diffusion (DD) method was used. The strains isolated in Hospital B were submitted to DNA analysis by pulsed-field gel electrophoresis (PFGE). The study showed high prevalence of ESBL-producing *K. pneumoniae* (25% in hospital A and 66.7% in hospital B), with high rates of antimicrobial resistance. The most active compound was imipenem (100% susceptibility *in vitro*). The PFGE test showed similiarity in five strains and variability in six strains.The high prevalence of ESBL-producing *Klebsiella* may be due to individual selection and to dissemination of a common strain.

Key words: Extended-spectrum β-lactamases (ESBL), *Klebsiella pneumoniae,* β-lactamases, bacterial resistance.

INTRODUCTION

Antimicrobial resistance has increased dramatically in both nosocomial and community settings (10). β-lactam antibiotics are the most widely used group of antimicrobial agents. The most common mechanism of resistance among gram-negative pathogens to β-lactam involves the synthesis of β-lactamases, especially extended-spectrum β-lactamases (ESBL). The emergence of ESBL-producing organisms is serious concern worldwide and it is increasingly related to nosocomial infections. It is particularly important in *Klebsiella pneumoniae* and *Escherichia coli*, which are able to produce large variety of these enzymes (4). ESBL are able to hydrolyse extended-spectrum cephalosporins and monobactams, but are inhibited by β- lactamases inhibitors. The ESBL-producing microoganisms can transmit this plasmid-mediated resistance to other species (3,9). ESBL-producing pathogens represent an important cause of morbidity, mortality, appearance of multidrug resistant outbreaks and increase in the hospital costs (13,25). The treatment of infections caused by ESBL-producing strains is a concern because few antimicrobial agents remain active against them. Even though the infections caused by the ESBL-producing strains are more often identified in Intensive Care Units (ICU) patients, they may be present in other hospital settings and even in communitary infections (1,14). Their prevalence is probably higher than recognized, because they are not readily detected by routine susceptibility tests used in clinical microbiology laboratories (11,27,32).

The spread of these multi-resistant microorganisms is associated to the selective pressure caused by the overuse of broad spectrum cephalosporins, mainly ceftazidime. The ESBL production is also associated to cross-resistance to other classes of drugs, leading to therapeutic failures (14,17). A better undestanding of the dissemination of bacterial resistance to antimicrobial agents is necessary to control the problem. This report focuses on the prevalence, susceptibility profile and

*Corresponding Author. Mailing address: Universidade Católica de Goiás. Rua 232 nº 128 - área V - 3º andar Setor Universitário CEP 74605-140. Goiânia, GO, Brasil. Tel.: (62) 3946- 1346. Fax (62) 3946-1114. E-mail: biomedico47@gmail.com

evaluation of the genetic correlation of ESBL-producing *K. pneumoniae* isolates, obtained from hospitalized patients in two hospitals in Goiânia/Brazil.

MATERIALAND METHODS

Bacterial strains: A total of 61 isolates were identified as *Klebsiella* spp., being 54 *Klebsiella pneumoniae* (88.5%) and 7 *Klebsiella oxytoca* (11.5%). The isolates were collected from hospitalized patients in two hospitals in Goiânia/Brazil, between January 2005 and May 2006. Only one isolate per patient was selected for the study. The strains were identified using the MicroScan WalkAway (Dade Behring, USA) in accordance to the manufacturer's instructions.

Susceptibility testing: Antimicrobial susceptibility testing was performed by the use of MicroScan Walkway (Dade Behring, USA) and the Neg-Urine Combo 32 panels. The strains were categorized as susceptible, intermediate or resistant, according to the Clinical and Laboratory Standards Institute (CLSI) breakpoints (15).

Confirmation phenotypes: The characterization of suspicious ESBL-producing isolates was performed according to the Clinical and Laboratory Standards Institute (CLSI) breakpoints (21). The confirmatory test was performed with 31 strains, using the Double-disk diffusion (DD) testing method, in which a disk containing amoxicillin/clavulanate was placed on the surface of the plate in a distance of 20mm from the disks containing the oxymino-β-lactam. An enhanced inhibition zone between any one of the β-lactam disks and the disk containing clavulanic acid was interpreted as a positive result.

Quality Control: *Escherichia coli* ATCC 25922 and *K. pneumoniae* ESBL ATCC700603.

DNA analysis: Selected multi-resistant isolates were tested by pulsed-field gel electrophoresis (PFGE) in order to evaluate the method of dissemination of ESBL-producing strains in hospital B in Goiânia. Macrorestriction analysis of chromossomal DNA was performed by PFGE in accordance to the published procedures (19) with Xba I (New England Biolabs, Boston, Mass.). PFGE was run in a CHEF-DR II apparatus (Bio-Rad Laboratories, Richmond, California) with pulses ranging from 5 to 60 seconds at a voltage of 6V/cm for 23 hours at 14ºC for 20 hours. Lambda ladder (48.5 Kb, Sigma) was used as molecular weight marker. Fragments were stained with ethidium bromide (Sigma) and photographed. Visual comparisons were made and the criteria of Pfaller (19) were used to establish the relationship among the isolates.

RESULTS

Forty *Klebsiella* spp. strains were isolated in Hospital A (65.6%) and 21 in hospital B (34.4%). The largest number of isolates was obtained in urinary tract infections (35 cases), followed by bloodstream (17 cases), respiratory tract (6 cases), marrow (1 case), pleural liquid (1 case) and wound infections (1 case). Table 1 compares the susceptibility test results for ESBL and non-ESBL-producing *K. pneumoniae* isolates. The susceptibility patterns of penicillins/β-lactamases inhibitors, cephalosporins and fluoroquinolones were higher among non-ESBL *K. pneumoniae* than among the ESBL-producing strains. Only imipenem was active against all ESBL- *Klebsiella* isolates. Activity of fluoroquinolones against the ESBL-producing strains was low (45.8%).

The prevalence of ESBL-producing *K. pneumoniae* among *Klebsiella* spp isolates collected was 25.0% in hospital A and 66.7% in Hospital B.

The DNA analysis was performed in 14 ESBL-producing strains collected from Hospital B, as the their prevalence in this hospital was higher than the national average (42%). A great variety of profiles was observed in five (35.7%) strains but six (42.9%) strains displayed similar macrorestriction patterns (Fig. 1). Three strains (21.4%) did not present PFGE resolution pattern, even after the test was redone.

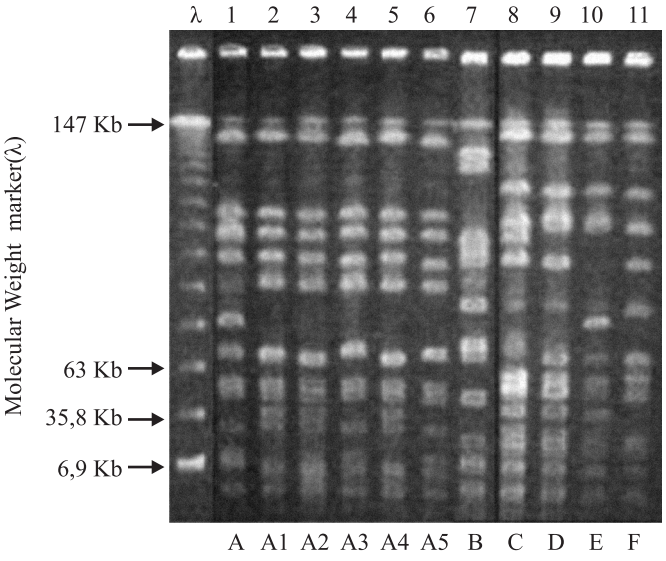

Figure 1. Macrorestriction analysis of DNA by PFGE of 11 ESBL *K. pneumoniae* isolates (column – isolate number): λ - molecular weight marker (λ = 48.5Kb) , 1- 206, 2 -227, 3 - 288, 4 - 314, 5 - 315, 6 - 350, 7 - 365, 8 - 381,9 - 416, 11 - 455, 12 - 567.

DISCUSSION

In general, the isolates presented high rates of resistance to different classes of antimicrobials, including cross-resistance to other classes of drugs. The ESBL-producing *K. pneumoniae* isolates showed higher resistance rates than the non-ESBL-

Table 1. *In vitro* susceptibility profile of ESBL and non-ESBL *Klebsiella pneumoniae* isolates collected in two hospitals in Goiânia from January 2005 to May 2006.

Antimicrobials	Non-ESBL strains N=30 (55.5%)			ESBL strains N=24 (44.5%)		
	S N (%)	I N (%)	R N (%)	S N (%)	I N (%)	R N (%)
Ampicillin	2(6.7)	8(26.7)	20(66.7)	-	-	24(100.0)
Ampicillin/sulbactam	27(90.0)	1(3.3)	2(6.7)	3(12.5)	-	21(87.5)
Piperacillin	20(66.7)	2(6.7)	8(26.7)	2(8.3)	1(4.2)	21(87.5)
Piperacillin -tazobactam	24(80.0)	1(3.3)	5(16.7)	11(45.8)	2(8.4)	11(45.8)
Amoxicillin-clavulanate	28(93.3)	-	2(6.7)	13(54.2)	11(45.8)	-
Ticarcillin-clavulanate	1(3.3)	28(93.3)	1(2.8)	5(20.8)	8(33.3)	11(45.8)
Cefazolin	24(80.0)	1(3.3)	5(16.7)	3(12.5)	-	21(87.5)
Cefalotin	22(73.3)	2(6.7)	6(20.0)	2(8.4)	-	22(91.7)
Cefuroxime	29(96.7)	-	1(3.3)	4(16.7)	-	20(83.3)
Cefpodoxime	30 (100.0)	-	-	-	-	24(100.0)
Ceftriaxone	29(96.7)	-	1(3.3)	5(20.8)	-	19(79.2)
Cefotaxime	26(86.7)	-	4(13.3)	3(12.5)	-	21(87.5)
Ceftazidime	27(90.0)	-	3(10.0)	6(25.0)	-	18(75.0)
Cefepime	26(86.7)	1(3.3)	3(10.0)	2(8.4)	2(8.4)	20(83.3)
Aztreonam	30 (100.0)	-	-	6(25.0)	-	18(75.0)
Imipenem	30(100.0)	-	-	24(100.0)	-	-
Amikacin	27(90.0)	-	3(10.0)	11(45.8)	6(25.0)	7(29.2)
Gentamicin	26(86.7)	1(3.3)	3(10.0)	7(29.2)	-	17(70.8)
Tobramycin	28(93.3)	-	2(6.7)	7(29.2)	-	17(70.8)
Levofloxacin	28(93.3)	2(6.7)	-	11(45.8)	1(4.2)	12(50.0)
Ciprofloxacin	25(83.3)	1(3.3)	4(13.3)	11(45.8)	2(8.4)	11(45.8)
Gatifloxacin	22 (73.3)	4(13.3)	4(13.3)	11(45.8)	1(4.2)	12(50.0)
Trimethoprim/Sulphametho-xazol	20(66.7)	-	10(33.3)	-	-	24(100.0)

producing ones. The broad spectrum antimicrobial resistance rates were similar to the ones identified by SENTRY - 1998 in other Brazilian hospitals located in Rio de Janeiro, Florianópolis and São Paulo (7,23). Brazilian rates are much higher than in most other parts of the world (29).

The combination of penicillins/ β-lactamases inhibitors resulted in low activity against ESBL-producing isolates, suggesting that other resistance mechanisms may be involved (4,16). Nevertheless, there are cases of successful treatments using a combination of penicillin/β-lactamases inhibitor, mainly piperacillin/tazobactam. These combinations may reduce the selective pressure and hinder the appearance and spread of resistant strains (12,17,22,24).

Imipenem was the most active drug against ESBL-producing isolates. This antimicrobial can easily enter the bacterial cell and is resistant to ESBL hydrolysis (5,16). However, its use must be based on results of the susceptibility test, because there are reports of carbapenems resistant *Klebsiella* spp (9,30).

In this study, the activity of cefepime against ESBL-producing *Klebsiella* was much lower than that reported in another Brazilian study (9). *K. pneumoniae* can become more resistant to this drug when another resistance mechanism is associated, as porin mutant (25). In this case, *K. pneumoniae* bacteremia treatment with cefepime may fail (29).

Some Brazilian studies have shown that fluoroquinolones are alternative drugs for treatment of ESBL-producing *K. pneumoniae* infections (9,13,24). However, this drug class was not active against the ESBL-producing isolates of the present study. This can be explained by resistance mechanism associated to alterations in target enzymes (DNA gyrase/ topoisomerase IV) or to impaired access to the target enzymes due to changes in porin expression (9,13,24).

The prevalence of ESBL-producing strains in increasing in hospital settings all over the world. Studies have demonstrated that the prevalence of ESBL-producing microorganisms in Brazil is higher than in other parts of the world (6,23). These

microorganisms are multidrug resistant and there is evidence that they are emerging and spreading in the community. These infections are also associated to extremely high mortality (2,13,18,25).

In this study, the prevalences of ESBL-strains in Hospital A and B were 25% and 67.7%, respectively. These differences in prevalence may be due to innadequate collection of clinical material and to failures in isolation or identification of the isolates. In addition, routine tests may be mislead, requiring expensive complementary tests (25,31). The affinity of ESBL-producing isolates to the different substrates is variable and makes their detection difficult (20). As a consequence**,** the prevalence of ESBL-producing microorganisms may be underestimated (11,27).

In order to evaluate the routes of dissemination of ESBL-producing *K. pneumoniae*, the macrorestriction of DNA by PFGE was performed. Results indicated clonal correlation among six strains and genotype diversity among five strains. *K. pneumoniae* infections may be either clonal or multiclonal (19). The clonal relation indicates transmission of a common strain among patients and the multiclonal infection indicates a selection of resistant strains.

In the treatment of ESBL- producing *K. pneumoniae* infections, some actions in order to modify the antimicrobial use in hospitals and implementation of antimicrobial surveillance programs are required. The criteria to establish the rational antimicrobial use must be based on the patient's clinical features and on the isolates epidemiological profile. Infection control measures, such as surveillance antimicrobial resistant strains and isolation and barriers to either colonized or infected patients, periodic distribution of data about bacterial susceptibility, educational campaigns, restriction of use of broad spectrum antimicrobials, mainly cephalosporins (ceftazidime), antimicrobial cycling and combined antimicrobial therapy are very important (22,26,28). This study emphasizes the importance of developing appropriate detection methods and the need of continuous preventive measures to control infections, mainly those caused by multi-resistant pathogens.

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RESUMO

Klebsiella pneumoniae produtoras de β-lactamases de espectro ampliado isoladas em dois Hospitais em Goiânia/Brasil: Detecção, Prevalência, Suscetibilidade Antimicrobiana e Tipagem Molecular

Este estudo foi desenvolvido para avaliar a prevalência de *Klebsiella pneumoniae* produtoras de ESBL em dois hospitais (A e B) de Goiânia, GO, Brasil. Os isolados foram analisados quanto à resistência a antibióticos através do MicroScan WalkAway (Dade Behring, USA). A correlação genética entre isolados produtores de ESBL também foi avaliada. O teste fenotípico para ESBL foi realizado através de teste de Disco de Difusão Dupla (DD) e a análise do DNA de *K. pneumoniae* produtoras de ESBL, isoladas no Hospital B, realizada por *Pulsed Field Gel Electrophoresis* (PFGE). O estudo demonstrou alta prevalência de *K. pneumoniae* produtora de ESBL (25,0% no hospital A e 66,7% no hospital B) com altas taxas de resistência aos antimicrobianos. O composto mais ativo foi o imipenem (100% de sensibilidade *in vitro*). O PFGE mostrou similaridade em cinco isolados e variabilidade em seis isolados. A alta prevalência *Klebsiella* produtora de ESBL pode ser devida à seleção individual e à disseminação de uma cepa comum.

Palavras chaves: Extended-spectrum β-lactamases (ESBL), *Klebsiella pneumoniae,* β-lactamases, resistência bacteriana.

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