

Full Length Research Paper

Prevalence of resistance and molecular characterization of extended spectrum beta-lactamase (ESBL)-producing bacteria isolated in a hospital in Southern Brazil

Geórgia Muccillo Dexheimer*, Johan Prediger, Luciana Weidlich and Adriane Pozzobon

Univates. 171 Avelino Tallini st, Universitário, Zip Code: 95900-000 - Lajeado, Rio Grande do Sul - Brazil.

Received 16 December, 2014; Accepted 2 February, 2015

Infectious diseases are a major cause of morbidity worldwide, and the indiscriminate use of antibiotics favors the development of bacterial resistance mechanisms, making it difficult to control infection. This study aimed to determine the prevalence of bacterial infection, their antibiotic resistance genes and extended spectrum beta-lactamase (ESBL)-producing genes. It was divided into two stages. The first stage was a retrospective analysis of infections by multidrug-resistant bacteria in a hospital, from January 2011 to June 2012. The most prevalent isolates were selected for verification of ESBL-producing related genes. The analysis of 374 samples showed that tracheal aspirates showed the highest infection rate, followed by urine and sputum. The most prevalent bacteria were *Acinetobacter* sp., *Klebsiella* sp., *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Bacteria resistant to a large number of antibiotics were *Klebsiella* sp., *Acinetobacter* sp., *Enterobacter* sp., *Escherichia coli*, *Serratia* sp., *Pseudomonas* sp. and *S. aureus*. Of the 62 isolates, 43.54% were classified as ESBL-producing. The second stage was molecular analysis, which showed that TEM gene was present in 70.96, 56.45% for SHV and 90.32% for CTX-M gene. As for CTX family groups, 22.58% were CTX-M1, 14.51% CTX-M2 and 24.19% CTX-M9. This study showed a high incidence of infection caused by multidrug-resistant bacteria and a high prevalence of CTX-M and TEM genes.

Key words: Drug resistance, antibiotics, bacterial infection, nosocomial infection, hospitals, beta-lactamases.

INTRODUCTION

Infectious diseases are a serious health problem, being one of the main causes of morbidity worldwide. After extensive and indiscriminate use of antibiotics, bacteria have developed resistance mechanisms, making it

difficult to control infections (Ahluwalia and Sharma, 2007). It is estimated that 17 to 20 million people die worldwide each year of infectious diseases. In addition, about 10 million hospital-acquired infections occur each year,

*Corresponding author. E-mail: georgiamuccillo@gmail.com. Tel: +55 51 3714-7000.

resulting in approximately 300,000 deaths (Ferrareze et al., 2007). The risk of nosocomial infection is related to the patient's length of stay in hospital, the patient's immunological and nutritional status, patient's age, the use of immunosuppressants and antibiotics and invasive procedures (Ferrareze et al., 2007; Santos, 2004).

In addition to the vulnerable state of patients, the conduct of health professionals is an important issue in the increase of nosocomial infection. The inappropriate use of resources for diagnosis and treatment can lead to increased risk of infections (Andrade et al., 2006).

The acquisition of resistance has a variety of mechanisms: presence of enzymes capable of disabling the antimicrobial agent; presence of an alternative enzyme for the enzyme inhibited by the antimicrobial agent; occurrence of a mutation in the target for the antimicrobial agent, affecting the connection between the target and antimicrobial agent. Furthermore, there may be a post-transcriptional or post-translational modification of the target of the antimicrobial agent, as well as active efflux of the antimicrobial agent or an overproduction of the antimicrobial agent targets (Fluit et al., 2001).

Outbreaks of nosocomial infections have been caused by *Pseudomonas aeruginosa* and *Acinetobacter* spp. worldwide (Cortes et al., 2009; Hosoglu et al., 2012), and, in Brazil, multidrug resistance of these isolates is likewise common (Rossi, 2011). Multidrug-resistant *P. aeruginosa*, for example, is one of the main causes of pneumonia related to nosocomial infection in various intensive care units (ICUs) in Brazil (Gales et al., 2003). *Acinetobacter baumannii* has emerged as an important pathogen in hospital settings related to pneumonia, mechanical ventilation, bacteremia, urinary tract infection and other infections related to large outbreaks since 1996 (Levin et al., 1996).

Extended spectrum beta-lactamase (ESBL) production is the most important mechanism of resistance to penicillin, cephalosporin and monobactam employed by Gram-negative bacteria (Sekar and Shanthi, 2009). ESBL is able to hydrolyse third and fourth generations of cephalosporins and aztreonam, and is inactivated by specific inhibitors, such as clavulanic acid, sulbactam and tazobactam (Silva and Lincopan, 2012). The beta-lactamase hydrolyzes the structure of the beta-lactam ring of the antibiotic, inactivating it (Tissera and Lee, 2013).

Generally, ESBL is derived from genes encoded as CTX-M, TEM-1, TEM-2, SHV-1 or mutations that allow a change of amino acids around the active site of beta-lactamases (Tissera and Lee, 2013; Paterson and Bonoma, 2005). For the most part, ESBL production is encoded in plasmid, and thus can be easily transferred between bacteria and hospitalized patients (Bradford, 2001). This is an important factor for the large increase and dissemination of ESBL producers. The identification of ESBL-producing bacteria and the correct prescription of antibiotics are essential to prevent their spread

(Moghaddam et al., 2012). TEM, SHV and CTX-M genes are most commonly found in Enterobacteriaceae, *Escherichia coli*, *Klebsiella pneumoniae*, *P. aeruginosa*, *Enterobacter* and *Acinetobacter baumannii* (Sekar and Shanthi, 2009).

Detection of TEM, SHV and CTX-M genes by molecular analysis in ESBL-producing bacteria and their resistance to antibiotics can contribute to information about their epidemiology and also help in the selection of the most appropriate antimicrobial therapy (Kaur and Aggarwal, 2013).

The first aim of this study was to determine the prevalence of bacterial infection and its resistance and multidrug resistance to antibiotics in a medium-sized hospital. The second aim of this study was to verify the presence of TEM, SHV and CTX-M genes, as well as the genes belonging to families CTX-M1, CTX-M2 and CTX-M9, related to bacterial resistance.

MATERIALS AND METHODS

This study was divided into two phases. The first phase consisted of a cross-sectional study based on retrospective analysis of antibiotic-resistant bacterial infections, in a medium-sized hospital in the interior of Rio Grande do Sul, in Southern Brazil, between January of 2011 and June of 2012. Data were collected from the Infection Control Service database of the hospital. The second phase entailed a prospective analysis of the most prevalent resistant isolates found in the first phase of the study. Samples were collected from June of 2012 to March of 2013 for the genotypic characterization through research of genes TEM, SHV and CTX-M. The bacteria used in the study were chosen according to the potential for ESBL-producing and involvement of patients during the collection period, not being possible to collect some potentially ESBL-producing species such as *Acinetobacter* sp. The bacteria studied were *Klebsiella* spp., *Escherichia coli*, *Pseudomonas* spp. and *Enterobacter* spp. due to their greater potential for ESBL production and involvement in patients in the collection period.

The bacteria in the study were collected from a group of hospitalized patients, who had procedures at the hospital, after their assessment and diagnosis. Most of the sampling sites were tracheal aspirate, surgical wound and urine, followed by sputum and blood cultures. The isolation and identification of bacteria were performed by a third-party laboratory, using the method of standardized susceptibility testing by the Clinical and Laboratory Standards Institute (CLSI, 2013). The disc diffusion method was used for antimicrobial susceptibility assessment, where a disc impregnated with an antimicrobial is placed on the surface of pre-seeded agar. The plates are incubated for 18 to 24 h at a temperature of 35°C, after which the inhibition zones of growth are measured.

For characterization of ESBL-producing bacteria, the method of approach discs was used. This method consists of bacteria suspension inoculation, adjusted to the standard 0.5 of the McFarland grade in MullerHinton agar. Amoxicillin and clavulanic acid discs were placed in the center of the plate. The antimicrobials aztreonam-ATM, cefotaxime-CTX, ceftazidime-CAZ and cefepime-CPM were positioned near the center disc, followed by incubation of the plate for 18 to 20 h at 35°C. After this, the researcher observed if there was a third zone between the clavulanic acid and a marker disc substrate, confirming production of ESBL (Jarlier et al., 1988).

In the method of approach discs, cephalosporins discs were placed at 30 mm from the disk with antibiotic inhibitor (clavulanate, tazobactam and sulbactam). In the case of synergism between the substrate and inhibitor, the production of ESBL was confirmed (Sekar and Shanthi, 2009).

TEM, SHV, CTX-M, CTX-M1, CTX-M2 and CTX-M9 genes analysis

The isolated microorganisms were maintained in a skim milk medium (distilled water and milk powder), and then subculture was performed in the MacConkey Agar, a selective medium for Gram-negative bacteria. The cultivation time was 24 h at 37±1°C.

The bacterial DNA was isolated using the kit PureLink Genomic DNA kit (Invitrogen, Carlsbad, USA) following the manufacturer's protocol. After DNA extraction, polymerase chain reaction (PCR) was performed using specific primers for TEM, Forward 5'-ATA AAA TTC TTG AAG ACG AAA-3' and Reverse 5'-GAC AGT TAC CAA TGC TTA ATC A-3' (1080pb) (Yao et al., 2007); SHV Forward 5'-GGG TTA GTC TTC TTT TTA GC-3' and Reverse 5'-TTA GGC TTG CTC CCA GTG-3' (930 bp) (Jain and Mondal, 2008), CTX-M1 Forward 5'-AAA CAC AAT TGC AGT GCC TC-3' and Reverse 5'-AGC TTA TTC ATC GCC ACG TT-3' (415 bp), CTX-M2 Forward 5'-CGA CGC TAC CCC TGC TAT T -3' and Reverse 5'-TCA CCA GCG TCA GAT TTT GG-3' (552 bp), CTX-M9 Forward 5'-AAC TGC AGA GAG GGA AAC TG-3' and Reverse 5'-GGA ATT TCA CAA CGT TCA CC-3' (205 bp) (Woodford et al., 2006), CTX-M Forward 5'-ATA GCT TTG GGC ACC TG-3' and Reverse 5'-CGC GGC ATG TTT TGC AG-3' (550 pb) (Ahmed et al., 2004).

The reaction conditions for TEM were 94°C for 5 min followed by 35 cycles of 94°C for 1 min, 52°C for 1 min and 72°C for 1 min. The final extension was at 72°C for 10 min. For SHV were 35 cycles of 94°C for 30 s, 55°C for 40 s and 72°C for 40 s. The final extension was 72°C for 10 min. CTX-M1 were 37 cycles of 94°C for 1 min, 49°C for 50 s and 72°C for 60 s. The final extension was 72°C for 6 min. CTX-M2 were 37 cycles of 94°C for 60 s, 49°C for 50 s and 72°C for 1 min. The final extension was 72°C for 6 min. CTX-M9 were 35 cycles of 94°C for 30 s, 50°C for 40 s and 72°C for 50 seconds. The final extension was 72°C for 6 min. CTX-M were 38 cycles of 94°C for 45 s, 54°C for 45 s and 72°C for 60 s. The final extension was 72°C for 5 min.

PCR reactions were performed in a thermal cycler TC-512 (Techne® Caterpillar Scientific, Stone, Staffordshire, UK) at a final volume of 50 µL. PCR buffer was used (20 mM Tris HCl pH 8.4 and 50mM KCl), 1.5 mM MgCl₂, 1.25 U of Taq DNA polymerase, 50 pmole/µL of sense and antisense primers and 0.2 mM dNTP mix. The fragments were analyzed by 1.5% agarose gel stained with ethidium bromide, subjected to electrophoresis.

The study used strains for the standardization of PCR programs and compared the band size with the isolates at the time of agarose gels analysis. The strains used were: ATCC IOC.- Fiocruz CCBH 4955 *Klebsiella pneumoniae* ATCC and 700603.- Fiocruz CCBH 3858 *K. pneumoniae*.

Ethical considerations

This study was approved by the Ethics Committee of Univates (No. 335 646 of 17, July 2013) and by the Hospital's Center for Teaching and Research. The hospital name was reserved.

Statistical analysis

The sensitivity and specificity were analyzed by Chi-square test (χ^2)

and Fisher's exact test. Statistical analyzes were performed using Prism 5 software (Graphpad®, California, USA), using a significance level 5%.

This test used the results of the presence of the genes by PCR assay, coupled with the result of disc approximation method. The presence of either gene alone will result in the production of ESBL. The disc approximation test may yield false-negative results due to interaction between enzymes, or low ESBL production.

RESULTS

The retrospective analysis of 374 samples obtained from January of 2011 to July of 2012 showed that tracheal aspirates were the samples with the highest infection rate (70.85%), followed by urine (6.95%) and sputum (6.68%). The least contaminated sample was hematopoietic (0.27%). The most prevalent bacteria were *Acinetobacter* sp., *Klebsiella* sp., *Pseudomonas aeruginosa* and *S. aureus* (Table 1). The bacteria that were resistant to a large number of antibiotics were *Klebsiella* sp., *Acinetobacter* spp., *Enterobacter* spp., *E. coli*, *Serratia* sp., *P. aeruginosa* and *S. aureus* (Table 2).

For this study, 62 isolates were collected: *Klebsiella* spp., *E. coli*, *Pseudomonas* spp. and *Enterobacter* spp. The disc approximation test was conducted only for *Klebsiella* spp. and *E. coli*. The disc approximation test was not applied to isolates of *Enterobacter* spp. and *Pseudomonas* spp., because bacterial enzyme interactions affect the test results.

Of the 62 isolates, 27 were considered extended spectrum beta-lactamase (ESBL)-producing by the disc approximation test. The molecular characterization showed 56 isolates with CTX-M gene, 44 for TEM gene, 35 for SHV, 15 for CTX-M9, 14 for CTX-M1 and 9 for CTX-M2 (Table 3).

Statistical analysis compared the two methods of identification of ESBL production capacity. The PCR was used as a standard, so the effectiveness of the discs approximation technique was compared. Table 4 shows the results of specificity and sensitivity.

DISCUSSION

In this study, the most prevalent bacteria in our samples were *Acinetobacter* sp., *Klebsiella* sp., *P. aeruginosa* and *S. aureus*. The bacteria that showed the highest resistance to antibiotics were *Klebsiella* sp., *Acinetobacter* sp., *Enterobacter* sp., *E. coli*, *Serratia* sp., *Pseudomonas* sp. and *S. aureus*. In a retrospective study in Brazil by Andrade et al. in 2006, there was a higher frequency of *Staphylococcus* sp., followed by *S. aureus*, *Acinetobacter baumannii*, *P. aeruginosa* and *Klebsiella* sp. in samples from patients with nosocomial infection. The sample with the highest rate of infection in the Andrade et al. (2006) study was blood culture, followed by urine culture, catheter, cerebrospinal fluid and surgical wounds.

Table 1. Prevalence of microorganism as the sample.

MO	No.	Tracheal aspirate	Urine	Sputum	Sternal fragment	Hemato-poietic	Broncho-alveolar lavage	Catheter tip	Secretion wound sacrum	Surgical wound	Blood culture	Abdominal wall	Pleural fluid	Cerebro-spinal fluid
<i>Acinetobacter</i> sp.	73	62	1	4	-	-	1	3	1	-	-	-	1	-
<i>Enterobacter</i> sp.	17	12	1	1	1	-	-	-	-	1	1	-	-	-
<i>Enterococcus</i> sp.	3	-	3	-	-	-	-	-	-	-	-	-	-	-
<i>Escherichia coli</i>	23	9	9	-	-	-	-	-	1	2	1	1	-	-
<i>Haemophilus</i> sp.	12	9	-	1	-	-	-	-	-	2	-	-	-	-
<i>Klebsiella</i> sp.	59	38	3	7	-	-	1	2	1	1	2	1	3	-
<i>Proteus mirabilis</i>	15	7	5	-	-	1	-	-	-	-	1	1	-	-
<i>Proteus vulgaris</i>	3	2	-	1	-	-	-	-	-	-	-	-	-	-
<i>Pseudomonas aeruginosa</i>	43	34	-	3	-	-	2	2	-	1	-	1	-	-
<i>Pseudomonas</i> sp.	7	6	-	-	-	-	-	-	-	1	-	-	-	-
<i>Serratia</i> sp.	8	6	-	-	-	-	2	-	-	-	-	-	-	-
<i>Staphylococcus aureus</i>	39	33	2	1	1	-	-	-	-	1	1	-	-	-
<i>Staphylococcus</i> sp.	20	6	1	2	-	-	-	5	-	5	1	-	-	-
<i>Stenotrophomonas maltophilia</i>	33	28	-	2	-	-	-	-	-	1	1	1	-	-
<i>Streptococcus pneumoniae</i>	16	11	1	3	-	-	-	-	-	-	-	-	-	1
<i>Neisseria meningitidis</i>	1	-	-	-	-	-	-	-	-	-	-	-	-	1
<i>Bacilos gram negative nonfermenters</i>	2	2	-	-	-	-	-	-	-	-	-	-	-	-
Total	374	265	26	25	2	1	6	12	3	15	8	5	4	2
Total %	100	70.85	6.95	6.68	0.53	0.27	1.60	3.20	0.80	4.01	2.14	1.34	1.07	0.53

MO = Microorganism, No. = isolates number.

A research study conducted in São Paulo with patients in the ICU for treatment of burns found that the most frequently isolated bacteria from the bloodstream were *Staphylococcus* sp., *A. baumannii*, *Pseudomonas* sp. and *Enterobacter* sp. Moreover, *Staphylococcus* sp., *A. baumannii*

and *Pseudomonas* sp. had a high antibiotic resistance index (Millan et al., 2012). According to Pondei et al. (2013) microbial cultures isolated from wound swabs showed a higher frequency for Gram-negative bacilli. *P. aeruginosa* was the most commonly found bacterium with a high level of

resistance to the tested antibiotics.

A 2013 study of Brazilian pediatric patients under 16 years of age showed a higher frequency of infections caused by coagulase-negative *Staphylococcus*, *Klebsiella* spp, *S. aureus* and *Acinetobacter* spp. The bacteria that showed

Table 2. Resistance percentage of isolated bacteria in relation to the tested antibiotics.

Antibiotic	<i>Acinetobacter</i> sp.	<i>Enterobacter</i> sp.	<i>Escherichia</i> <i>coli</i>	<i>Pseudomonas</i> <i>aeruginosa</i>	<i>Serratia</i> sp.	<i>Klebsiella</i> sp.	<i>Staphylococcus</i> <i>aureus</i>
Amoxicilin/clavulanate	1.37	88.23	21.73	-	62.5	49.15	28.2
Cefepim	75.34	5.88	21.73	4.00	37.5	45.76	-
Ciprofloxacin	72.6	35.29	30.43	52.00	37.5	35.59	20.51
Tobramicin	57.53	-	-	-	-	-	-
Levofloxacin	71.23	-	-	-	-	-	-
Gentamicin	72.6	29.42	30.43	42.00	37.5	38.98	23.07
Piperacilin	71.23	-	-	12.00	-	-	-
Piperacilin/tazobactam	23.28	-	4.34	4.00	12.5	22.03	-
Ampicilin/sulbactam	19.17	-	-	-	-	-	-
Ceftazidim	57.53	29.42	21.73	2.00	37.5	47.45	-
Sulfametoxazol/trimetoprim	75.34	23.52	43.47	-	37.5	42.37	10.25
Nalidixic acid	-	5.88	8.69	-	-	5.08	-
Ampicilin	-	100	43.47	-	100.00	98.3	-
Amicacin	72.6	17.64	4.34	32.00	12.5	5.08	-
Cefalotin	-	94.11	26.08	-	100.00	64.4	-
Ceftriaxon	-	29.41	21.73	2.00	37.5	54.23	-
Cefuroxim	-	64.7	21.73	-	100.00	55.93	-
Norfloxacin	-	5.88	8.69	-	-	3.39	2.56
Cloranfenicol	-	-	-	-	-	-	5.12
Nitrofurazon	-	5.88	-	-	-	5.08	-
Perfloxacin	-	-	-	2.00	-	-	-
Aztreonam	-	-	-	14.00	-	-	-
Clindamicin	-	-	-	-	-	-	25.64
Eritromicin	-	-	-	-	-	-	46.15
Oxacilin	-	-	-	-	-	-	12.82
Ticarciclin	-	-	-	46.00	-	-	-
Imipenem	2.74	-	-	-	-	-	-
Meropenem	1.37	-	-	-	-	-	-

Table 3. Bacteria classification of the ESBL-producing, the presence of different genes and sample site.

Bacteria	N	ESBL	TEM	SHV	CTX-M	CTX-M1	CTX-M2	CTX-M9	Sample site
<i>Klebsiella</i> spp.	35	19	27	23	32	9	6	4	Tracheal aspirate, surgical wound and urine
<i>Escherichia coli</i>	24	8	16	10	21	4	3	11	Urine and surgical wound
<i>Pseudomonas</i> spp.	2	ND	1	2	2	1	0	0	Tracheal aspirate and surgical wound
<i>Enterobacter</i> spp.	1	ND	0	0	1	0	0	0	Tracheal aspirate
Total	62	27	44	35	56	14	9	15	-
%	100	43.55	70.96	56.45	90.32	22.58	14.51	24.19	-

N= number of isolated, ND = not done.

higher antimicrobial resistance indices were *S. aureus*, *P. aeruginosa* and *Klebsiella* spp. (Pereira et al., 2013), a result comparable to results obtained in this research. Recent research in Cuba showed that in tracheal aspirate samples, the most common bacterium was *A. baumannii*,

followed by *Pseudomonas* sp., *Serratia*, *Klebsiella* and *E. coli* (Medell et al., 2013).

In this study, nearly half of the bacterial isolates were classified as ESBL-producing, with the most frequent bacteria *Klebsiella* sp. followed by *E. coli*. Most ESBL-

Table 4. Sensitivity and specificity results of the comparison of the PCR technique and the discs approximation technique.

Gene	Sensitivity	Specificity
TEM	0.8519	0.4000 (p= p=0.0470)
SHV	0.4571	0.5926 (p=0.9262)
CTX-M1	0.4667	0.5745 (p=0.9616)
CTX-M2	0.5556	0.5849 (p=0.7344)
CTX-M9	0.3571	0.5417 (p=0.7979)
CTX-M	0.4464	0.6667 (p=0.8685)

producing bacteria are classified in the family *Enterobacteriaceae*, particularly *Klebsiella* spp, *E. coli*, and *Enterobacter* spp. (Tissera and Lee, 2013). A study in Saudi Arabia noted that *K. pneumoniae* was the ESBL-producing species most commonly found in hospitalized patients (Hassan et al., 2013).

This research assessed the presence of genes related to ESBL production by PCR. The results indicate that the CTX-M gene was the most prevalent followed by TEM gene, and were compared with the study by Tissera and Lee (2013), who used the multiplex PCR technique for genotypic characterization of bacteria and found a higher prevalence of CTX-M gene, followed by TEM gene. Another study showed a higher prevalence of CTX-M gene (94.6%), followed by TEM gene (56.8%) in the same bacterial genus (Moghaddam et al., 2012). Anago et al. (2015) found that TEM gene was most prevalent in ESBL and non ESBL isolates, with 72.4 and 80%, respectively. SHV occurred in 24.1% ESBL strains and in 18.1% of non ESBL isolates.

The presence of the gene does not ensure the production of ESBL, because the gene can be silenced, or may produce some other enzyme inhibiting the production of ESBL, such as cAMP enzyme. However, it is important to investigate these genes, due to the ease of transmission between bacteria by plasmids. Thus, the production of ESBL may be an aggravating factor in conjunction with other types of multi-resistance (Bradford, 2001).

The production of beta-lactamases is the most important mechanism of resistance to beta-lactam antibiotics in Gram-negative organisms. These enzymes limit hospital treatment. They are mainly present in the family *Enterobacteriaceae* (Hernández et al., 2003). The high frequency of multi-resistant bacteria containing CTX-M gene may be the result of a coexistence in the same plasmid of CTX-M genes with genes that confer resistance to other classes of antibiotics (Cantón and Coque, 2006).

It should be noted that there is the possibility of detecting these genes in bacteria not characterized as ESBL-producing, as seen in the present study. According to Hassan et al. (2013), the gene has been detected in

49% of all ESBL-producing bacteria, but not all enzymes encoded by the TEM gene may be considered ESBL, because the presence of this gene alone was also associated with non-confirmed strains of ESBL. This can be explained by the presence of the enzyme inhibitor-resistant TEM or the presence of other enzymes encoded by the same gene (Murnier et al., 2010).

Currently, ESBLs represent the largest group of beta-lactamases studied worldwide and have been the subject of extensive microbiological, biochemical, genetic and epidemiological investigations. The increased use of broad-spectrum antibiotics and the high incidence of ESBL-producing bacteria have worried hospital infection control teams. Hence, the molecular characterization of these microorganisms can contribute to their identification and prevent hospital infection outbreaks. Several techniques have been proposed to identify ESBL production, but most effort is directed to the detection of bacteria that have low beta-lactamase activity with chromosomal origin, such as AmpC enzymes, which mask the presence of ESBL. This fact hinders the establishment of a gold standard of identification, because we can have false-positive or false-negative results; therefore, a combination of tests is recommended (Filho et al., 2003).

Various methods of molecular typing of bacterial pathogens and the study of nosocomial infections have been used and described as effective for molecular typing of pathogens belonging to the family *Enterobacteriaceae* and others (Singh et al., 2006). For molecular typing of *K. pneumoniae*, the Pulsed field gel electrophoresis (PFGE) technique is considered the gold standard due to its excellent discriminatory capacity (Pfaller and Segreti, 2006). However, PFGE requires several days for its execution, is expensive, and cannot be used for some strains due to DNA degradation. As an alternative to PFGE, PCR-based methods have been widely used (Silbert et al., 2003).

Conclusion

This study demonstrated the high incidence of nosocomial infection by resistant bacteria, in addition to the high prevalence of TEM gene associated with the presence of ESBL. Even with existing biochemical methods for the identification of resistant bacteria, the best method for the detection and surveillance of ESBL production by micro-organisms has not yet been determined.

Measures such as hand washing, proper use of laboratory coats and awareness of the prudent use of antibiotics are also important factors in this control of hospital infection. Furthermore, studies that assess genotypic diversity through resistance gene analysis can be useful in the identification of these micro-organisms as well as the choice of appropriate treatment.

Research to assess the presence of other genes

related to the production of ESBL may achieve a more complete profile of the bacteria studied, and thus contribute to the understanding of resistance mechanisms.

Conflict of interests

The authors did not declare any conflict of interest.

REFERENCES

- Ahluwalia G, Sharma SK (2007). Philanthropy and medical science: at last a new dawn for tuberculosis also. *Indian J. Chest Dis. Allied Sci.* 49:71-73.
- Ahmed AM, Nakano H, Shimamoto T (2004). The first characterization of extended-spectrum β -lactamase-producing *Salmonella* in Japan. *J. Antimicrob. Chemother.* 54:283-284.
- Anago E, Ayi-Fanou L, Akpovi CD, Hounkpe WB, Tchiboza MA, Bankole HS, Sanni A (2015). Antibiotic resistance and genotype of beta-lactamase producing *Escherichia coli* in nosocomial infections in Cotonou, Benin. *Ann. Clin. Microbiol. Antimicrob.* 14(1):5.
- Andrade D, Leopoldo VC, Haas VJ (2006). Multiresistant bacteria infections in Intensive Care Center Emergency Hospital. *Rev. Bras. Ter Intensiva.* 18(1):27-33.
- Bradford PA (2001). Extended spectrum beta-lactamases in the 21st century: characterization, epidemiology and the detection of this important resistance threat. *Clin. Microbiol. Rev.* 14:933-951.
- Cantón R, Coque TM (2006). The CTX-M β -lactamase pandemic. *Curr. Opin. Microbiol.* 9:1-10.
- CLSI (Clinical and Laboratory Standards Institute) (2013). Performance Standards for Antimicrobial Susceptibility Testing. M100-S23. Wayne, PA, USA.
- Cortes JA, Cuervo SI, Urdaneta AM, Potdevin G, Arroyo P, Bermúdez D, Correa A, Villegas MV (2009). Identifying and controlling a multiresistant *Pseudomonas aeruginosa* outbreak in a Latin-American cancer centre and its associated risk factors. *Braz. J. Infect. Dis.* 13(2):99-103.
- Ferrareze MVG, Leopoldo VC, Andrade D, Silva MFI, Haas VJ (2007). Multiresistant *Pseudomonas aeruginosa* in intensive care unit: challenges preceding? *Acta Paul Enferm.* 20(1):7-11.
- Filho JRC, Pereira AS, Sader HS, Tognim B (2003). Evaluation of the accuracy of laboratory tests for the detection of *Klebsiella pneumoniae* producing extended-spectrum beta-lactamase. *J. Bras. Patol. Med. Lab.* 39:4.
- Fluit AC, Visser MR, Schmitz FJ (2001). Molecular detection of Antimicrobial Resistance. *Clin. Microbiol. Rev.* 14 (4):836-871.
- Gales AC, Menezes LC, Silbert S, Sader HS (2003) Dissemination in distinct Brazilian regions of an epidemic carbapenem-resistant *Pseudomonas aeruginosa* producing SPM metallo-beta-lactamase. *J. Antimicrob. Chemother.* 52:699-702.
- Hassan MI, Alkharsah KR, Alzahrani, AJ, Obeid OE, Khamis AH, Diab A (2013). Detection of extended spectrum beta-lactamases-producing isolates and effect of AmpC overlapping. *J. Infect. Dev. Ctries.* 7(8):618-629.
- Hernández JR, Pascual A, Cantón R, Martínez-Martínez L (2003). *Escherichia coli* y *Klebsiella pneumoniae* productores de betalactamasas de espectro extendido em hospitales españoles. *Enferm. Infecc. Microbiol. Clin.* 21:77-82.
- Hosoglu S, Hascuhadar M, Yasar E, Uslu S, Aldudak B (2012). Control of an *Acinetobacter baumannii* outbreak in a neonatal ICU without suspension of service: a devastating outbreak in Diyarbakir, Turkey. *Infection* 40(1):11-18.
- Jain A, Mondal R (2008). TEM & SHV genes in extended spectrum beta-lactamase producing *Klebsiella* species beta their antimicrobial resistance pattern. *Indian J. Med. Res.* 128(6):759-764.
- Jarlier V, Nicolas G, Fournier A, Philippon A (1988). Extended broadspectrum β -lactamases conferring transferable resistance to newer β -lactam agents in Enterobacteriaceae: hospital prevalence and susceptibility patterns. *Rev. Infect. Dis.* 10:867-878.
- Kaur M, Aggarwal A (2013). Occurrence of the CTX-M, SHV and the TEM Genes Among the Extended Spectrum β -Lactamase Producing Isolates of Enterobacteriaceae in a Tertiary Care Hospital of North India. *J. Clin. Diagn. Res.* 7(4):642-645.
- Levin AS, Mendes CM, Sinto SI, Sader HS, Scarpitta CR, Rodrigues E, Saaia N, Boulos M (1996). An outbreak of multiresistant *Acinetobacter baumannii* in a university hospital in Sao Paulo, Brazil. *Infect. Control Hosp. Epidemiol.* 17:366-368.
- Medell M, Hart M, Duquesne A, Espinosa F, Valdés R (2013). Nosocomial ventilator-associated pneumonia in Cuban intensive care units: bacterial species and antibiotic resistance. *MEDICC Rev.* 15(2):26-29.
- Millan LS, Benedette CEM, Maximo LZ, Almeida PCC, Gomes DS, Gempelli R, Ferreira MC (2012). Bloodstream infections by multidrug-resistant bacteria in burn treatment ICU: 4 years' experience. *Rev. Bras. Cir. Plast.* 27(3):374-8.
- Moghaddam MN, Forghanifard MM, Moshrefi S (2012). Prevalence and molecular characterization of plasmid-mediated Extended-Spectrum β -Lactamase genes (*bla*_{TEM}, *bla*_{CTX} and *bla*_{SHV}) Among Urinary *Escherichia coli* Clinical Isolates in Mashhad, Iran. *J. Basic Med. Sci.* 15(3):833-839.
- Munier GK, Johnson CL, Snyder JW, Moland ES, Hanson ND, Thomson KS (2010). Positive extended-spectrum-beta-lactamase (ESBL) screening results may be due to AmpC beta-lactamases more often than to ESBLs. *J. Clin. Microbiol.* 48:673-674.
- Paterson DL, Bonoma RA (2005). Extended-spectrum β -lactamases: a clinical update. *Clin. Microbiol. Rev.* 18:657-686.
- Pereira CAP, Marra AR, Camargo LFA, Pignatari ACC, Sukiennik T, Behar PRP, Medeiros EAS, Ribeiro J, Girão E, Correa L, Guerra C, Carneiro I, Brites C, Reis M, Souza MA, Tranchesi R, Barata CU, Edmond MB (2013). Nosocomial Bloodstream Infections in Brazilian Pediatric Patients: Microbiology, Epidemiology and Clinical Features. *PLoS One* 8:7.
- Pfaller MA, Segreti J (2006). Overview of the epidemiological profile and laboratory detection of extended-spectrum beta-lactamases. *Clin. Infect. Dis.* 42:153-63.
- Pondei K, Fente BG, Oladapo O (2013). Current microbial isolates from wound swabs, their culture and sensitivity pattern at the niger delta university teaching hospital, Okolobiri, Nigeria. *Trop. Med. Health* 41(2):49-53.
- Rossi F (2011). The challenges of antimicrobial resistance in Brazil. *Clin. Infect. Dis.* 52(9):1138-1143.
- Santos NQ (2004). Bacterial resistance in the context of hospital infection. *Contexto Enferm.* 13:64-70.
- Sekar U, Shanthi M (2009). The expanding family of beta-lactamases. *J. Acad. Clin. Microbiol.* 11(2):72-86.
- Silbert S, Boyken L, Hollis RJ, Pfaller MA (2003). Improving typeability of multiple bacterial species using pulsed-field gel electrophoresis and thiourea. *Diagn. Microbiol. Infect. Dis.* 47(4):619-621.
- Silva KC, Lincopan N (2012). Epidemiology of extended-spectrum beta-lactamases in Brazil: clinical impact and implications for agribusiness. *J. Bras. Pat. Med. Lab.* 48(2):91-99.
- Singh A, Goering RV, Simjee S, Foley SL, Zervos MJ (2006). Application of Molecular Techniques to the Study of Hospital Infection. *Clin. Microbiol. Rev.* 19 (3):512-530.
- Tissera S, Lee SM (2013). Isolation of Extended Spectrum β -lactamase (ESBL) Producing Bacteria from Urban Surface Waters in Malaysia. *Malays. J. Med. Sci.* 20(3):14-22.
- Woodford N, Fagan E, Ellington MJ (2006). Multiplex PCR for rapid detection of genes encoding CTX-M extended-spectrum β -lactamases; *J. Antimicrob. Chemother.* 57: 154-155.
- Yao F, Qian Y, Chen S, Wang P, Huang Y (2007). Incidence of extended-Spectrum β -Lactamases and Characterization of Integrons in Extended-Spectrum- β -Lactamase-producing *Klebsiella pneumoniae* Isolated in Shantou, China. *Acta Biochim. Biophys. Sin.* 39(7):527-532.