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REVIEW ARTICLE

A Global Overview of β-lactam Resistance Genes in *Klebsiella pneumoniag*

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Abstract: *Klebsiella pneumoniae* is a gram-negative bacillus of the *Enterobacteriaceae* family, commonly associated with nosocomial infections. This pathogen is a serious public health problem as some of its strains are resistant to about 95% antimicrobials of the pharmaceutical market. This resistance is promoted by the production of the β-lactamase extended spectrum (ESBL) enzymes, one of the major causes of therapeutic failure. This review evaluated the incidence and distribution of resistance genes from *Klebsiella pneumoniae* to β-lactams worldwide. Our study was conducted with the subject the organism *K. pneumoniae* and β-lactamic resistance. The most reported genes were bla_{SHV-12}, $bla_{CTX-M-2}$ and bla_{SHV-5}; with bla_{SHV-12} being the most described. The last two were present in all continents, characterizing its cosmopolitan profiles. The greatest genetic diversity was observed in the Asian and Oceania, where 41 different genes were isolated. Additionally, our review points out the coexistence of different classes of β-lactamases in a single bacterial isolate. Finally, knowledge of mechanisms associated with resistance of *K. pneumoniae* is of great public interest and the verification of resistance genes shows a variation over time and location highlights the importance of evaluating the mechanisms or strategies by which these variations occur.

Keywords: Antibiotic resistance, Enterobacteria, β-lactamase, Global distribution, Antimicrobial Resistance, Klebsiella Preumonia.

1. INTRODUCTION

Klebsiella pneumoniae is a microorganism found in humans and other mammals, colonizing intestine, skin, nasopharynx and, several environmental niches. A constituent of the human intestinal microbiota, this bacterium, considered harmless, has gained notoriety by being identified as an opportunistic pathogen. This is responsible for a variety of diseases in humans and animals, such as urinary tract infection, pneumoniae, intra-abdominal infection, bloodstream infection, meningitis, pyogenic liver abscess and is a prominent nosocomial pathogen [1, 2]. Moreover, the occurrence and global spread of hypervirulent and MDR (multidrug-resistant) *K. pneumoniae* are increasingly reported in community-acquired and nosocomial infections [3], and the use of antimicrobials in the treatment of infections has become progressively more difficult [4, 5]. However, despite there has been an increase in the number of vulnerable populations to MDR pathogens, in addition to a renewed interest in the *Klebsiella pneumoniae*, many questions about this pathogen are still unknown, as to how resistance genes spread out and how they reach humans [6].

Over time, bacteria have developed several mechanisms by which they can withstand antimicrobials, rendering the action of these substances ineffective [3]. The resistance mechanisms of a gram-negative bacterium include: efflux pump; membrane permeability; production of β-lactamases; modification of antibiotic targets; and, acquisition of alternative metabolic pathways to those inhibited by the antibiotic [4, 6]. One of the most important mechanisms of resistance in bacteria is the Extended Spectrum β-lactamase (ESBL) production [7]. The first β-lactamase with the ability to hydrolyze β-lactam was described in 1983 in a *K. ozaenae* isolated in Germany and designated as SHV-2 (Sulfhydryl variable) [8, 9]. However, the initial outbreaks were related only in 1985 in France and the late '80s in the United States. Currently, over one hundred and fifty different ESBLs types have been identified. These classes of enzymes have a distinct spectrum of action and are classified as: Extended Spectrum β-lactamase (ESBL); Metalo-β-lactamase (MBL); and, β-lactamase Class C (AmpC) [8].

The definition of ESBLs is not consensual, however, some authors propose that an ESBL is any β-lactamase, generally acquired and not inherent in a species, that hydrolyzes rapidly or confers resistance to oxyimino-β-lactam chain, broad-spectrum cephalosporins, penicillins, and monobactams and is inhibited by clavulanate, sulbactam and tazobactam [10, 11].

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Since the discovery of these enzymes, an increase of *K. pneumoniae* resistant to several antimicrobials has been reported such as aminoglycosides and even carbapenems [12, 13]. These latter are considered to be one of the most important antibiotics classes in the fight against ESBL-producing microorganisms due to their stability against the hydrolytic activity of this enzyme and also for the ability to penetrate the bacterium [14].

β-lactam antibiotics interact with diverse groups of bacterial enzymes. The enzymes, such as TEM-1 (Temoniera), TEM-2 and SHV-1, encoded by plasmids and widely distributed among enterobacteria, can hydrolyze a wide variety of penicillins and third generation of cephalosporins, which were originally developed as drugs capable of overcoming bacterial resistance. These enzymes have emerged as one of the most successful resistance mechanisms, which limit therapeutic options for treating various human infections, including *Klebsiella* [15, 16].

Given the therapeutic limitations caused by resistance mechanisms, several research groups have followed the distribution and susceptibility profile of the genus *Klebsiella* spp., to monitor possible outbreaks and set up the sensitivity profile in different regions [12, 13]. Based on the global dissemination of ESBL-producing *Klebsiella pneumoniae* and limitations of the current treatments, the implementation of prevention and control measures has become extremely important [13].

Considering the recognition of the genus *Klebsiella* spp. as an important pathogen involved in infections, its variations in the antibiotic resistance profile, and its emerging mechanisms of resistance, reinforces the need to know the local or regional characteristics of this genus, as well as the evolution of its resistance. Thus, monitoring of the resistance and prevalence of antimicrobial resistance determinants are urgently needed and may contribute to better targeting and use of antimicrobials. In this context, the present review focuses on the main aspects related to the distribution of resistance genes to β-lactam antibiotics associated with *Klebsiella pneumoniae* found in the scientific literature.

2. MATERIALS AND METHOD

The searches were conducted between February and June of 2018 in online health databases, and date delimitation was set as between 2000 and 2018. Editorials, letters, and Ph.D. theses were excluded. The following databases were used for the research: PubMed, SCOPUS, Science Direct, Latin American and Caribbean Literature in Health Sciences (LILACS), Virtual Electronic Scientific Library (SCIELO) and Portal of Periodicals of the Coordination for the Upper Level (Capes Newspapers). Additionally, the descriptors to perform the searches were: *Klebsiella pneumoniae*, *Klebsiella pneumoniae* resistance factors, *Klebsiella pneumoniae* with resistance to β-lactam antimicrobials, broad-spectrum β-lactamases, and extended-spectrum β-lactamase. There was no restriction of sample size for evaluation of publications and the articles selected were only in the English language. Exclusion criteria were: studies that exclusively investigated resistance to non-β-lactam antibiotics and non-human studies. Some studies

considered essential to compose the review not included in any of the research bases were added (i.e., articles addressing pathologies in humans, characteristics of the bacterium and, some studies published earlier than the year 2000 due to the importance of it to a specific country and to better contextualize a finding).

3. RESULTS AND DISCUSSION

The descriptors and databases used allowed the identification of 84 scientific articles that fully met the inclusion criteria of the study. It should be noted that several studies found despite the treatment of β-lactam antibiotic resistance associated with *K. pneumoniae* did not present all relevant information for our review, *e.g.*, resistance genes and/or sites where the microorganism was isolated, which consequently had to be excluded. Finally, not all the countries that constitute the different continents were considered since until the conclusion of our review no results were published between 2000 and 2018.

3.1. Antimicrobial Resistance

Antimicrobial resistance is a growing worldwide iatrogenic complication, which currently constitutes a major public health problem and is responsible for a significant increase in patients' morbidity and mortality [10, 17].

As commented above, the *Enterobacteriaceae* family has several mechanisms of resistance recognized. This group has also received special attention in recent years as being among the most isolated microorganisms in the laboratory routine [12]. Belonging to this family, one of the important pathogens for humans, with well-known and powerful mechanisms of resistance, is the genus *Klebsiella* spp [12, 13].

In recent years, increased antibiotic resistance in *Enterobacteriaceae* has become a major concern worldwide. Although β-lactam antibiotics have been widely used as the first treatment of severe infections, resistance to these drugs due to the acquisition of resistance genes has emerged and spread worldwide since the early 2000s, thus increasing concern about the occurrence of infections of hospital origin where these bacteria are widespread [18].

The progression of resistance of *Klebsiella pneumoniae* to antibiotics has caused great concern since the 1980s with the emergence of *K. pneumoniae* ESBL-producing [19]. Since them, other β-lactamases were discovered, for example, the carbapenemase. This enzyme was discovered in the United States in 2001 in *K. pneumoniae* and has expanded remarkably, being able to confer resistance to all β-lactams, even those associated with β-lactamase inhibitors [20]. Another important β-lactamase is the MBL, which degrades all β-lactams except aztreonam (monobactam) *in vitro*, whereas AmpC, in turn, can hydrolyze penicillins, monobactams, and cephalosporins until the third generation [21].

The β-lactamase type enzymes can be classified into two distinct ways, the molecular classification of Ambler [22] and the functional classification Bush & Jacoby [23]. The first classifies the β-lactamases into four classes (A, B, C, and D), grouped according to the similarity between the amino acid
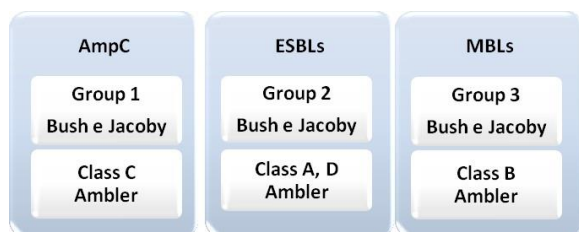
sequences. Classes A, C, and D are considered serine β-lactamase because they have the site of action composed of serine. Class B, also called MBL, uses zinc (Zn) as a cofactor [24].

The most current classification is from Bush & Jacoby, based on the activity of the enzyme, that is, on functional groups and correlates the properties of each enzyme with the resistance profile of each isolate [23, 24]. In this way, enzymes are classified into three groups: group 1, which is the group of cephalosporinases. Enzymes belonging to molecular class C, from Ambler, which are more active against cephalosporins and are not inhibited by β-lactamase inhibitors, such as clavulanic acid and tazobactam. Group 2 comprises the serine β-lactamases, enzymes belonging to group A and D, of Ambler, represented by the largest group of β-lactamases. It is divided into subgroups (2a and 2b) according to the action spectrum of each enzyme. Group 3 includes the enzymes that require the zinc ion at its site of action to produce antimicrobial activity (Fig. **1**). They are the MBLs that can hydrolyze carbapenems and are inhibited by ion chelators, such as EDTA [23, 24].

The AmpC-type β-lactamases are chromosomal β-lactamases but can be found on both the bacterial chromosome and inserted into mobile plasmids. There are various types of plasmid-mediated AmpC β-lactamases: the CMY, MIR, MOX, LAT, FOX, DHA, ACT, ACC and CFE. They are capable of hydrolyzing penicillins, monobactams, and cephalosporins of up to the third generation, with resistance to cefoxitin being the main marker of AmpC expression. AmpC-producing bacteria are commonly also resistant to non-β-lactam drugs, such as aminoglycosides, chloramphenicol, quinolones, sulfonamides, tetracyclines and trimethoprim [21, 23].

Chromosomal origin AmpC can occur in two forms: inducible and constitutive. Thus, the exposure of bacteria to β-lactam antibiotics leads to an increase in the production of enzymes. This stimulus may occur at different levels of β-lactamase production, depending on the type of antimicrobial. In both cases, hyperproduction or hyperinduction of the enzyme can be attributed to a mutation in the AmpC regulatory genes [21, 23, 25].

Another important factor to be highlighted is the ability to transfer genes that determine antimicrobial resistance. The genes encoding ESBLs are usually located on plasmids that are highly mobile and can harbor resistance genes to several other unrelated classes of antimicrobials [26]. Carattoli [27] and Zhang [28] identified in *Klebsiella pneumoniae* isolates, transconjugants of $bla_{CTX-M-type}$ antibiotic resistance genes, bla_{SHV}, bla_{VIM}, bla_{DHA}. Of these, about 67.7% of the transconjugants harbored at least two resistance classes and 32.2% identified three classes of resistance determinants.

The family of SVH-type enzymes appears to be closely related to *Klebsiella* spp. SHV-1 isthe parent of the SHV-type enzyme class, and most of these enzymes are found in strains of *K. pneumoniae* [8, 29]. There are a few derivatives of SHV-1, and the changes that occurred in the gene to give rise to its variations were characterized by the substitution of serine for glycine [8].

TEM-1 enzyme is the β-lactamase most commonly found in gram-negative bacteria. Its first derivative is TEM-2, where the only difference is the amino acid substitution from the original β-lactamase. The amino acid substitutions occurring within the TEM enzyme occur in a limited number of positions. Combinations of these amino acid changes result in several subtle alterations in ESBL phenotypes, such as the ability to hydrolyze specific oxyimino-cephalosporins, such as ceftazidime and cefotaxime, or a change in isoelectric points [30]. More than 150 different variants of TEM and SHV that generate resistant have been described in the literature. Among these genes, TEM-3 causes a high rate of resistance concomitant with such antibiotics, being the most reported. The CTX-M enzymes have been recognized as the most prevalent among *Enterobacteriaceae* and other ESBLs, such as GES, PER, and EBV, have been described as having a lower prevalence [31].

During the 1990s, TEM and SHV were dominant; however, the last decade was marked by the rapid and massive spread of β-lactamases of the CTX-M type, which are currently the most prevalent ESBL in *Enterobacteriaceae* in Europe and other parts of the world [32]. The existence of the CTX-M enzyme was discovered at the end of the '80s and, more than 100 variations have been identified [33]. Its origin is different from the TEM, and SHV enzymes, the ESBL of CTX-M were acquired by horizontal transfer of genes from other bacteria by translocation or conjugative plasmid, whereas TEM and SHV ESBLs originate from the substitution of the amino acids of parental enzymes [34, 35]. They are classified into five groups based on their amino acid sequence: CTXM-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25 [33]. The diversity of CTX-M enzymes from clinical isolates, described today, grossly underestimate the diversity that exists in nature. The rapid global spread of the CTX-M enzyme poses a threat to current antimicrobial therapy strategies, which rely heavily on third-generation cephalosporins to control gram-negative bacterial infections [33].

3.2. Epidemiology

Our results indicate that the population of *K. pneumoniae* is highly diversified and that in some cases resistant strains seem to have spread throughout the country over time. In addition, we found evidence that some strains independently acquired antimicrobial resistance genes, presumably in

Fig. (1). β-lactamases of major clinical importance according to the classification of Ambler and Bush & Jacoby. AmpC: β-lactamases resistant to extended-spectrum of cephalosporins. ESBLs: Extended-spectrum β-lactamase. MBLs: Metallo-β-lactamases.

response to antimicrobial treatment [5].

The spectrum of infections and the epidemiology of *K. pneumoniae* changed drastically from the 1970s when this microorganism was identified in a hospital environment. In addition to the considerable colonization efficiency, the antimicrobial resistance allowed *K. pneumoniae* to spread rapidly in health care settings [14]. The frequency of the association of the bacteria with cases of hospital infections, as well as the high mortality rates caused by *K. pneumoniae* justify their monitoring [36]. There are reports in the literature of the increasing number of genes associated with the production of ESBLs in *K. pneumoniae*. Among these, the precursors of SHV and TEM enzymes, as well as CTX-M are isolated in numerous countries, alerting to the occurrence of more than one resistance gene in the same strain [37]. In this study, $bla_{CTX-M-15}$ and bla_{SHV-5} genes were identified in 4 of the 7 (57%) of the European countries that were part of this research, both of which were identified in France, Italy, Spain, and Portugal. With featured on France and Spain, where the gene $bla_{CTX-M-15}$ was isolated twice in rectal swab in both countries (Table **1**). Concomitant with the use of antibiotic, the prevalence of *Klebsiella pneumoniae* strains producing CTX-M-type ESBLs has increased worldwide [46]. The $bla_{CTX-M-15}$ gene is predominantly present on the European continent due to the spread of isolates initially identified in France and Italy, which have spread to other countries [46].

The β-lactamase CTX-M-15, first detected in 1999 in India, is currently recognized as the most widely distributed CTX-M β-lactamase in the world [46]. CTX-M enzymes, especially CTX-M-15, are related to various epidemiological situations and have spread throughout the continents due to epidemic plasmids and/or epidemic strains [47]. Corroborating this statement, in the present study, the $bla_{CTX-M-15}$ gene was found in *K. pneumoniae* in all continents evaluated Table(**1-4**). Many of the emerging antimicrobial resistance problems of the nineties have been characterized by difficulty in recognition of resistance in the laboratory, particularly by rapid susceptibility-test methods. The emergence of plasmid-encoded ESBLs is a significant evolution in antimicrobial resistance. Outbreaks due to the dissemination of ESBL-producing bacterial strains and to the dissemination of ESBL-encoding plasmids among different species of the family *Enterobacteriaceae* have been described in hospitals and other health care facilities [46, 47].

Some authors identified antimicrobial resistance genes in *K. pneumoniae* and demonstrated the CTX-M-15 and SHV-5 domain isolated from Portuguese hospitals [45]. The production of the SHV-type ESBLs in *K. pneumoniae* is associated with an increased tendency to invade epithelial cells and expression of fimbrial adhesions [48]. The propagation of these genes is influenced by the amplification of the epidemic and international clones of *K. pneumoniae* and specific types of plasmids. The same authors also highlight the potential for antibiotic resistance and virulence plasmid dissemination [45]. The origin of the $bla_{CTX-M-15}$ gene identified in *K. pneumoniae* in Portugal is clone ST336, and the most recent occurrence in France, described in the literature, reported a clonal outbreak involving *K. pneumoniae* isolates recovered from a single hospital in the Picardie region of northern France [45, 49]. This

outbreak was caused by a resistance to colistin with OXA-48 and CTXM-15 which produced *K. pneumoniae* type ST11 that was susceptible only to cefoxitin. The first identification of the ST11 clone in *K. pneumoniae* occurred in 1997 in France [49]. This clone has been associated to the acquisition of resistance genes to a broad spectrum of antimicrobial agents, as well as to the dissemination of OXA-48, in addition to VIM (Verona Imipenemase), NDM (New Delhi Metallo-β-lactamase) and KPC [40, 49].

The most recent occurrence of $bla_{CTX-M-15}$ in other European countries was described by Rodrigues and Brañas [40, 45]. The first author related finding of the increased incidence of CTX-M-15, SHV and several other types ESBL were observed in a Portuguese hospital associated with the increase of MDR *K. pneumoniae* epidemic clones, the second reported bla_{OXA-48} and $bla_{CTX-M-15}$ as responsible for a major outbreak involving 44 patients at a hospital in Madrid, Spain, 2009 to 2014 [40, 45].

In the United Kingdom and Sweden, the only resistance genes identified were NDM-type, with bla_{NDM-1} being isolated in both countries (Table **1**). Among the NDM isolates identified in the United Kingdom, ST231 was the most common type along with ST147 and ST273. In the case of Sweden, the isolates belonged to four different sequence types, as they were imported from various regions of the world such as India and Iraq [43]. Strains of *K. pneumoniae* NDM-1 positive can exhibit relatively high clonal diversity. In Sweden and the United Kingdom clone ST14 was the most frequently observed type. Clone *K. pneumoniae* ST14 has been described as a host for the NDM-1 enzyme and is also a frequent host of CTX-M enzymes. In addition, ST14 is a single locus variant (SLV) of ST15, which often encodes the CTX-M type ESBLs. The production of the NDM-1 enzyme by isolates belonging to clone ST11 and complex ST147-ST273 is very relevant information since clone ST11 is a frequent host of CTX-M and KPC and is also an SLV of ST258, making it fundamental for its dissemination [43, 50].

Of the European countries correlated in this study, Spain stands out as the site where the largest number of different genes (15 in total) was identified, which shows the genetic diversity in relation to the occurrence of *K. pneumoniae* with resistance to the antibiotic β-lactams (Table **1**). This characteristic, from the point of view of the therapeutic of the infection, is a difficult factor, considering that the variation of resistance genes decreases the antimicrobial therapy options.

The country where the lowest gene diversity occurred was Greece, where the only isolated gene was bla_{KPC-2} in two outbreak cases (Table **1**). Considering that the outbreaks were of monoclonal origin and that there was no identification of a common source or an environmental reservoir, the main dissemination mechanism of the bla_{KPC-2} in these cases was the transmission from patient to patient [41, 42]. The smallest genetic diversity in Greece, identified in this study, may be related to a low number of publications in this country.

Table 1. Association between resistance genes to β-lactams, geographical areas of occurrence and clinical isolates in the European continent.

Europe			
Country	**Genes (N. reports)***	**Isolated (N. reports)***	**References**
France	$bla_{CTX-M-15}$ (2) $bla_{CTX-M-1}$ $bla_{CTX-M-40}$ bla_{SHV-5} bla_{SHV-12} bla_{SHV-2} bla_{TEM-3} bla_{CMY-2} bla_{OXA-48}	Rectal swab (2) Catheter Urine Wound exudate Respiratory tract	(Baraniak *et al.* 2013)[38]
Italy	$bla_{CTX-M-2}$ $bla_{CTX-M-15}$ bla_{TEM-3} $bla_{CTX-M-1}$ bla_{SHV-5}	Rectal swab	(Baraniak *et al.* 2013)[38]
Spain	$bla_{CTX-M-1}$ $bla_{CTX-M-3}$ $bla_{CTX-M-9}$ $bla_{CTX-M-10}$ $bla_{CTX-M-15}$ (2) bla_{SHV-2} (2) bla_{SHV-2a} bla_{SHV-5} bla_{SHV-12} bla_{DHA-1} bla_{TEM-3} bla_{TEM-4} bla_{TEM-25} $bla_{TEM-133}$ bla_{OXA-48} bla_{VIM-1}	Bloodstream Bronchial aspirates Clinical isolates Non-surgical wound Rectal swabs (2) Sputum Surgical wound Urine Vascular catheters	(Hernández *et al.* 2005)[39] (Baraniak *et al.* 2013)[38] (Brañas *et al.* 2015)[40]
Greece	bla_{KPC-2} (2)	Respiratory tract Catheter tip surgical site Bloodstream (2) Feces Bronchial secretions Pus Central venous catheter	(Maltezou *et al.* 2009)[41] (Souli *et al.* 2010)[42]
United Kingdom	bla_{NDM} bla_{NDM-1}	Urine (2) Respiratory tract	(Giske *et al.* 2012)[43] (Jain *et al.* 2014)[44]
Portugal	$bla_{CTX-M-15}$ bla_{SHV-12} $bla_{SHV-106}$ bla_{SHV-28} bla_{SHV-55} bla_{SHV-2} bla_{SHV-5}	Urine Bloodstream Exudate Sputum	(Rodrigues *et al.* 2014)[45]
Sweden	bla_{KPC} bla_{NDM-1}	Urine Respiratory tract	(Giske *et al.* 2012)[43]

*Number of times the gene was identified.

In Asia and Oceania, 41 different resistance gene types have been identified in the literature. Of these, the greatest diversity occurred in China, where 14 of the 41 genes were described, corresponding to 41% of the total of related genes in Table **2**. The variety of genes in China can be justified by its high population density, associated with intense migrations.

The second country with the greatest diversity of genes was Israel (12 different genes), followed by Turkey (11 genes) and Iran (10 genes). The lowest variety of β-lactam resistance genes occurred in Pakistan (2 genes), according to data in

Table **2**. Despite the lower diversity, the genes identified in Pakistan, bla_{KPC}, and bla_{NDM-1}, are of great importance for tracking the control of microbial resistance. The first gene is characterized by its wide plasmodial spread, and the second has broad relationships with the globalization and population mobility [63, 64]. The bla_{NDM-1} gene was first identified in 2009 in a Swedish patient of Indian origin, who acquired a urinary tract infection in New Delhi, caused by a strain of *K. pneumoniae* that showed resistance to β-lactam antibiotics [64, 65].

Table 2. Association between resistance genes to β-lactams, geographical areas of occurrence and clinical isolates in Asia and Oceania.

Country	Genes (N. reports)*	Isolated (N. reports)*	References
China	bla_{CMY-2}	Bloodstream	(Giske *et al.* 2012) [43]
	bla_{CTX-M}	Urine (2)	(Ginn *et al.* 2014) [51]
	$bla_{CTX-M-1}$	Respiratory tract	(Qi *et al.* 2015) [52]
	$bla_{CTX-M-15}$		
	$bla_{CTX-M-9}$		
	bla_{DHA}		
	bla_{IMP-4}		
	bla_{KPC}		
	bla_{KPC-2}		
	bla_{NDM-1}		
	bla_{OXA-10}		
	bla_{SHV-12}		
	bla_{SHV-5}		
	bla_{TEM}		
Israel	bla_{CMY-2}	Swab retal	(Leavitt *et al.* 2007) [53]
	$bla_{CTX-M-10}$	Urine	(Baraniak *et al.* 2013) [58]
	$bla_{CTX-M-14}$	Body fluids	
	$bla_{CTX-M-2}$	Wounds	
	$bla_{CTX-M-39}$	Catheter tips	
	bla_{KPC-2}	Bloodstream	
	bla_{KPC-3}	Respiratory tracts	
	bla_{OXA-4}		
	bla_{SHV-12}		
	bla_{SHV-27}		
	bla_{SHV-5}		
	bla_{TEM-1}		
	bla_{TEM-3}		
India	bla_{NDM-1}	SENTRY	(Castanheira *et al.* 2011) [54]
	$bla_{OXA-181}$	Antimicrobial	
	bla_{VIM-5}	Surveillance Program	
		(hospital patients)	

Asia and Oceania			
Country	**Genes** **(N. reports)***	**Isolated** **(N. reports)***	**References**
Singapore	bla_{CMY-2}	Bloodstream	(Ginn *et al.* 2014) [51]
	$bla_{CTX-M-1}$	Urine	
	$bla_{CTX-M-9}$		
	bla_{DHA}		
	bla_{SHV-12}		
	bla_{SHV-5}		
	bla_{TEM}		
Australia	$bla_{CTX-M-3}$	Bloodstream	(Paterson *et al.* 2003) [55]
	bla_{SHV-2}		
	bla_{TEM-1B}		
Turkey	$bla_{CTX-M-2}$ (2)	Bloodstream (4)	(Paterson *et al.* 2003) [55]
	$bla_{CTX-M-15}$	Urine (3)	(Bali *et al.* 2010) [56]
	bla_{PER-1}	Sputum	(Carrer *et al.* 2010) [57]
	bla_{SHV-2} (2)	Wound (3)	
	bla_{SHV-5} (2)	Abscesses	
	bla_{SHV-12} (3)	Catheter (3)	
	bla_{TEM-1B}	Peritoneum fluid	
	bla_{TEM-1} (2)	Cerebrospinal fluid	
	bla_{TEM-2}	Endotracheal aspirate (2)	
	bla_{OXA-48}	Pus (2)	
	bla_{OXA-9}	Ascites (2)	
	bla_{OXA-1}		
Taiwan	$bla_{CTX-M-14}$	Nationwide	(Ling Ma *et al.* 2009) [58]
	$bla_{CTX-M-15}$	surveillance	
	$bla_{CTX-M-3}$	program	
	bla_{SHV-1}		
	bla_{SHV-11}		
	bla_{SHV-12}		
	bla_{TEM-31}		
Iran	bla_{CTX-M}	Abscess	(Nasehi *et al.* 2010) [59]
	$bla_{CTX-M-15}$	Abdominal exudates	(Feizabadi *et al.* 2010) [60]
	bla_{PER}	Bloodstream (2)	
	bla_{SHV}	Bronchial	
	bla_{SHV-1}	Eyes exudates	
	bla_{SHV-12}	Sputum	
	bla_{SHV-31}	Stool	
	bla_{TEM}	Trachea	
	bla_{TEM-1}	Umbilical exudates	
	bla_{TEM-79}	Urine (2)	
		Wound (2)	
Lebanon	$bla_{CTX-M-15}$	Stool sample	(Tokajian *et al.* 2015 [62])
	bla_{OXA-1}		
	bla_{SHV-11}		
	bla_{TEM-1b}		
Pakistan	bla_{KPC-2}	Pus	(Sattar *et al.* 2016) [61]
	bla_{NDM-1}	Urine	
		Catheter tip	
		Sputum	
		Wound	

[1]*Number of times the gene was identified.

Of the isolates of *K. pneumoniae* from Singapore, the occurrence of bla_{CTX} type genes predominated, with the $bla_{CTX\text{-}M\text{-}1}$ group being more numerous than the others. Literature data report transport of methylase genes in isolated MDR *K. pneumoniae* strains in Chinese hospitals [52]. In most countries of Asia and Oceania, the $bla_{CTX\text{-}M}$ gene, in particular, $bla_{CTX\text{-}M\text{-}15}$, was detected in *K. pneumoniae*, as shown in Table **2**. A study published in 2015 reported, for the first time, the isolation of *K. pneumoniae* CTX-M-15, SHV-11, and KPC belonging to ST336 in Lebanon [62]. The contributors to the dissemination of the $bla_{CTX\text{-}M\text{-}15}$ gene highlight the clonal complex 17, among them ST336 [45] is related to the presence of this gene in clinical isolates in several countries of the world. In the present study, the occurrence of the $bla_{CTX\text{-}M\text{-}15}$ gene is reported in all continents evaluated, except in the African continent, revealing its endemicity and, highlighting the dissemination of this gene at a global level through human mobility. This clone was isolated for the first time from a patient (nurse), 62 years old, with an abdominal tumor and who had recurrent urinary infections. The ST336 epidemic clone belongs to CG17, and studies revealed the prevalence of this strain in France, Germany, Italy, USA and Greece [38, 66]

In this study, *K. pneumoniae* with the presence of the bla_{KPC} gene was isolated in all the evaluated continents, as described in Tables 1, 2, 3 and 4. Although it occurs in all continents evaluated, the $bla_{KPC\text{-}2}$ type gene was more prominent in the Asian and American continents (Tables **2** and **3**), and is described as the second most frequent gene. The most prevalent β-lactamase in the family *Enterobacteriaceae* is the type of KPC, which was found in *Klebsiella pneumoniae*, especially in the United States, Asia, United Kingdom, Israel and Europe [65]. The propagation of the bla_{KPC} gene to other countries is associated with cases imported from countries where they are endemic [66]. Besides, population mobility is recognized as a major factor in the spread of microorganisms resistant to antimicrobial drugs. Thus, the emergence of *K. pneumoniae* producing bla_{KPC} in the United States in 2001 may be associated with the onset of travel-related outbreaks in other countries [63].

In the literature, the first identification of the $bla_{OXA\text{-}48}$ gene in a *K. pneumoniae* isolate occurred in Istanbul, Turkey, in 2001, since then the β-lactamase OXA-48 has become an important carbapenemase associated with the *Enterobacteriaceae* family in Europe, North Africa, and Asia [35, 72].

In the Americas, the highest diversity of β-lactam resistance genes occurred in Brazil, where 12 of the 25 different types of genes identified on the continent were reported, representing 48% of the total genes present in this continent (Table **3**). Secondly, in the diversity of resistance genes, the United States of America (USA) presented 10 of the 25 genes identified; followed by Canada (9 different genes) and Argentina (6 different genes). The lowest resistance gene diversity occurred in Mexico (3 different genes), according to data described in Table **3**. The smallest variety of genes in Mexico can be justified by the low number of publications on the subject in this country.

The occurrence of β-lactamase gene diversity produced by *K. pneumoniae* may be closely related to the variety of antimicrobials used in the treatment of infection over the decades. With the evolution of antimicrobial resistance in bacteria, it is necessary to continually re-evaluate the laboratory detection of ESBLs and potential treatment options for organisms that produce ESBLs [1].

Among the resistance genes identified in the American continent, $bla_{CTX\text{-}M\text{-}2}$, $bla_{SHV\text{-}1}$, $bla_{SHV\text{-}5}$, and $bla_{SHV\text{-}2}$ were isolated from *K. pneumoniae* in 60% of the American countries listed in this study. The genes $bla_{CTX\text{-}M\text{-}15}$, $bla_{CTX\text{-}M\text{-}1}$, $bla_{SHV\text{-}11}$, $bla_{SHV\text{-}12}$, $bla_{TEM\text{-}1}$, $bla_{TEM\text{-}10}$ and $bla_{KPC\text{-}2}$ were the second most frequent in the Americas, being found in 40% (Table **3**).

TEM-10 was ESBL type TEM which is most frequently detected in *K. pneumoniae* isolates, being found in different geographic regions (United States, South Africa, and Argentina). The identification of genes encoding the production of TEM-10 β-lactamase isolated from *K. pneumoniae* in Argentina in 2003 represented the first report of ESBL-type TEM-10 in South America [55]. In Argentina, most ESBLs belong to the CTX-M class, referring to a growing family of ESBLs that shares a single phenotype that confers *in vitro* resistance to cefotaxime, ceftriaxone and many times to cefepime [74].

Table 3. Association between genes of resistance to β-lactams, geographical areas of occurrence and clinical isolates, in American continent.

America			
Country	**Genes**	**Isolated**	**References**
	(N. reports)*	**(N. reports)***	
Argentina	$bla_{TEM\text{-}10}$	Bloodstream (2)	(Paterson *et al.* 2003) [55]
	$bla_{SHV\text{-}5}$	Surgical site	(Pasteran *et al.* 2008) [67]
	$bla_{SHV\text{-}2}$		
	$bla_{TEM\text{-}12}$		
	$bla_{CTX\text{-}M\text{-}2}$		
	$bla_{KPC\text{-}2}$		

(Table 3) contd.....

Brazil	bla_{KPC-2} (2)	Bloodstream (3)	(Peirano *et al.* 2009) [68]
	bla_{SHV-1}	Urine (3)	(Pereira *et al.* 2013) [69]
	bla_{SHV-11}	Abdominal fluid	(Tollentino *et al.* 2011) [70]
	bla_{SHV-12} (2)	Respiratory tract secretion	(Andrade *et al.* 2014) [48]
	bla_{SHV-31}	Tracheal secretion (2)	
	bla_{SHV-38}	Venous catheter	
	$bla_{CTX-M-2}$ (2)		
	$bla_{CTX-M-15}$		
	bla_{OXA-9}		
	bla_{TEM-1} (2)		
	$bla_{CTX-M-1}$		
	$bla_{CTX-M-8}$		
Canada	$bla_{CTX-M-2}$	Bloodstream	(Denisuik *et al.* 2013) [71]
	bla_{SHV-11}	Urine	
	$bla_{CTX-M-3}$	Respiratory tract	
	$bla_{CTX-M-14}$	Secretions	
	bla_{SHV-1}		
	bla_{TEM-1}		
	$bla_{CTX-M-15}$		
	bla_{OXA-1}		
	bla_{SHV-2}		
Mexico	bla_{SHV-1}	Bloodstream	(Miranda *et al.* 2004) [73]
	bla_{SHV-2}	Cerebrospinal fluid	
	bla_{SHV-5}		
United States	$bla_{CTX-M-1}$	Bloodstream	(Ginn *et al.* 2014) [51]
	$bla_{CTX-M-9}$	Urine	(Mathers *et al.* 2013) [72]
	bla_{SHV}	Perirectal swab (2)	
	bla_{SHV-5}	Fluid from abdominal drain	
	bla_{SHV-12}		
	bla_{TEM-10}		
	bla_{TEM} (2)		
	bla_{OXA-10}		
	bla_{OXA-48}		
	$bla_{OXA-181}$		

[1]*Number of times the gene was identified.

In Brazil, the first reported case of KPC occurred in 2009. Data from a survey published in 2005 pointed out that the number of ESBL positive strains is increasing worldwide from 30% to 60% of strains of *Klebsiella* [37]. A major concern emerged in Brazil after the isolation and identification of *Klebsiella pneumoniae* with concomitant expression of MBL IMP-1 and ESBL CTX-M, since the production of these enzymes together gives the microorganism resistance to all antibiotics available for treatment, including carbapenems [78]. A study on the antimicrobial resistance of *Klebsiella* spp., in Brazil, reported the trend of resistance growth in most of the antibiotics tested, indicating the need for new studies to monitor both the evolution of resistance and the emergence of new resistance mechanisms. The rapid spread of ESBL-producing *K. pneumoniae* is a major clinical and public health concern. These broad-spectrum β-lactamases are increasing in new locations around the world, indicating this to be a continuous process [79].

The literature data are still scarce in reporting the problem of ESBLs produced by *K. pneumoniae* in Africa, especially in sub-Saharan Africa [65, 80]. In the present study, the presence of *K. pneumoniae* presenting bla_{NDM-1} resistance gene, as described in (Table **4**), was identified in Kenya and South Africa. NDM-1 is a type of MBL whose denomination is related to the city of origin, being a common practice in the case of transferable MBLs [81]. Isolates from the *Enterobacteriaceae* family harboring the bla_{NDM-1} gene are responsible for causing a variety of infections including urinary tract infections, septicemia, pulmonary infections, diarrhea, peritonitis, device-associated infections, and soft tissue infections [82]. The bla_{NDM-1} gene is a genetic element found in different plasmids. This gene is characterized by a large capacity of duplication migrating from bacterium to bacterium, which explains its rapid dissemination [64]. Also, bla_{NDM-1} is easily disseminated between different bacterial species by lateral transfer. These characteristics make globalization a

favorable aspect of gene dissemination, where travel represents a high risk of a global pandemic among *Enterobacteriaceae*. Since these plasmids also harbor genes that confer resistance to almost all antibiotics, making rapid their spread in clinically relevant bacteria a serious threat to therapy [64, 81]. The results of the present study report the occurrence of the *K. pneumoniae* isolated bla_{NDM-1} gene in all continents, except in the Americas (Table **3**).

Although the bla_{NDM-1} gene isolated from *K. pneumoniae* in the American continent was not related to the present study (because the articles found did not meet the inclusion criteria), a study identified the first isolate of this gene in South America [83]. However, data from the literature mainly demonstrate the presence of the gene in the Americas isolated from other enterobacteria other than *K. pneumoniae*. This occurrence demonstrates the ease of dissemination of this gene among different bacterial species by lateral transfer, especially in the *Enterobacteriaceae* family, as previously described [84].

The propagation of NDM-1 shows some problems related to the occurrence of MBLs, which include: the absence of a routine standardized phenotypic test for MBL detection; the consequent high prevalence of unrecognized asymptomatic carriers, allowing international dissemination of these bacteria; the scarcity of effective antibiotics available today; and the possibility of their spread in many different gram-negative bacteria [81]. In addition to NDM-1 β-lactamase, other resistance genes have been identified in South Africa, namely: blaSHV-5, bla_{SHV-2}, bla_{TEM-10}, bla_{TEM12}, $bla_{CTX-M-2}$ and bla_{KPC-2}, as described in (Table **4**). In 2012, the occurrence of *K. pneumoniae* in South Africa with reduced susceptibility to carbapenems due to ESBL CTX-M was reported. In this study, the $bla_{CTX-M-2}$ gene was identified in South Africa [55, 76]. However, there was no isolation in Kenya (Table **4**), as well as other types of CTX-M, originating from *K. pneumoniae* were not reported.

4. CONCLUSION

The content of the present study characterizes the relevance/importance of epidemiological knowledge   related to mechanisms of resistance of β-lactam antibiotics associated

Table 4. Association between genes of resistance to β-lactams, geographical areas of occurrence and clinical isolates in Africa.

Africa			
Country	Genes (N. reports)*	Isolated (N. reports)*	References
Kenya	bla_{NDM-1}	Urine Urethral pus	(Poirel *et al.* 2011) [75]
South Africa	bla_{TEM-10} bla_{SHV-5} bla_{SHV-2} bla_{TEM-12} $bla_{CTX-M-2}$ bla_{NDM-1} bla_{KPC-2}	Bloodstream Urine Sputum	(Paterson *et al.* 2003) [55] (Brink *et al.* 2012) [76]
Tanzania	$bla_{CTX-M-15}$ $bla_{TEM-104}$ bla_{SHV-11} $bla_{TEM-176}$	Bloodstream Wound swab Urine Pus	(Mshana *et al.* 2013) [77]

[1]*Number of times the gene was identified.

with the microorganism *Klebsiella pneumoniae* from the analysis of scientific production. One of the results of great importance in this study is the coexistence of different classes of β-lactamases in the same strain, representing not only a therapeutic challenge but also a challenge in the diagnosis of infectious disease. Among the microbial resistance genes originating from *K. pneumoniae* and identified in this study, bla_{SHV-12} – which codes one of the most prevalent ESBLs - was the most frequent in Americas, Europe, and Asia, except in Africa. Otherwise, $bla_{CTX-M-2}$ and bla_{SHV-5} genes, the second most reported, were found in all continents, characterizing their cosmopolitan distribution. The highest genetic diversity was observed in Asian and Oceania continents, where 41 different types of genes were reported. Additionally, the verification of resistance genes shows a variation over time and location, which highlights the importance of evaluating the mechanisms or strategies by which these variations occur. Finally, understanding the occurrence and distribution of these genes

may generate possibilities of future interference in this process with the production of new drugs which are more efficient and specific, thereby providing results that allow assisting the health team in the most appropriate therapy.

CONSENT FOR PUBLICATION

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The author declares no conflict of interest, financial or otherwise.

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REFERENCES

[1]   Yu VL, Hansen DS, Ko WC, *et al.* Virulence characteristics of *Klebsiella* and clinical manifestations of *K. pneumoniae* bloodstream infections. Emerg Infect Dis 2007; 13(7): 986-93.
[http://dx.doi.org/10.3201/eid1307.070187] [PMID: 18214169]

[2]   Brisse S, Fevre C, Passet V, *et al.* Virulent clones of Klebsiella pneumoniae: identification and evolutionary scenario based on genomic and phenotypic characterization. PLoS One 2009; 4(3)e4982
[http://dx.doi.org/10.1371/journal.pone.0004982] [PMID: 19319196]

[3]   El Salabi A, Walsh TR, Chouchani C. Extended spectrum β-lactamases, carbapenemases and mobile genetic elements responsible for antibiotics resistance in Gram-negative bacteria. Crit Rev Microbiol 2013; 39(2): 113-22.
[http://dx.doi.org/10.3109/1040841X.2012.691870] [PMID: 22667 455]

[4]   Matzov D, Bashan A, Yonath A. A bright future for antibiotics? Annu Rev Biochem 2017; 86(7): 1–7-.17.
[http://dx.doi.org/10.1146/annurev-biochem-061516-044617]

[5]   Moradigaravand D, Martin V, Peacock SJ, Parkhill J. Evolution and epidemiology of multidrug-resistant *Klebsiella pneumoniae* in the United Kingdom and Ireland. MBio 2017; 8(1): 01976-16.
[http://dx.doi.org/10.1128/mBio.01976-16] [PMID: 28223459]

[6]   Broberg CA, Palacios M, Miller VL. *Klebsiella*: a long way to go towards understanding this enigmatic jet-setter. F1000Prime Rep 2014; 6: 64-76.
[http://dx.doi.org/10.12703/P6-64] [PMID: 25165563]

[7]   Güzel M, Genç Y, Aksoy A, *et al.* Antibiotic resistance and metallo-beta-lactamase positivity in carbapenem-resistant non-fermentative Gram negative bacilli. Turk Hij Deney Biyol Derg 2015; 72: 131-8.

[8]   Bradford PA. Extended-spectrum beta-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clin Microbiol Rev 2001; 14(4): 933-51.
[http://dx.doi.org/10.1128/CMR.14.4.933-951.2001] [PMID: 11585 791]

[9]   Kliebe C, Nies BA, Meyer JF, Tolxdorff-Neutzling RM, Wiedemann B. Evolution of plasmid-coded resistance to broad-spectrum cephalosporins. Antimicrob Agents Chemother 1985; 28(2): 302-7.
[http://dx.doi.org/10.1128/AAC.28.2.302] [PMID: 3879659]

[10]   Tal Jasper R, Coyle JR, Katz DE, Marchaim D. The complex epidemiology of extended-spectrum β-lactamase-producing *Enterobacteriaceae.* Future Microbiol 2015; 10(5): 819-39.
[http://dx.doi.org/10.2217/fmb.15.16] [PMID: 26000652]

[11]   Shaikh S, Fatima J, Shakil S, Rizvi SM, Kamal MA. Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. Saudi J Biol Sci 2015; 22(1): 90-101.
[http://dx.doi.org/10.1016/j.sjbs.2014.08.002] [PMID: 25561890]

[12]   Souli M, Galani I, Giamarellou H. Emergence of extensively drug-resistant and pandrug-resistant Gram-negative bacilli in Europe. Euro Surveill 2008; 13(47): 19045.
[PMID: 19021957]

[13]   EARSS – European Antimicrobial Resistance Surveillance System EARSS Annual report 2008. 2008.

[14]   Tzouvelekis LS, Markogiannakis A, Psichogiou M, Tassios PT, Daikos GL. Carbapenemases in *Klebsiella pneumoniae* and other *Enterobacteriaceae*: an evolving crisis of global dimensions. Clin Microbiol Rev 2012; 25(4): 682-707.
[http://dx.doi.org/10.1128/CMR.05035-11] [PMID: 23034326]

[15]   Campos AC, Albiero J, Ecker AB, *et al.* Outbreak of *K pneumoniae* carbapenemase–producing: A systematic review. Am J Infect Control 2016; 44: 1374-80.
[http://dx.doi.org/10.1016/j.ajic.2016.03.022] [PMID: 27156198]

[16]   Antimicrobial resistance: global report on surveillance. World Health Organization 2014.

[17]   Albrich WC, Angstwurm M, Bader L, Gärtner R. Drug resistance in intensive care units. Infection 1999; 27(Suppl. 2): S19-23.
[http://dx.doi.org/10.1007/BF02561665] [PMID: 10885822]

[18]   Miriagou V, Cornaglia G, Edelstein M, *et al.* Acquired carbapenemases in Gram-negative bacterial pathogens: detection and surveillance issues. Clin Microbiol Infect 2010; 16(2): 112-22.
[http://dx.doi.org/10.1111/j.1469-0691.2009.03116.x] [PMID: 20085 605]

[19]   Boszczowski I, Nicoletti C, Puccini DM, *et al.* Outbreak of extended

spectrum beta-lactamase-producing *Klebsiella pneumoniae* infection in a neonatal intensive care unit related to onychomycosis in a health care worker. Pediatr Infect Dis J 2005; 24(7): 648-50.
[http://dx.doi.org/10.1097/01.inf.0000168844.55898.8f] [PMID: 15999012]

[20]   McGettigan SE, Hu B, Andreacchio K, Nachamkin I, Edelstein PH. Prevalence of CTX-M beta-lactamases in Philadelphia, Pennsylvania. J Clin Microbiol 2009; 47(9): 2970-4.
[http://dx.doi.org/10.1128/JCM.00319-09] [PMID: 19587301]

[21]   Philippon A, Arlet G, Jacoby GA. Plasmid-determined AmpC-type β-lactamases. Antimicrob Agents Chemother 2002; 46(1): 1-11.
[http://dx.doi.org/10.1128/AAC.46.1.1-11.2002] [PMID: 11751104]

[22]   Ambler RP. The structure of β-lactamases. Philos Trans R Soc Lond B Biol Sci 1980; 289(1036): 321-31.
[http://dx.doi.org/10.1098/rstb.1980.0049] [PMID: 6109327]

[23]   Jacoby GA. AmpC β-lactamases. Clin Microbiol Rev 2009; 22(1): 161-82.
[http://dx.doi.org/10.1128/CMR.00036-08] [PMID: 19136439]

[24]   Bush K, Jacoby GA. Updated functional classification of β-lactamases. Antimicrob Agents Chemother 2010; 54(3): 969-76.
[http://dx.doi.org/10.1128/AAC.01009-09] [PMID: 19995920]

[25]   Ghafourian S, Sadeghifard N, Soheili S, Sekawi Z. Extended spectrum beta-lactamases: definition, classification and epidemiology. Curr Issues Mol Biol 2015; 17: 11-21.
[PMID: 24821872]

[26]   Baudry PJ, Nichol K, DeCorby M, *et al.* Mechanisms of resistance and mobility among multidrug-resistant CTX-M-producing *Escherichia coli* from Canadian intensive care units: the 1st report of QepA in North America. Diagn Microbiol Infect Dis 2009; 63(3): 319-26.
[http://dx.doi.org/10.1016/j.diagmicrobio.2008.12.001] [PMID: 19216943]

[27]   Carattoli A. Resistance plasmid families in *Enterobacteriaceae.* Antimicrob Agents Chemother 2009; 53(6): 2227-38.
[http://dx.doi.org/10.1128/AAC.01707-08] [PMID: 19307361]

[28]   Zhang X, Lü X, Zong Z. *Enterobacteriaceae* producing the KPC-2 carbapenemase from hospital sewage. Diagn Microbiol Infect Dis 2012; 73(2): 204-6.
[http://dx.doi.org/10.1016/j.diagmicrobio.2012.02.007] [PMID: 22463 869]

[29]   Livermore DM. Fourteen years in resistance. Int J Antimicrob Agents 2012; 39(4): 283-94.
[http://dx.doi.org/10.1016/j.ijantimicag.2011.12.012] [PMID: 22386 741]

[30]   Liebana E, Batchelor M, Hopkins KL, *et al.* Longitudinal farm study of extended-spectrum β-lactamase-mediated resistance. J Clin Microbiol 2006; 44(5): 1630-4.
[http://dx.doi.org/10.1128/JCM.44.5.1630-1634.2006] [PMID: 16672 386]

[31]   Walsh TR. Combinatorial genetic evolution of multiresistance. Curr Opin Microbiol 2006; 9(5): 476-82.
[http://dx.doi.org/10.1016/j.mib.2006.08.009] [PMID: 16942901]

[32]   Belbel Z, Chettibi H, Dekhil M, Ladjama A, Nedjai S, Rolain JM. Outbreak of an *armA* methyltransferase-producing ST39 *Klebsiella pneumoniae* clone in a pediatric Algerian Hospital. Microb Drug Resist 2014; 20(4): 310-5.
[http://dx.doi.org/10.1089/mdr.2013.0193] [PMID: 24475919]

[33]   Bonnet R. Growing group of extended-spectrum beta-lactamases: the CTX-M enzymes. Antimicrob Agents Chemother 2004; 48(1): 1-14.
[http://dx.doi.org/10.1128/AAC.48.1.1-14.2004] [PMID: 14693512]

[34]   Olson AB, Silverman M, Boyd DA, *et al.* Identification of a progenitor of the CTX-M-9 group of extended-spectrum beta-lactamases from Kluyvera georgiana isolated in Guyana. Antimicrob Agents Chemother 2005; 49(5): 2112-5.
[http://dx.doi.org/10.1128/AAC.49.5.2112-2115.2005] [PMID: 15855 541]

[35]   Poirel L, Héritier C, Tolün V, Nordmann P. Emergence of oxacillinase-mediated resistance to imipenem in *Klebsiella pneumoniae.* Antimicrob Agents Chemother 2004; 48(1): 15-22.
[http://dx.doi.org/10.1128/AAC.48.1.15-22.2004] [PMID: 14693513]

[36]   Landman D, Bratu S, Kochar S, *et al.* Evolution of antimicrobial resistance among Pseudomonas aeruginosa, Acinetobacter baumannii and Klebsiella pneumoniae in Brooklyn, NY. J Antimicrob Chemother 2007; 60(1): 78-82.
[http://dx.doi.org/10.1093/jac/dkm129] [PMID: 17490999]

[37]   Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: a clinical update. Clin Microbiol Rev 2005; 18(4): 657-86.
[http://dx.doi.org/10.1128/CMR.18.4.657-686.2005] [PMID: 16223

952]

[38]   Baraniak A, Izdebski R, Fiett J, *et al.* Comparative population analysis of Klebsiella pneumoniae strains with extended-spectrum β-lactamases colonizing patients in rehabilitation centers in four countries. Antimicrob Agents Chemother 2013; 57(4): 1992-7.
[http://dx.doi.org/10.1128/AAC.02571-12] [PMID: 23403 417]

[39]   Hernández JR, Martínez-Martínez L, Cantón R, Coque TM, Pascual A. Nationwide study of *Escherichia coli* and *Klebsiella pneumoniae* producing extended-spectrum β-lactamases in Spain. Antimicrob Agents Chemother 2005; 49(5): 2122-5.
[http://dx.doi.org/10.1128/AAC.49.5.2122-2125.2005] [PMID: 15855 544]

[40]   Brañas P, Villa J, Viedma E, Mingorance J, Orellana MA, Chaves F. Molecular epidemiology of carbapenemase-producing *Klebsiella pneumoniae* in a hospital in Madrid: Successful establishment of an OXA-48 ST11 clone. Int J Antimicrob Agents 2015; 46(1): 111-6.
[http://dx.doi.org/10.1016/j.ijantimicag.2015.02.019] [PMID: 25914088]

[41]   Maltezou HC, Giakkoupi P, Maragos A, *et al.* Outbreak of infections due to KPC-2-producing *Klebsiella pneumoniae* in a hospital in Crete (Greece). J Infect 2009; 58(3): 213-9.
[http://dx.doi.org/10.1016/j.jinf.2009.01.010] [PMID: 19246099]

[42]   Souli M, Galani I, Antoniadou A, *et al.* An outbreak of infection due to β-Lactamase *Klebsiella pneumoniae* Carbapenemase 2-producing *K. pneumoniae* in a Greek University Hospital: molecular characterization, epidemiology, and outcomes. Clin Infect Dis 2010; 50(3): 364-73.
[http://dx.doi.org/10.1086/649865] [PMID: 20041768]

[43]   Giske CG, Fröding I, Hasan CM, *et al.* Diverse sequence types of *Klebsiella pneumoniae* contribute to the dissemination of bla_{NDM-1} in India, Sweden, and the United Kingdom. Antimicrob Agents Chemother 2012; 56(5): 2735-8.
[http://dx.doi.org/10.1128/AAC.06142-11] [PMID: 22354295]

[44]   Jain A, Hopkins KL, Turton J, *et al.* NDM carbapenemases in the United Kingdom: an analysis of the first 250 cases. J Antimicrob Chemother 2014; 69(7): 1777-84.
[http://dx.doi.org/10.1093/jac/dku084] [PMID: 24769387]

[45]   Rodrigues C, Machado E, Ramos H, Peixe L, Novais Â. Expansion of ESBL-producing Klebsiella pneumoniae in hospitalized patients: a successful story of international clones (ST15, ST147, ST336) and epidemic plasmids (IncR, IncFIIK). Int J Med Microbiol 2014; 304(8): 1100-8.
[http://dx.doi.org/10.1016/j.ijmm.2014.08.003] [PMID: 25190354]

[46]   Pfeifer Y, Cullik A, Witte W. Resistance to cephalosporins and carbapenems in Gram-negative bacterial pathogens. Int J Med Microbiol 2010; 300(6): 371-9.
[http://dx.doi.org/10.1016/j.ijmm.2010.04.005] [PMID: 20537585]

[47]   Ruppé E, Woerther PL, Diop A, *et al.* Carriage of CTX-M-15-producing *Escherichia coli* isolates among children living in a remote village in Senegal. Antimicrob Agents Chemother 2009; 53(7): 3135-7.
[http://dx.doi.org/10.1128/AAC.00139-09] [PMID: 19364858]

[48]   Andrade LN, Vitali L, Gaspar GG, *et al.* Expansion and evolution of a virulent, extensively drug-resistant (polymyxin b-resistant), QnrS1, CTX-M-2, and KPC-2-producing Klebsiella pneumoniae ST11 international high-risk clone. J Clin Microb 2014; pp. 2530-5.

[49]   Jayol A, Poirel L, Dortet L, Nordmann P. National survey of colistin resistance among carbapenemase-producing *Enterobacteriaceae* and outbreak caused by colistin-resistant OXA-48-producing *Klebsiella pneumoniae*, France, 2014. Euro Surveill 2016; 21(37): 30339.
[http://dx.doi.org/10.2807/1560-7917.ES.2016.21.37.30339] [PMID: 27685388]

[50]   Woodford N, Zhang J, Warner M, *et al.* Arrival of Klebsiella pneumoniae producing KPC carbapenemase in the United Kingdom. J Antimicrob Chemother 2008; 62(6): 1261-4.
[http://dx.doi.org/10.1093/jac/dkn396] [PMID: 18812425]

[51]   Ginn AN, Wiklendt AM, Zong Z, *et al.* Prediction of major antibiotic resistance in *Escherichia coli* and *Klebsiella pneumoniae* in Singapore, USA and China using a limited set of gene targets. Int J Antimicrob Agents 2014; 43(6): 563-5.
[http://dx.doi.org/10.1016/j.ijantimicag.2014.02.010] [PMID: 24721 234]

[52]   Qi Y, Wei Z, Ji S, Du X, Shen P, Yu Y. ST11, the dominant clone of KPC-producing *Klebsiella pneumoniae* in China. J Antimicrob Chemother 2011; 66(2): 307-12.
[http://dx.doi.org/10.1093/jac/dkq431] [PMID: 21131324]

[53]   Leavitt A, Navon-Venezia S, Chmelnitsky I, Schwaber MJ, Carmeli Y.

Emergence of KPC-2 and KPC-3 in carbapenem-resistant *Klebsiella pneumoniae* strains in an Israeli hospital. Antimicrob Agents Chemother 2007; 51(8): 3026-9.
[http://dx.doi.org/10.1128/AAC.00299-07] [PMID: 17562800]

[54]   Castanheira M, Deshpande LM, Mathai D, Bell JM, Jones RN, Mendes RE. Early dissemination of NDM-1- and OXA-181-producing *Enterobacteriaceae* in Indian hospitals: report from the SENTRY Antimicrobial Surveillance Program, 2006-2007. Antimicrob Agents Chemother 2011; 55(3): 1274-8.
[http://dx.doi.org/10.1128/AAC.01497-10] [PMID: 21189345]

[55]   Paterson DL, Hujer KM, Hujer AM, *et al.* Extended-spectrum β-lactamases in *Klebsiella pneumoniae* bloodstream isolates from seven countries: dominance and widespread prevalence of SHV- and CTX-M-type β-lactamases. Antimicrob Agents Chemother 2003; 47(11): 3554-60.
[http://dx.doi.org/10.1128/AAC.47.11.3554-3560.2003] [PMID: 14576 117]

[56]   Bali EB, Açik L, Sultan N. Phenotypic and molecular characterization of SHV, TEM, CTX-M and extended-spectrum-lactamase produced by *Escherichia coli, Acinetobacter baumannii* and *Klebsiella pneumoniae* isolates in a Turkish hospital. Afr J Microbiol Res 2010; 4: 650-4.

[57]   Carrër A, Poirel L, Yilmaz M, *et al.* Spread of OXA-48-encoding plasmid in Turkey and beyond. Antimicrob Agents Chemother 2010; 54(3): 1369-73.
[http://dx.doi.org/10.1128/AAC.01312-09] [PMID: 20086157]

[58]   Ma L, Lin CJ, Chen JH, *et al.* Widespread dissemination of aminoglycoside resistance genes *armA* and *rmtB* in *Klebsiella pneumoniae* isolates in Taiwan producing CTX-M-type extended-spectrum β-lactamases. Antimicrob Agents Chemother 2009; 53(1): 104-11.
[http://dx.doi.org/10.1128/AAC.00852-08] [PMID: 18936188]

[59]   Nasehi L, Shahcheraghi F, Nikbin VS, *et al.* PER, CTX-M, TEM and SHV beta-lactamases in clinical isolates of *Klebsiella pneumoniae* Isolated from Tehran, Iran. Iran J Basic Med Sci 2010; 13: 111-8.

[60]   Feizabadi MM, Mahamadi-Yeganeh S, Mirsalehian A, *et al.* Genetic characterization of ESBL producing strains of *Klebsiella pneumoniae* from Tehran hospitals. J Infect Dev Ctries 2010; 4(10): 609-15.
[http://dx.doi.org/10.3855/jidc.1059] [PMID: 21045352]

[61]   Sattar H, Toleman M, Nahid F, Zahra R. Co-existence of bla_{NDM-1} and bla_{KPC-2} in clinical isolates of *Klebsiella pneumoniae* from Pakistan. J Chemother 2016; 28(4): 346-9.
[http://dx.doi.org/10.1179/1973947814Y.0000000223] [PMID: 25407 219]

[62]   Tokajian S, Eisen JA, Jospin G, Farra A, Coil DA. Whole genome sequencing of extended-spectrum β-lactamase producing *Klebsiella pneumoniae* isolated from a patient in Lebanon. Front Cell Infect Microbiol 2015; 5: 32.
[http://dx.doi.org/10.3389/fcimb.2015.00032] [PMID: 25905047]

[63]   Navon-Venezia S, Leavitt A, Schwaber MJ, *et al.* First report on a hyperepidemic clone of KPC-3-producing *Klebsiella pneumoniae* in Israel genetically related to a strain causing outbreaks in the United States. Antimicrob Agents Chemother 2009; 53(2): 818-20.
[http://dx.doi.org/10.1128/AAC.00987-08] [PMID: 19029323]

[64]   Yong D, Toleman MA, Giske CG, *et al.* Characterization of a new metallo-beta-lactamase gene, $bla_{(NDM-1)}$, and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. Antimicrob Agents Chemother 2009; 53(12): 5046-54.
[http://dx.doi.org/10.1128/AAC.00774-09] [PMID: 19770275]

[65]   Nordmann P, Poirel L, Toleman MA, Walsh TR. Does broad-spectrum beta-lactam resistance due to NDM-1 herald the end of the antibiotic era for treatment of infections caused by Gram-negative bacteria? J Antimicrob Chemother 2011; 66(4): 689-92.
[http://dx.doi.org/10.1093/jac/dkq520] [PMID: 21393184]

[66]   MacPherson DW, Gushulak BD, Baine WB, *et al.* Population mobility, globalization, and antimicrobial drug resistance. Emerg Infect Dis 2009; 15(11): 1727-32.
[http://dx.doi.org/10.3201/eid1511.090419] [PMID: 19891858]

[67]   Pasteran FG, Otaegui L, Guerriero L, *et al.* *Klebsiella pneumoniae* Carbapenemase-2, Buenos Aires, Argentina. Emerg Infect Dis 2008; 14(7): 1178-80.
[http://dx.doi.org/10.3201/eid1407.070826] [PMID: 18598660]

[68]   Peirano G, Seki LM, Val Passos VL, Pinto MC, Guerra LR, Asensi MD. Carbapenem-hydrolysing β-lactamase KPC-2 in *Klebsiella pneumoniae* isolated in Rio de Janeiro, Brazil. J Antimicrob Chemother 2009; 63(2): 265-8.
[http://dx.doi.org/10.1093/jac/dkn484] [PMID: 19028717]

[69]   Pereira PS, de Araujo CF, Seki LM, Zahner V, Carvalho-Assef AP, Asensi MD. Update of the molecular epidemiology of KPC-2-producing *Klebsiella pneumoniae* in Brazil: spread of clonal complex 11 (ST11, ST437 and ST340). J Antimicrob Chemother 2013; 68(2): 312-6.
[http://dx.doi.org/10.1093/jac/dks396] [PMID: 23070735]

[70]   Tollentino FM, Polotto M, Nogueira ML, *et al.* High prevalence of *bla*$_{CTX-M}$) extended spectrum beta-lactamase genes in *Klebsiella pneumoniae* isolates from a tertiary care hospital: first report of *bla*$_{SHV-12}$, *bla*$_{SHV-31}$, *bla*$_{SHV-38}$, and *bla*$_{CTX-M-15}$) in Brazil. Microb Drug Resist 2011; 17(1): 7-16.
[http://dx.doi.org/10.1089/mdr.2010.0055] [PMID: 20795871]

[71]   Denisuik AJ, Lagacé-Wiens PR, Pitout JD, *et al.* Molecular epidemiology of extended-spectrum β-lactamase-, AmpC β-lactamase- and carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae* isolated from Canadian hospitals over a 5 year period: CANWARD 2007-11. J Antimicrob Chemother 2013; 68(Suppl. 1): i57-65.
[http://dx.doi.org/10.1093/jac/dkt027] [PMID: 23587779]

[72]   Mathers AJ, Hazen KC, Carroll J, *et al.* First clinical cases of OXA-48-producing carbapenem-resistant *Klebsiella pneumoniae* in the United States: the "menace" arrives in the new world. J Clin Microbiol 2013; 51(2): 680-3.
[http://dx.doi.org/10.1128/JCM.02580-12] [PMID: 23175248]

[73]   Miranda G, Castro N, Leaños B, *et al.* Clonal and horizontal dissemination of *Klebsiella pneumoniae* expressing SHV-5 extended-spectrum β-lactamase in a Mexican pediatric hospital. J Clin Microbiol 2004; 42(1): 30-5.
[http://dx.doi.org/10.1128/JCM.42.1.30-35.2004] [PMID: 14715728]

[74]   Quinteros M, Radice M, Gardella N, *et al.* Extended-spectrum beta-lactamases in *enterobacteriaceae* in Buenos Aires, Argentina, public hospitals. Antimicrob Agents Chemother 2003; 47(9): 2864-7.
[http://dx.doi.org/10.1128/AAC.47.9.2864-2867.2003] [PMID: 12936986]

[75]   Poirel L, Revathi G, Bernabeu S, Nordmann P. Detection of NDM-1-producing *Klebsiella pneumoniae* in Kenya. Antimicrob Agents Chemother 2011; 55(2): 934-6.
[http://dx.doi.org/10.1128/AAC.01247-10] [PMID: 21115785]

[76]   Brink AJ, Coetzee J, Clay CG, *et al.* Emergence of New Delhi metallo-β-lactamase (NDM-1) and *Klebsiella pneumoniae* carbapenemase (KPC-2) in South Africa. J Clin Microbiol 2012;

50(2): 525-7.
[http://dx.doi.org/10.1128/JCM.05956-11] [PMID: 22116157]

[77]   Mshana SE, Hain T, Domann E, Lyamuya EF, Chakraborty T, Imirzalioglu C. Predominance of *Klebsiella pneumoniae* ST14 carrying CTX-M-15 causing neonatal sepsis in Tanzania. BMC Infect Dis 2013; 13: 466.
[http://dx.doi.org/10.1186/1471-2334-13-466] [PMID: 24099282]

[78]   Lincopan N, McCulloch JA, Reinert C, Cassettari VC, Gales AC, Mamizuka EM. First isolation of metallo-beta-lactamase-producing multiresistant *Klebsiella pneumoniae* from a patient in Brazil. J Clin Microbiol 2005; 43(1): 516-9.
[http://dx.doi.org/10.1128/JCM.43.1.516-519.2005] [PMID: 15635033]

[79]   Cuzon G, Naas T, Truong H, *et al.* Worldwide diversity of Klebsiella pneumoniae that produce β-lactamase blaKPC-2 gene. Emerg Infect Dis 2010; 16(9): 1349-56.
[http://dx.doi.org/10.3201/eid1609.091389] [PMID: 20735917]

[80]   Blomberg B, Jureen R, Manji KP, *et al.* High rate of fatal cases of pediatric septicemia caused by gram-negative bacteria with extended-spectrum beta-lactamases in Dar es Salaam, Tanzania. J Clin Microbiol 2005; 43(2): 745-9.
[http://dx.doi.org/10.1128/JCM.43.2.745-749.2005]

[81]   Rolain JM, Parola P, Cornaglia G. New Delhi metallo-beta-lactamase (NDM-1): towards a new pandemia? Clin Microbiol Infect 2010; 16(12): 1699-701.
[http://dx.doi.org/10.1111/j.1469-0691.2010.03385.x]

[82]   Kumarasamy KK, Toleman MA, Walsh TR, *et al.* Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infect Dis 2010; 10(9): 597-602.
[http://dx.doi.org/10.1016/S1473-3099(10)70143-2] [PMID: 20705517]

[83]   Escobar Pérez JA, Olarte Escobar NM, Castro-Cardozo B, *et al.* Outbreak of NDM-1-producing *Klebsiella pneumoniae* in a neonatal unit in Colombia. Antimicrob Agents Chemother 2013; 57(4): 1957-60.
[http://dx.doi.org/10.1128/AAC.01447-12] [PMID: 23357776]

[84]   Pereira PS, Albano RM, Asensi MD, Carvalho-Assef AP. Draft genome sequences of three NDM-1-producing *Enterobacteriaceae* species isolated from Brazil. Mem Inst Oswaldo Cruz 2015; 110(4): 580-2.
[http://dx.doi.org/10.1590/0074-02760150081] [PMID: 26061151]