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Occurrence and sensitivity profile of extended spectrum beta-lactamase-producing Enterobacteriaceae at a tertiary hospital in Southern Brazil

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ABSTRACT

Introduction: Nosocomial infections are closely associated with antimicrobial drug resistance. One of the most important mechanisms of resistance to β-lactam antibiotics is the production of extended spectrum β-lactamases (ESBLs). The objective of the present study was to evaluate the prevalence and antimicrobial susceptibility profile of ESBL-producing strains and to assess the evolution of antimicrobial drug resistance between 2007 and 2013 at the *Hospital São Vicente de Paulo*, Passo Fundo, State of Rio Grande do Sul, Brazil. **Methods:** We conducted a descriptive, observational, cross-sectional study. Bacterial culture was performed from January to December 2013. The antimicrobial susceptibility profile of these cultures was determined using the disk diffusion method. Phenotypic screening for ESBL production was performed using the disk approximation method. **Results**: We analyzed a total of 19,112 cultures, 11.5% of which were positive for Enterobacteriaceae. Of these, 30.3% of the isolates were positive for ESBL production, and the most prevalent species was *Klebsiella* sp. (37.5%). Over 95% of these isolates showed reduced susceptibility to all cephalosporins, aztreonam, and amoxicillin/clavulanic acid. The isolates also showed high sensitivity to the following antimicrobials: amikacin, meropenem, and piperacillin/tazobactam. Overall, the resistance rates among ESBL-producing Enterobacteriaceae decreased from 2007 to 2013. **Conclusions**: In our hospital, the increased sensitivity to certain antimicrobial agents seems to be directly related to the implementation of improvements in the methods to prevent and control nosocomial infections in addition to the natural development of other resistance mechanisms.

Keywords: Antimicrobial profile. Drug resistance. Extended spectrum beta-lactamase. Nosocomial infections.

INTRODUCTION

Nosocomial infections are closely related to antimicrobial drug resistance, which is a threat to global public health because of its great impact on morbidity and mortality, length of hospital stay, and costs of diagnosis and treatment[1] [2]. The most significant nosocomial infections are usually caused by multi-resistant microorganisms that show various mechanisms of resistance against the common treatments used to control infectious diseases. Enterobacteriaceae are the most frequent pathogens causing nosocomial infections. Some members of this family produce extended spectrum β-lactamases (ESBLs), which is one of the most prevalent antibiotic resistance mechanisms[2] [3]. Even these strains most frequently cause infections that occur in hospital intensive care units (ICUs), they have also been reported in other hospital settings (such as in wards) as well as in association with community-acquired infections[4] [5].

Extended spectrum β-lactamase-producing microorganisms are resistant to the action of third-generation cephalosporins (except for cephamycins) and monobactams (such as aztreonam). These microorganisms may also hydrolyze carbapenems (such as meropenem, imipenem, and ertapenem) through *Klebsiella pneumoniae* carbapenemase (KPC) or via association between ESBL and chromosomal AmpC-type β-lactamases[6] [7] [8]. The hydrolytic action of these enzymes is usually blocked by serine β-lactamase inhibitors such as clavulanic acid, sulbactam, and tazobactam[9].

Because the prevalence, phenotypic characteristics, and antimicrobial susceptibility profile of ESBL-producing strains may vary from one region to another, epidemiological studies are urgently needed to establish appropriate therapeutic management strategies and methods to prevent infections caused by these multi-resistant microorganisms[10] [11].

Toward this end, the objective of this study was to evaluate the prevalence and antimicrobial susceptibility profile of ESBL-producing strains in isolates from patients admitted to the *Hospital São Vicente de Paulo* (HSVP), located in Passo Fundo, State of Rio Grande do Sul, Brazil. We also compared our results with data collected from the same hospital in 2007 in order to assess the trend in antimicrobial evolution in recent years.

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METHODS

Study center

The present study was conducted at the Laboratory of Clinical Analyses of the HSVP, located in Passo Fundo, State of Rio Grande do Sul, Brazil. The hospital has 614 beds and 123 recovery beds, in a building with an area of approximately 50,000m². There are nearly 32,000 admissions per year.

Study design and data collection

We conducted a descriptive, observational, cross-sectional study involving the patients admitted to the HSVP who had bacterial cultures performed from January to December 2013.

Data were collected from the computer system of the HSVP using the software at the Laboratory of Clinical Analyses. We collected the following data: age (group I: 0-2 years; group II: 3-12 years; group III: 13-25 years; group IV: 26-45 years; group V: 46-60 years; group VI: >61 years), sex, inpatient unit, patient's laboratory record number, type of biological material, result of culture, and antibiogram.

Data from patients with positive cultures for Enterobacteriaceae and ESBL-producing microorganisms were analyzed in further detail. For epidemiological purposes, negative cultures were also evaluated.

Definitions

The antimicrobial susceptibility profile was determined using the disk diffusion method according to the standards of the Clinical and Laboratory Standards Institute (CLSI) [12]. The antimicrobials tested for Enterobacteriaceae were as follows: amikacin (30μg), amoxicillin/clavulanic acid (20/10μg), ampicillin (10μg), aztreonam (30μg), cephalothin (30μg), cefepime (30μg), ceftazidime (30μg), ceftriaxone (30μg), ciprofloxacin (5μg), gentamicin (10μg), meropenem (10μg), nitrofurantoin (300μg), norfloxacin (10μg), piperacillin/tazobactam (100/10μg), and sulfamethoxazole/trimethoprim (1.25/23.75μg). The phenotypic screening for ESBL producers was conducted using the disk approximation method in compliance with the standards of the CLSI[12].

Statistical analysis

The frequency of positive cultures was calculated. Based on this result, we also calculated the frequency of infections caused by Enterobacteriaceae. The percentage of ESBL-producing strains was calculated considering only the cultures positive for Enterobacteriaceae. Data from the positive cultures and ESBL-producing strains were compared with respect to the type of biological sample, antimicrobial susceptibility profile, and demographic data (sex, age, and inpatient unit) using the Statistical Package for the Social Sciences (SPSS) 22.

Ethical considerations

Experiments with performed in accordance with the resolution of the National Council of Ethics in Research (CONEP) 466/12 concerning research with human beings, and in keeping with the Helsinki Declaration of 1964, as revised in 1975, 1983, 1989, 1996, and 2000. The study was approved by the Research Ethics Committee of the HSVP and by the Research Ethics Committee of the *Universidade de Passo Fundo*, which is affiliated to the CONEP, under the number 33281914.7.0000.5342.

RESULTS

We evaluated data from a total of 19,112 cultures. Overall, 11.5% (2,197/19,112) of the cultures were positive for Enterobacteriaceae. Among these, 30.3% (666/2,197) of the bacterial isolates had a positive screening test for ESBL production. The most prevalent species was *Klebsiella* sp. (37.5%; 250/666), followed by *Escherichia coli* (34.4%; 229/666), *Enterobacter* sp. (18.3%; 122/666), *Citrobacter* sp. (3.6%; 24/666), *Proteus mirabilis* (2.7%; 18/666), *Edwardsiella* sp. (1.5%; 10/666), *Proteus vulgaris* (1.1%; 7/666), *Serratia* sp. (0.3%; 2/666), *Providencia* sp. (0.3%; 2/666), and *Proteus* sp. (0.3%; 2/666).

With respect to demographic variables among the patients with a positive culture result, men were more frequently infected with ESBL-producing isolates than women (62.5%; 416/666 vs 37.5%; 250/666). In terms of age, the mean age was 53.7 ± 26.6 years, and the patients were divided as follows into groups I, II, III, IV, V, and VI: 10.6%, 4.5%, 7%, 11.7%, 17.6%, and 48.7%, respectively. Therefore, the majority of infected patients were elderly (>61 years).

ESBL-producing isolates were mainly found in the urine (46.8%; 312/666), tracheal aspirate (11.9%; 79/666), stool (7.1%; 47/666), and mucocutaneous lesions (5%; 33/666). These isolates were less frequently found in other types of biological samples such as sputum, bronchial lavage and aspirates, peritoneal and pleural fluids, catheters, blood, surgical and non-surgical wound discharge, and eye secretions.

With respect to different parts of the hospital, patients in the wards showed the highest prevalence of ESBL-producing strains (81.2%; 541/666), followed by the central ICU (9.3%; 62/666), with lower percentages found in the neonatal, pediatric, and cardiology ICUs. The wards include 17 admission centers, recovery rooms, the emergency department, maternity ward, surgical center, dialysis unit, and ICU/nursing (ICU/N).

Extended spectrum β-lactamase-producing strains showed >95% reduced susceptibility to all cephalosporins, aztreonam, and amoxicillin/clavulanic acid. However, these antimicrobials proved to be effective for non-ESBL-producing strains **(Table 1)**. In particular, among these non-ESBL-producing strains, there was remarkably reduced susceptibility of *Enterobacter* sp. to amoxicillin/clavulanic acid (57.1%), *Escherichia coli* to ampicillin (59.1%) and cephalothin (54.8%), and *Klebsiella* sp. to cephalothin (52.8%).

A high percentage of the ESBL-producing isolates of *Klebsiella* sp. showed reduced susceptibility to ciprofloxacin (73.5%) and gentamicin (64.4%), as well as to the combination sulfamethoxazole/trimethoprim (69.6%), which were higher than

TABLE 1 - Percentage of reduced susceptibility to tested antimicrobials among ESBL-producing and non-producing strains of *Enterobacter* sp., *Escherichia coli*, and *Klebsiella* sp.

	Reduced susceptibility (%)					
	Enterobacter sp.		*Escherichia coli*		*Klebsiella* sp.	
	ESBL	non-ESBL	ESBL	non-ESBL	ESBL	non-ESBL
AMI	5.7	0.6	4.8	0.7	4.4	3.8
AMC	87.5	57.1	97.8	13.4	98.8	23.0
AMP	NR	NR	98.7	59.5	NR	0.0
ATM	97.5	7.8	96.9	1.8	99.2	10.6
CFL	NR	NR	99.6	54.8	99.2	52.8
CPM	96.7	9.6	96.5	1.7	97.2	12.4
CAZ	95.9	7.8	95.2	1.4	98.4	10.0
CRO	99.2	11.6	99.6	2.4	98.8	12.4
CIP	57.4	11.9	66.1	19.8	73.5	16.1
GEN	59.0	10.1	50.0	7.5	64.4	11.8
MER	NR	NR	0.9	0.0	1.2	1.2
NIT	54.8	18.6	31.8	5.8	71.1	25.5
NOR	76.2	23.3	68.5	23.1	87.8	33.9
PIT	14.2	3.6	9.2	0.3	11.7	3.1
SUT	50.9	17.6	53.7	32.5	69.6	16.1

ESBL: extended spectrum β-lactamases; **AMI:** amikacin; **AMC:** amoxicillin/clavulanic acid; **AMP:** ampicillin; **ATM:** aztreonam; **CFL:** cephalothin; **CPM:** cefepime; **CAZ:** ceftazidime; **CRO:** ceftriaxone; **CIP:** ciprofloxacin; **GEN:** gentamicin; **MER:** meropenem; **NIT:** nitrofurantoin; **NOR:** norfloxacin; **PIT:** piperacillin/tazobactam; **SUT:** sulfamethoxazole/trimethoprim; **NR**: not rated.

the corresponding rates for *Enterobacter* sp. and *Escherichia coli* isolates **(Table 1)**. The ESBL-producing isolates showed only low resistance to amikacin, meropenem, and piperacillin/tazobactam.

Nitrofurantoin and norfloxacin were only tested in urine samples, and a high percentage of the ESBL-producing isolates showed reduced susceptibility to norfloxacin, especially *Enterobacter* sp., *Escherichia coli*, and *Klebsiella* sp. **(Table 1)**.

Comparison of the data related to antimicrobial drug resistance at the same hospital in 2007 and 2013 for ESBL-producing strains revealed that *Enterobacter* sp., *Escherichia coli*, and *Klebsiella* sp. showed an increased sensitivity to piperacillin/tazobactam, nitrofurantoin, meropenem, gentamicin, and amikacin in 2013. Nevertheless, all microorganisms showed increased resistance to ceftriaxone, with a greater increase among the strains of *Klebsiella* sp. **(Figure 1, Figure 2** and **Figure 3)**.

For the genera *Enterobacter* sp. and *Klebsiella* sp., there was a decrease in the percentage of isolates showing reduced susceptibility to norfloxacin in 2013 when compared with the data from 2007 **(Figure 1** and **Figure 3)**. Conversely, *Escherichia coli* showed an overall pattern of increased resistance to norfloxacin **(Figure 2)**.

DISCUSSION

Extended spectrum β-lactamase phenotypic screening of Enterobacteriaceae is important for epidemiological purposes, as well as to understand and properly manage the development of resistance mechanisms[13]. In our study, ESBL production was detected in different genera, with an overall prevalence of 30.3% in the strains tested and the majority of positive isolates belonging to *Enterobacter* sp., *Escherichia coli,* and *Klebsiella* sp. Similar percentages were reported in other studies conducted by Seki et al.[14] (40.2%), Lenhard-Vidal et al.[15] (22%), and Lago et al.[16] (24.8%) on the prevalence of ESBL-producers among Enterobacteriaceae. These studies analyzed data acquired in Brazilian hospitals between September 2007 and September 2008, January 2004 and December 2009, and July and December 2007, respectively. The study by Lago et al.[16] was conducted at the same hospital as the present study.

The main risk factors for colonization by ESBL-producing isolates include invasive procedures such as use of a central venous line or arterial catheter, length of hospital stay (especially in ICUs), urinary catheter, mechanical ventilation, severity of disease, and previous exposure to antibiotics[4] [17].

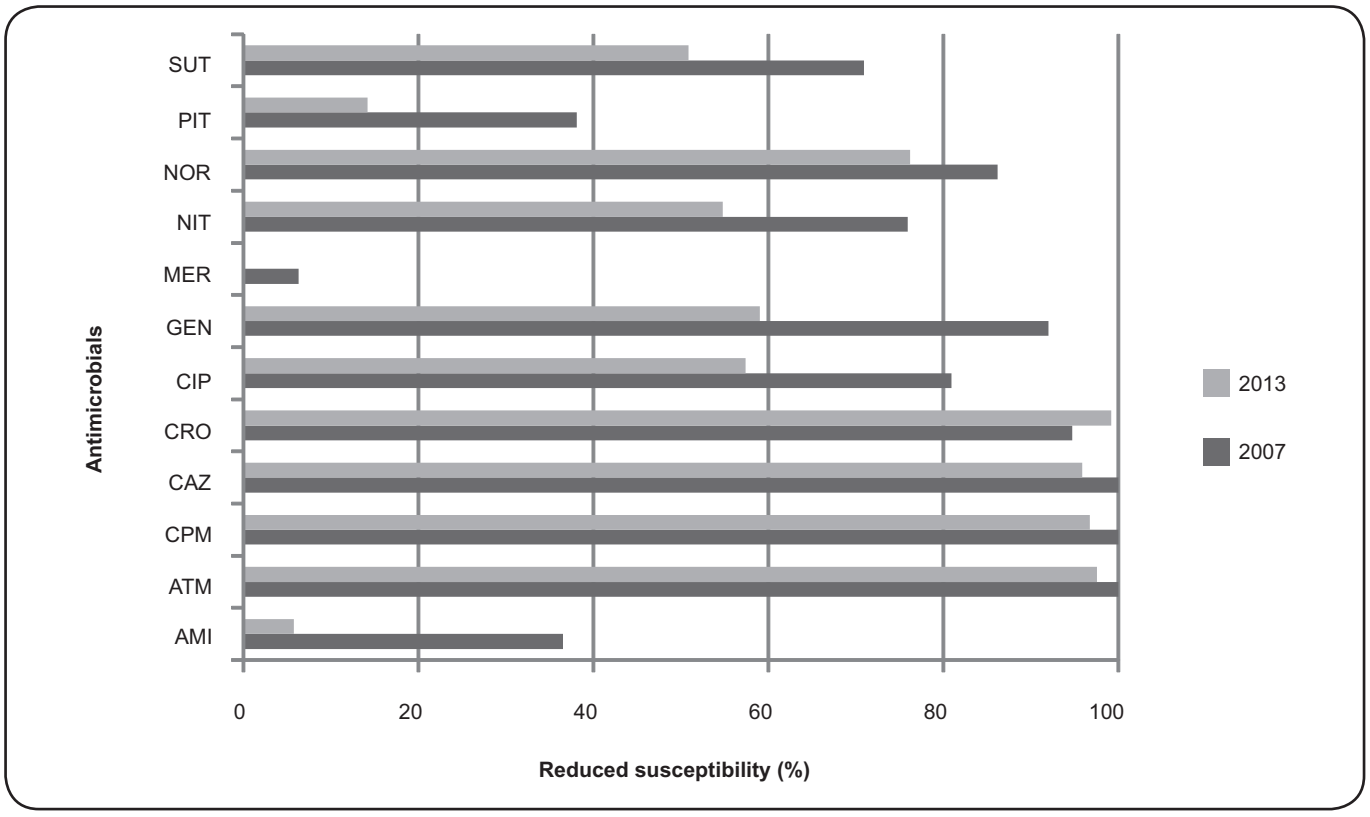

FIGURE 1 - Percentage of reduced susceptibility among strains of *Enterobacter* sp. between 2007 and 2013. SUT: sulfamethoxazole/trimethoprim; **PIT:** piperacillin/tazobactam; **NOR:** norfloxacin; **NIT:** nitrofurantoin; **MER:** meropenem; **GEN:** gentamicin; **CIP:** ciprofloxacin; **CRO:** ceftriaxone; **CAZ:** ceftazidime; **CPM:** cefepime; **ATM:** aztreonam; **AMI:** amikacin.

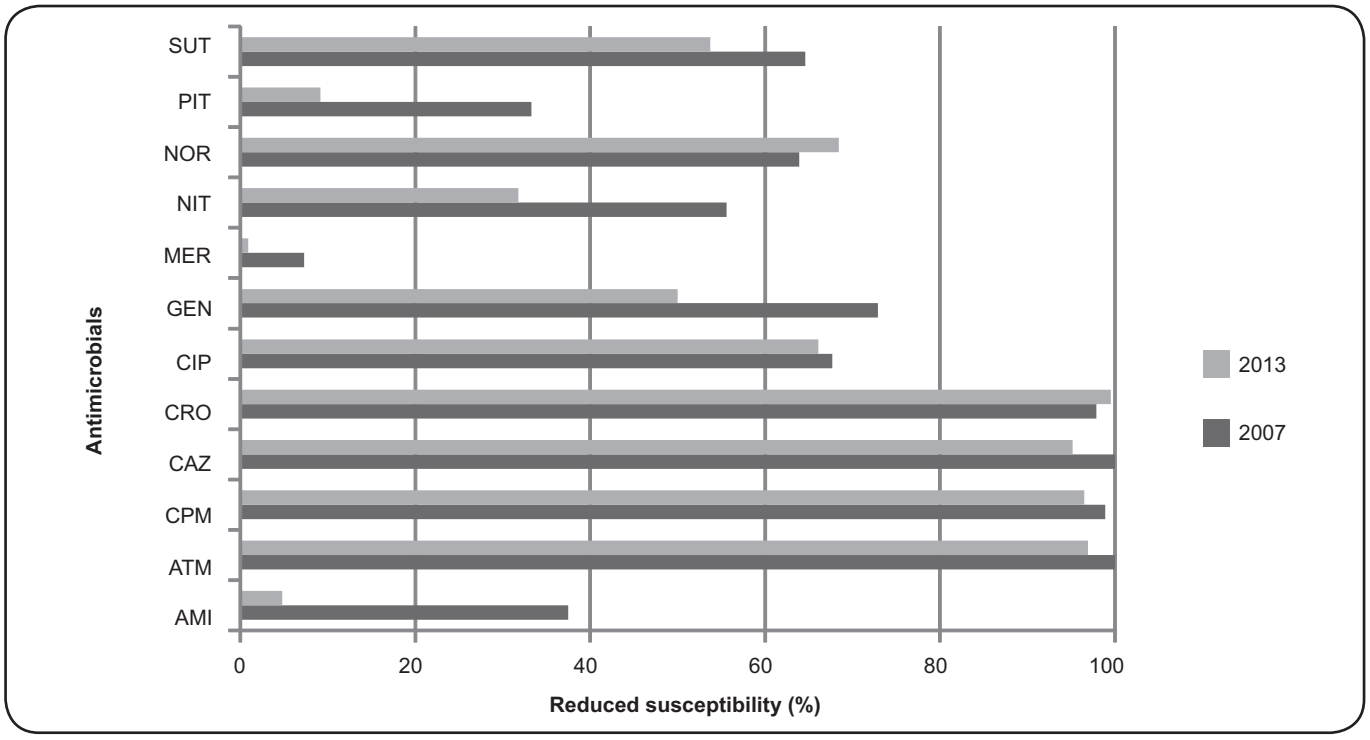

FIGURE 2 - Percentage of reduced susceptibility among strains of *Escherichia coli* between 2007 and 2013. **SUT:** sulfamethoxazole/trimethoprim; **PIT:** piperacillin/tazobactam; **NOR:** norfloxacin; **NIT:** nitrofurantoin; **MER:** meropenem; **GEN:** gentamicin; **CIP:** ciprofloxacin; **CRO:** ceftriaxone; **CAZ:** ceftazidime; **CPM:** cefepime; **ATM:** aztreonam; **AMI:** amikacin.

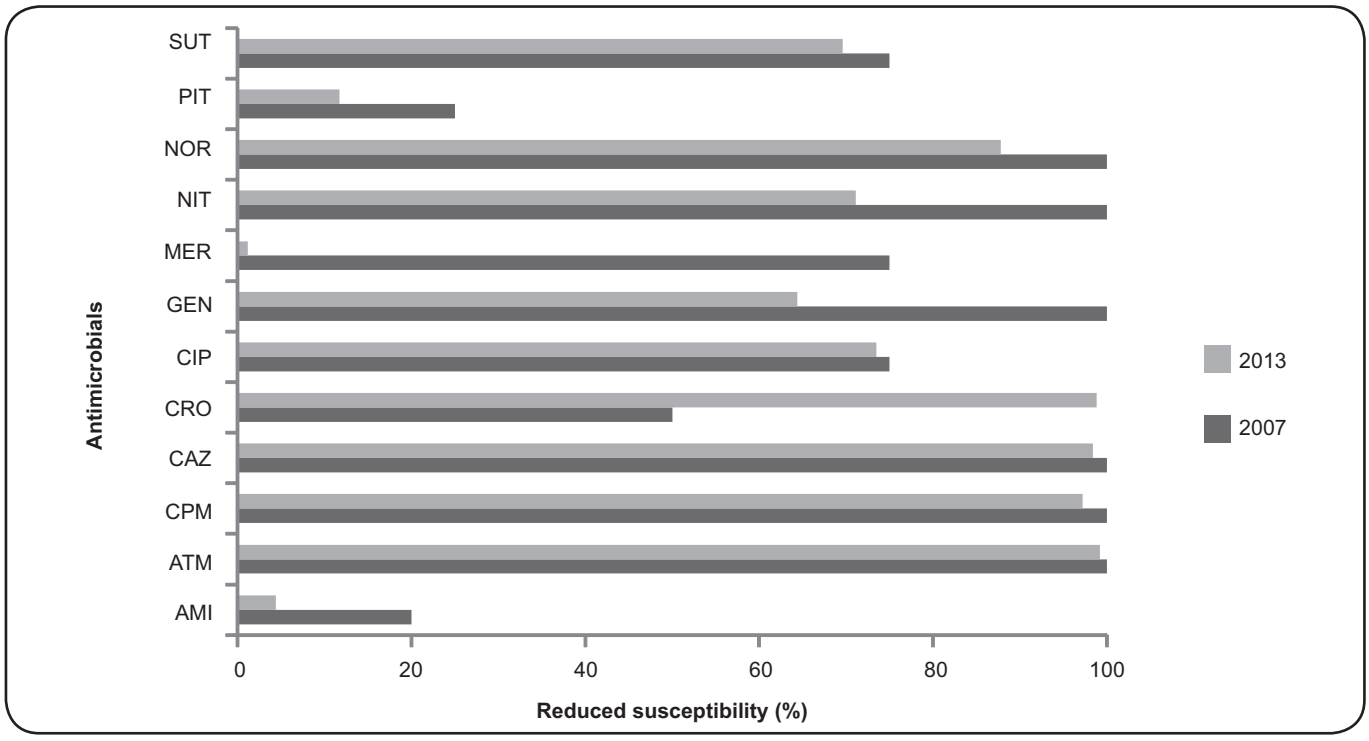

FIGURE 3 - Percentage of reduced susceptibility among strains of *Klebsiella* sp. between 2007 and 2013. SUT: sulfamethoxazole/trimethoprim; **PIT:** piperacillin/tazobactam; **NOR:** norfloxacin; **NIT:** nitrofurantoin; **MER:** meropenem; **GEN:** gentamicin; **CIP:** ciprofloxacin; **CRO:** ceftriaxone; **CAZ:** ceftazidime; **CPM:** cefepime; **ATM:** aztreonam; **AMI:** amikacin.

The highest incidence of ESBL-producing bacteria was found in the wards, especially in the ICU/N. This finding may be explained by the unique and variable characteristics of this unit, which receives chronic patients who stay for an indeterminate length of time, are often treated with broad-spectrum antibiotics for a long time, and have many debilitating underlying diseases.

ESBL-producing isolates of *Klebsiella* sp. and, to a less degree, of *Enterobacter* sp. and *Escherichia coli* were resistant to gentamicin, an aminoglycoside-class antibiotic. Such resistance may have developed owing to the fact that this drug is used in large amounts, since these same isolates showed high sensitivity to amikacin.

Based on our findings, the *in vitro* drugs that proved to be most effective to treat infections caused by ESBL-producing multidrug-resistant microorganisms were amikacin, meropenem, and a combination of piperacillin with tazobactam (a serine β-lactamase inhibitor). *Enterobacter* sp., *Escherichia coli*, and *Klebsiella* sp. were found to be highly resistant to the combination of amoxicillin and clavulanic acid, which is unusual for ESBL producers. The chromosomal β-lactamases (AmpC) constitute another group of enzymes with a serine active site that are able to hydrolyze cephalosporins, and also confer microorganisms with resistance to inhibitors such as clavulanate, sulbactam, and tazobactam[18]. Drawz and Bonomo[19] reported that piperacillin is relatively resistant to hydrolysis through certain plasmid-mediated β-lactamases as compared with amoxicillin or ampicillin, which makes its combination with a β-lactamase inhibitor more effective. Furthermore, the AmpC resistance mechanism is often associated with the presence of ESBL.

Quinolones are highly effective for treating acute cystitis. However, because of their side effects and the possibility of resistance in subsequent uses to treat more severe infections such as pyelonephritis, they should be used with caution and careful consideration[20]. In the present study, we found that isolates of *Escherichia coli* had increased resistance to norfloxacin, which may be explained by the high incidence of urinary tract infections caused by this microorganism. This finding is likely related to the broad use of quinolones in medical practice, or may be caused by the presence of another resistance mechanism such as efflux pumps[21] [22]. Another mechanism, which is more prevalent for gram-negative bacilli, is plasmid-mediated quinolone resistance (PMQR). This mechanism is mainly related to mutations in the chromosomal genes encoding topoisomerase II, the quinolone target site. Walsh and Rogers[23] demonstrated the relatively high prevalence of PMQR determinants among ESBL producers in Enterobacteriaceae, which may be another mechanism to explain the increased resistance to quinolones observed in the present study.

Comparing the data from 2007 and 2013 of our hospital, we found no evidence for increased antimicrobial resistance in the isolates of Enterobacteriaceae, except in the case of ceftriaxone. Another important trend observed was the increased sensitivity of *Enterobacter* sp., *Escherichia coli*, and *Klebsiella* sp. to piperacillin/tazobactam, nitrofurantoin, meropenem,

gentamicin, and amikacin. This suggests that improvements have been implemented in the hospital with respect to the prevention and control of nosocomial infection. In addition, there might have been changes to the primary therapeutic approaches used in the hospital during the period of analysis. Another important factor may have been the recent emergence of other resistance mechanisms such as KPC, metallo-β-lactamases, and AmpC.

The continuous isolation of ESBL-producing strains and the risk of treatment failure due to the administration of cephalosporins have resulted in greater use of carbapenems. The resistance to carbapenems is conferred by carbapenemases such as KPC and metallo-β-lactamases, which are more common in *Klebsiella* sp.[24]. This is a cause for concern because *Klebsiella* sp. are often resistant to different classes of drugs commonly used in the treatment of infections involving multidrug-resistant Enterobacteriaceae, but show especially high resistance rates to carbapenems[21] [25].

Our results suggest that, in the HSVP, broad-spectrum quinolones, aminoglycosides, and carbapenems should be used only when other drugs cannot be prescribed in order to avoid inducing increased resistance. Furthermore, genotypic screening studies are warranted to investigate other resistance mechanisms and their association with ESBL so that more effective interventions can be implemented.

It is important that (a) multidisciplinary teams are aware of the resistance profile of the microorganisms in their healthcare facilities and (b) to highlight the importance of adhering to nosocomial infection prevention measures such as proper hand washing, use of personal protective equipment, and, above all, appropriate prescription of antibiotics in compliance with the protocols established by the Nosocomial Infection Control Committee. The empirical choice of antimicrobial therapy should be based on knowledge of the local prevalence of microorganisms and their *in vitro* susceptibility, since the resistance patterns vary from one region to another.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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