

High prevalence rate of extended-spectrum beta-lactamases (ESBL) among *Enterobacteriaceae* in a small Brazilian public hospital

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The production of extended-spectrum beta-lactamases (ESBL) is considered one of the most important resistance mechanisms that impair antimicrobial treatment of infections caused by *Enterobacteriaceae*. Data on culture and susceptibility tests were collected from the Clinical Analyses and Research Laboratory charts reporting on patients admitted to the University Hospital of Maringá (HUM) from January 2004 to December 2009. The following *Enterobacteriaceae* were selected: *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Enterobacter* spp. and *Proteus mirabilis*. All tests were performed according to the recommendations of the Clinical and Laboratory Standards Institute (biochemical identification; susceptibility tests; initial screening and phenotypic confirmatory tests for ESBL). For *Enterobacter* spp. isolates, a disk approximation test was carried out, adding a cefepime disk. Seven hundred samples were analyzed, and *E. coli* was the most prevalent bacteria ($n=356$). ESBLs were detected phenotypically in 7.3% of *E. coli*, 61.7% of *K. pneumoniae*, 33.3% of *K. oxytoca*, 7.1% of *P. mirabilis*, and 13.4% of *Enterobacter* spp samples. Overall ESBL prevalence reached 22% when all producers were taken together. Although HUM is considered a small-sized hospital, it showed high levels of resistance to antimicrobial agents, similar to those observed in bigger hospitals, which demonstrated the need for careful epidemiological surveillance.

Uniterms: Extended-spectrum β -lactamases. *Enterobacteriaceae*. Bacterial resistance. β -lactams.

A produção de beta-lactamases de espectro ampliado (ESBL) é considerada um dos mais importantes mecanismos de resistência aos antimicrobianos, o que dificulta o tratamento de infecções causadas por enterobactérias. Dados sobre cultura e testes de sensibilidade foram coletados das fichas do Laboratório de Ensino e Pesquisa de Análises Clínicas de pacientes atendidos no Hospital Universitário de Maringá (HUM), de janeiro de 2004 a dezembro de 2009. As enterobactérias escolhidas foram: *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Enterobacter* spp. e *Proteus mirabilis*. Os testes foram realizados de acordo com as recomendações do CLSI (identificação bioquímica; testes de suscetibilidade; triagem e confirmação fenotípica de produção de ESBL). Para isolados de *Enterobacter* spp., utilizouse a técnica de disco aproximação, acrescentando um disco de cefepima. Setecentas amostras foram analisadas e *E. coli* foi a bactéria mais prevalente ($n=356$). ESBLs foram detectadas fenotipicamente em 7,3% das amostras de *E. coli*, 61,7% das de *K. pneumoniae*, 3,3% das de *K. oxytoca*, 7,1% das de *P. mirabilis* e em 13,4% das de *Enterobacter* spp. A prevalência geral de ESBL chegou a 22%, somando-se todos os isolados produtores. O HUM, mesmo sendo considerado um hospital de pequeno porte, apresenta níveis altos de resistência a antimicrobianos, semelhantes àqueles observados em hospitais maiores, demonstrando a necessidade de cuidadosa vigilância epidemiológica.

Unitermos: β -lactamases de espectro estendido. *Enterobacteriaceae*. Resistência bacteriana. β -lactâmicos.

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INTRODUCTION

An important emerging resistance mechanism among *Enterobacteriaceae* is the production of extended-spectrum beta-lactamases (ESBL), which turn these bacilli highly efficient in inactivating third-generation cephalosporins, monobactams, and penicillins (Hawkey, 2008). *Escherichia coli* and *Klebsiella pneumoniae* are the main ESBL-producing bacteria, even though other members of the *Enterobacteriaceae* family, namely *Proteus mirabilis*, *Enterobacter* spp., *Salmonella* spp., and *Serratia* spp., may show such resistance, albeit with less frequency (Thomson, 2001; Chaudhary, Aggarwal, 2004).

ESBL-producing organisms are clinically relevant and remain an important cause of therapy failure when cephalosporins are used, even when the bacteria appear to be sensitive to these agents by routine susceptibility testing (Pateron, Bonomo, 2005). Further, ESBL-producing bacteria are frequently resistant to many other non-beta-lactam antibiotics, limiting the available therapeutic options (Rice, Bonomo, 2007). In addition, owing to the resistance mechanism in plasmidial genes, patients colonized by or infected with ESBL-producing bacteria should be placed under contact supervision to avoid transmission of hospital infection (Siegel *et al.*, 2006).

ESBL-producing *Enterobacteriaceae* have been a problem in most hospitals worldwide. Very high rates have been reported in Asia, especially in China (55%) and India (79%), including isolates from the community (Pitout, 2010). Although the frequency of isolation of these microorganisms in Brazil (10% for *E. coli* and 17% for *Klebsiella* spp.) is lower than that found in Asian countries, it has been higher than in the United States and Europe (Rossi, 2011).

The presence of this enzyme implies an increase in the use of carbapenems in patient's treatment, so there is a greater likelihood of the emergence of carbapenemase-producing bacteria, enzymes capable of hydrolyzing carbapenems (Pitout, 2010). The presence of *Enterobacteriaceae* which produce carbapenemases is already a reality in most hospitals (Nordmann *et al.*, 2009). However, the emergence of such resistance in bacteria from community patients, as already reported, surely hampers its control (Nordmann *et al.*, 2011).

During the last few years, some studies have demonstrated that the breakpoints for cephalosporins, used by most laboratories, failed to detect many ESBL-producing *Enterobacteriaceae* (Wong-Beringer *et al.*, 2002; Pateron, 2006). The European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI) have recently

revised breakpoints for third-generation cephalosporin (Kahlmeter, 2008; CLSI, 2010). Until 2009, it had been recommended that, if ESBL production was confirmed, the laboratory must report all penicillins, cephalosporins and aztreonam as resistant, even though the susceptibility test showed sensitive results *in vitro* (CLSI, 2009). However, using the new breakpoints as recommended by CLSI, ESBL detection may be restricted for epidemiological or infection control purposes (CLSI, 2010).

Several phenotypic confirmatory tests for ESBL producers have been described in the literature. All methods utilize the characteristics of ESBL production inhibition by clavulanic acid. The CLSI recommended test consists of an initial screening test and a phenotypic confirmatory test. Susceptibilities to ceftazidime, ceftriaxone, cefotaxime, cefpodoxime and aztreonam in the initial screening test are evaluated by disk diffusion or by the broth dilution method. A decrease in susceptibilities to one or more antibiotics tested may indicate production of ESBLs and require the subsequent confirmatory test. This test is carried out using disk diffusion or broth dilution methods, and the performance of cefotaxime and ceftazidime in the presence and absence of clavulanate is evaluated (CLSI, 2010).

Resistance in *Enterobacteriaceae* remains a major problem in most hospitals in spite of the current debate on the relationship between minimum inhibitory concentrations (MICs), detection of ESBL, and clinical results when third-generation cephalosporins are used.

The present study reports on ESBL production frequency among the commonest isolated *Enterobacteriaceae* in a public university hospital in Maringá (PR), Brazil, during a six-year period.

METHODS

Data on culture and susceptibility tests were collected from the Clinical Analyses and Research Laboratory (LEPAC) charts reporting on patients admitted to the University Hospital of Maringá (HUM) from January 2004 to December 2009. HUM is a small size hospital, with 120 beds, of which 20 belong to the intensive care units (ICUs), and the others are distributed among general, surgical, obstetric and pediatric units. Since 2003, HUM participates in the *Projeto Hospital Sentinela* and in the *Rede de Monitoramento de Resistência – Agência Nacional de Vigilância Sanitária*.

No personal data were retrieved from patients so that privacy could be warranted and law transgressions concerning research with human beings avoided (Resolution 196/96 Brazil National Health Council, Health Ministry).

The present study was submitted to and approved by the HUM's Regulatory Commission of Academic Activities and Voluntary Services.

The *Enterobacteriaceae* selected for this study were: *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Enterobacter* spp., and *Proteus mirabilis*. These species were chosen since they are more frequent among the *Enterobacteriaceae* commonly detected in hospitals around the world (Baquero *et al.*, 2009). Only one isolate from each bacterial species was selected per patient.

As part of LEPAC Clinical Bacteriology Sector routine, the bacterial isolates were biochemically identified and submitted to susceptibility tests, using AUTO-SCAN-4 automated system (Siemens Microscan, Inc., Deerfield, IL, USA). The antimicrobials tested and the interpretation criteria were the ones recommended by the CLSI (CLSI, 2004-2010).

The selected *Enterobacteriaceae* that had MICs suggestive of ESBL production were submitted to confirmatory tests by double disk diffusion (CLSI, 2004-2010). The disks used were of ceftazidime (30 µg), cefpodoxime (10 µg) and cefotaxime (30 µg) combined with clavulanate (10 µg), and disks containing only the referred cephalosporins (Oxoid, Basingstoke, England). A ≥ 5 mm increase in growth inhibition zone for any antimicrobial associated with clavulanate in comparison with the inhibition zone of the antimicrobial tested alone confirmed ESBL production. For *Enterobacter* spp. isolates, a disk approximation test was carried out (Tumbarello *et al.*, 2004), adding a cefepime disk (30 µg; Oxoid Basingstoke, England).

RESULTS AND DISCUSSION

Increasing number of reports on bacteria resistant to beta-lactam antibiotics is a great concern since these drugs are frequently the primary arsenal for the treatment of bacterial infections. The detection of ESBL-producing bacteria isolated from clinical samples is highly important, since physicians may choose a probably more efficient treatment. Besides their resistance to penicillins, cephalosporins and aztreonam, these pathogens are also frequently resistant to other classes of antimicrobial agents and thus limit therapeutic options (Rice & Bonomo, 2007).

Seven hundred *Enterobacteriaceae* isolated from clinical samples of patients admitted to different HUM units were studied, of which 356 (50.9%) were identified as *E. coli*, 167 (23.9%) as *K. pneumoniae*, 134 (19.1%) as *Enterobacter* spp., 28 (4.0%) as *P. mirabilis*, and 15 (2.1%) as *K. oxytoca*. When isolates from all species are taken into consideration, urine was the most frequent clinical source (Figure 1).

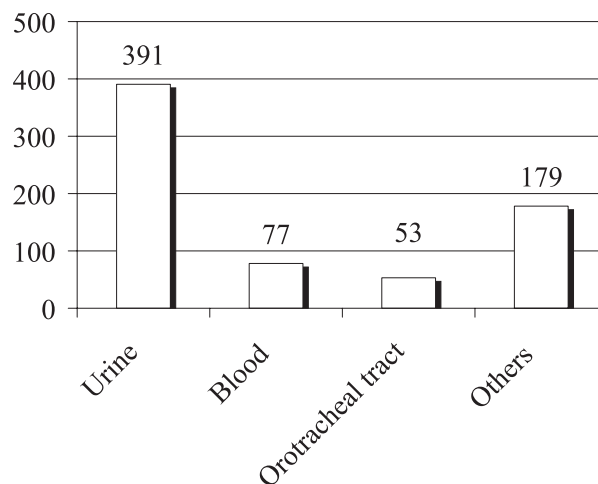


FIGURE 1 - Number of bacterial isolates from HUM per type of biological source, between January 2004 and December 2009.

There was an overall prevalence of 22% of ESBL production among the bacteria studied. Although this number may be considered high, it is close to that demonstrated in other Brazilian studies (Mendes *et al.*, 2000; Nogueira *et al.*, 2006).

The prevalence of ESBL-producing *K. pneumoniae* is high in many countries and of special concern in South American countries (Shah *et al.*, 2004; Nogueira *et al.*, 2006; Reinert *et al.*, 2007). *K. pneumoniae* isolates had the highest positiveness level for ESBL production in HUM (61.7%). The prevalence was high in almost every sector (Figure 2). One of the possible reasons for such high prevalence could be the fact that almost half of all *K. pneumoniae* isolated came from samples of ICU patients, including adult and pediatric ICUs. ESBL-producing bacteria were detected as important agents of bloodstream infection in pediatric ICU patients and contributed to high mortality rates recorded in these patients (Prabhu *et al.*, 2010). Although the prevalence of ESBL-producing *K. pneumoniae* remained steady for the past few years (with no significant increase), its high prevalence in the hospitalized population becomes a constant threat due to the subsequent narrowest choice of effective antimicrobials.

Although few *K. oxytoca* were isolated (15), 5 (33.3%) revealed ESBL-production, with 1 isolate from the general unit, 2 from the ICU for adults and 2 in the pediatric ICU. High prevalence of ESBL-producing *K. oxytoca*, close to 30%, as seen in this and other studies (Peloso *et al.*, 2003; Minarini *et al.*, 2008), has been rare. Lower prevalence is usually observed, with values close to 10% (Rossi *et al.*, 2006; Coque *et al.*, 2008). In fact, few *K. oxytoca* were isolated, and since 46% of all samples came from the ICUs, such high rate is justified.

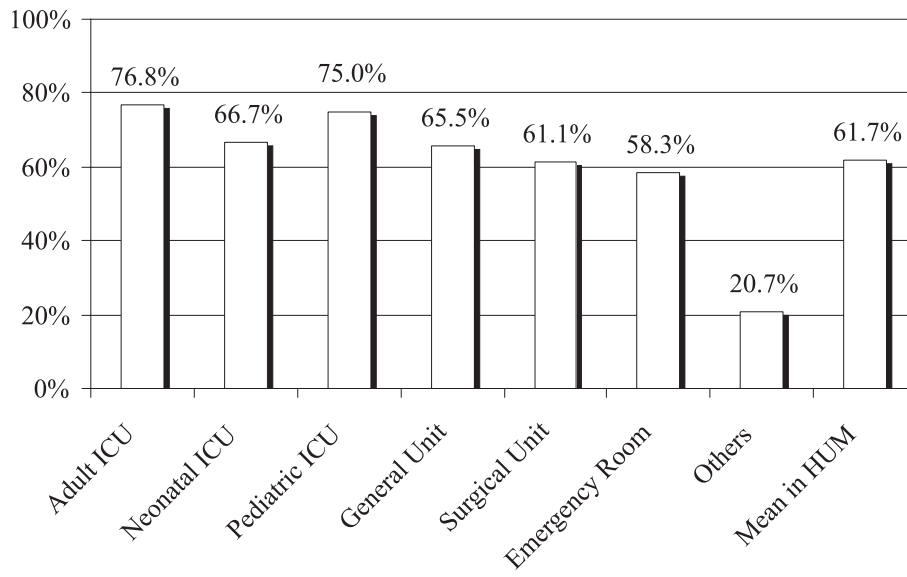


FIGURE 2 - Percentage of isolates of ESBL-producing *K. pneumoniae*, per admittance unit in HUM, from January 2004 to December 2009.

Among *Enterobacteriaceae*, *E. coli* is one of the commonest species isolated in nosocomial infections (Baquero *et al.*, 2009). Most *E. coli* were isolated from urine samples of outpatients attended at the emergency room (ER), with a total of 26 (7.3%) showing ESBL production. These patients stayed at the ER for a variable period of time until treatment was defined, and then were discharged. The pediatric ICU had the highest proportion of *E. coli* ESBL-producers and non-producers (Figure 3). The prevalence of ESBL-producing *E. coli* varies widely among countries, being <1% in some regions (Bradford, 2001; Coque *et al.*, 2008); however, higher rates, similar to

those found in the present study, have also been reported, especially in Latin American countries (Reinert *et al.*, 2007; Baquero *et al.*, 2009).

The high isolation rates of ESBL-producing *E. coli* in many countries, and hence the increasing use of carbapenems, highlight another public health issue: the emergence of carbapenemase-producing bacteria (Pitout, Laupland, 2008). Since *E. coli* is an important member of the human microbiota and agent of several kinds of nosocomial and community infections, it is essential to detect and control these mechanisms of resistance (Nordmann *et al.*, 2011)

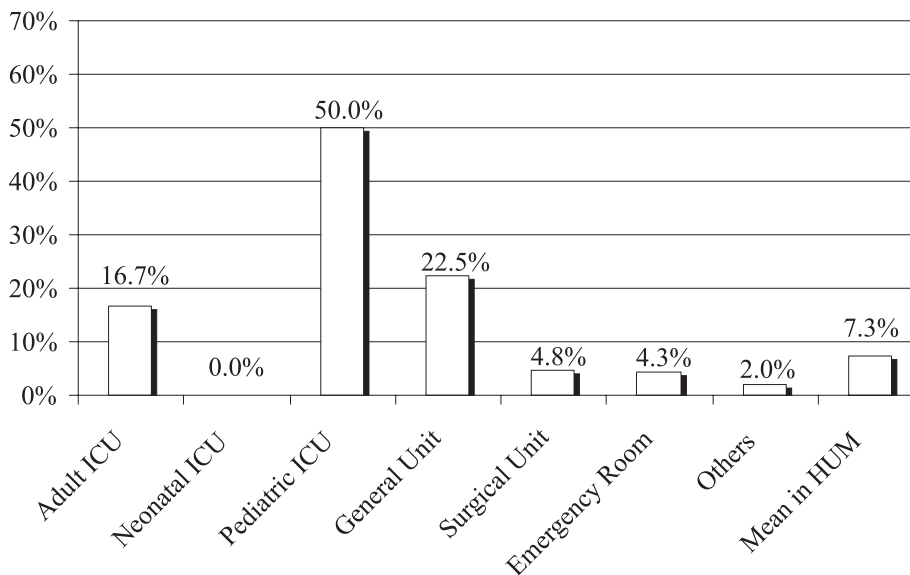


FIGURE 3 - Percentage of isolates of ESBL-producing *E. coli*, per admittance unit in HUM, from January 2004 to December 2009.

During the study period, *Enterobacter* spp. was isolated from 134 patients of all units, with urine as the commonest source (51.5%). The most prevalent species was *E. cloacae* (60.4%). Among the isolates, 8 *E. aerogenes* and 10 *E. cloacae* were ESBL-producers, of which 9 isolates came from patients admitted to the ICU for adults. The high number of ESBL-producing *Enterobacter* spp. found in the literature (Crowley, Ratcliffe, 2003; Rossi *et al.*, 2006) demonstrates how important it is to suspect a resistance mechanism also in this genus. Nevertheless, it is more difficult to identify ESBL production in *Enterobacter* spp. In fact, the genus may also present AmpC chromosomal enzyme, which, when induced by clavulanate, causes cephalosporin hydrolysis, as does ESBLs. Consequently, cefepime, a fourth-generation cephalosporin, is recommended in confirmatory tests, since this drug is weakly degraded by AmpC chromosomal enzyme but destroyed by ESBLs (Crowley, Ratcliffe, 2003; Tumbarello *et al.*, 2004; Rossi *et al.*, 2006). Although there are no CLSI recommendations for the detection of ESBL in *Enterobacter* spp., the present study demonstrated the importance to detect this kind of enzyme also in this genus, since 13.4% of the isolates were ESBL positive.

In the case of *P. mirabilis*, CLSI recommends ESBL screening tests only when it is clinically relevant, as a bacteremic isolate. At LEPAC, any lower sensitivity was investigated, even in less noble clinical specimens; even though, only 2 ESBL-producing bacteria were found among a total of 28 isolates: one was isolated from a pleural fluid sample from a patient in the general unit and the other was from an operated wound from a patient who had returned to the ER. This generated a prevalence of 7.1%, as observed in other studies in Brazil (Peloso *et al.*, 2003; Nogueira *et al.*, 2006) and in other Latin American countries (Dalmarco *et al.*, 2006).

Although CLSI recommends the detection of ESBL only for *K. pneumoniae*, *K. oxytoca*, *E. coli*, and occasionally *P. mirabilis*, it is known that other genera from the *Enterobacteriaceae* family, such as *Shigella*, *Morganella*, *Citrobacter*, and *Serratia*, may carry ESBL coding genes (Bonnet, 2004). It is thus important to look out for lower sensitivity levels to cephalosporins in other species, with particular attention to hospitalized patients. Reports of ESBL production in other CLSI's non-recommended *Enterobacteriaceae* species demonstrate the need to standardize detection techniques for this enzyme in other bacteria too.

Clinical results show that, when third-generation cephalosporins in ESBL-producing bacteria are used, a better evaluation is required, due to the alteration in susceptibility breakpoints for these antimicrobial substances.

With regard to DNA analysis of ESBL positive isolates, it is interesting to find out whether the high prevalence reported in the present study was due to the dissemination of one or a few mutant clones, or whether it was due to an individual selection of resistant strains.

CONCLUSION

Epidemiological studies similar to the present one turn out to be important to evaluate an institution's local status and to formulate a policy of empiric therapy. Although HUM is considered a small-sized hospital, it presents high levels of antimicrobial resistance, similar to those observed in bigger hospitals; therefore, careful epidemiological surveillance is clearly mandatory. Unfortunately, that may also be the case of many other Brazilian hospitals.

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Received for publication on 15th February 2011

Accepted for publication on 06th June 2011

