Advances in Clinical and Medical Microbiology
Received: Feb 23, 2016, Accepted: Apr 14, 2016, Published: Apr 18, 2016
Adv Clin Med Microbiol, Volume 1, Issue 1
http://crescopublications.org/pdf/acmm/ACMM-1-003.pdf
Article Number: ACMM-1-003

Research Article Open Access

Surveillance of ESBL-Producing Isolates Causing Urinary Tract Infections, in Elderly, in Aveiro, Portugal

S. Magalhães[1,2], I. Roxo[2], E. Ramalheira[3] and S. Ferreira[3,4*]

[1]Chemistry Department, University of Aveiro, Aveiro, Portugal
[2]Health Department, University of Aveiro, iBiMED, Aveiro, Portugal
[3]Centro Hospitalar do Baixo Vouga EPE, Aveiro, Portugal
[4]Instituto de Educação e Cidadania, Mamarrosa, Portugal

***Corresponding Author**: Sónia Ferreira, Ph.D, Instituto de Educação e Cidadania, Mamarrosa, 3770-033, Portugal; Tel: +351 234757110, E-mail: scnferreira@gmail.com

Citation: S. Magalhães, I. Roxo, E. Ramalheira and S. Ferreira (2016) Surveillance of ESBL-Producing Isolates Causing Urinary Tract Infections, in Elderly, in Aveiro, Portugal. Adv Clin Med Microbiol 1: 003.

Abstract

Urinary tract infections (UTIs) are one of the most prevalent community-acquired infections and represent a huge annual cost in health care. Elderly are more susceptible to have infections and in this age group UTIs are associated with a poor prognosis and higher mortality rates. In this type of infections, extended spectrum beta-lactamases (ESBLs) producing isolates are an increasing cause of resistance to antibiotics. Therefore the main aim of this study was to access the prevalence of ESBL-producing isolates collected from urine of elderly attending the Emergency Room (ER) during a 4 years period, in Aveiro. The percentage of *Escherichia coli* has been increased since 2011 and the percentage of *Klebsiella pneumoniae* has been significantly decreasing in the same period. The percentage of ESBL-producing *K. pneumoniae* is always higher than ESBL-producing *E. coli*. The percentage of ESBL-producers has been significantly reduced in the last years, with a slight increase in 2014 however, the values are still high. Considering the criteria used to select the isolates we can conclude that they are disseminated in community, mainly in nursing homes, so it is necessary to adopt strict policies to avoid the spread of these resistant isolates.

Keywords: Elderly; Urinary tract infection; ESBL.

Introduction

Urinary tract infections (UTI) are one of the most prevalent infections in community worldwide [1] and are more frequent in female patients, mostly due to anatomic characteristics of female reproductive system [2]. Although most UTIs do not require hospitalization, the costs associated with this type of infection are tremendous [3]. The developments in biotechnology and life sciences along with an improvement in life conditions, allowed the increase of average life expectancy is in both genders, leading to an increase in the elderly population worldwide [4, 5]. However, in this population infectious diseases are a serious health problem, accounting for one third of deaths [4]. UTIs are one of the most common, whether acquired in the community or associated to health care and are associated with high rates of mortality and morbidity [6, 7]. The geriatric population is in itself more susceptible to develop an UTI due to complications associated with old age, such as fragility of immune system, limited mobility, incontinence, poor nutrition, diabetes, dementia and strokes, which lead to carelessness in hygiene care [4]. In addition, in the elderly, due to functional and structural changes, most UTIs are complicated and have atypical symptoms, which difficult an accurate diagnosis [8].

The massive use of β-lactam antibiotics for the treatment of infections, including UTIs, led to the development of resistance, mainly bacteria of *Enterobacteriaceae* family. In recent years, the production and dissemination of extended spectrum β-lactamases (ESBL), mainly in Europe, has been a major concern for health professionals. This class of enzymes, which degrade penicillin, cephalosporin and monobactam's, is very large and diversified, limiting available therapeutic options [9]. Therefore, conducting studies of epidemiological surveillance in order to monitoring these multirresistant strains and determine their distribution in the community is crucial. Thus, the aims of this study were determine which microorganisms are more frequently responsible for urinary tract infections in the community served by the Hospital Infante D. Pedro and to infer in the epidemiology of ESBL-producing bacteria in elderly patients attending Emergency Room with a primary diagnosis of an UTI.

Material and Methods

Bacterial Isolates

The clinical isolates were isolated from urine collected from patients during the timeframe of this study, January 2011 to December 2014. The isolates were selected according to the following criteria: primary diagnosis of an UTI, patients older than 65 years (inclusive) and from Emergency Room (ER). The population analyzed was non-repetitive and only a single isolate/patient was included in the study. Urine samples were cultured in CLED (Cystine Lactose Electrolyte Deficient) media and incubated aerobically during 18-24h at 35ºC. Samples with $\geq 10^5$ colony-forming units per mL of urine (CFU/mL) and with a pure culture were considered as "significant" and were further studied.

Bacterial Identification, Susceptibility Testing and ESBL Production

All bacteria occurring in samples with $\geq 10^5$ colony-forming units per mL of urine (CFU/mL) were identified using the VITEK 2 system and Advanced Expert System (AES) (bioMérieux, Marcy L´Étoile, France). The antimicrobial susceptibility of the strains was carried out according to guidelines of CLSI standards [10]. ESBL production highlighted by the Advanced Expert System was confirmed by manual method E-test® (BioMérieux, Marcy L´Étoile, France), with cefotaxime/ cefotaxime + clavulanic acid (CT/CTL), ceftazidime/ ceftazidime + clavulanic acid (TZ/TZL) and cefpime/ cefpime + clavulanic acid (PM/PML) strips according to the manufacturer's instructions.

Statistical Analysis

The results obtained were analyzed using the software Microsoft Office Excel 2013®, Microsoft Corporation©. To the statistical analysis it was used simple descriptive statistics, such as mean, standard deviation and frequencies. In the course of this work we also used software VITEK®2 Advanced Expert System (BioMérieux, Marcy-L'Etoile, France) and computer system Appolo, for laboratory management of the Clinical Pathology department of our hospital, which give us information on the history of microbiological data, conducting various statistical analysis and management of patients and requests.

Results

Bacterial Isolates

During the timeframe studied 1720 urine isolates were collected following the criteria listed above. As expected, the number of UTIs was significantly higher in females. Also expected, was the fact of *Escherichia coli* and *Klebsiella pneumoniae* isolates were the most prevalent Gram negative. Nevertheless, the percentage of *E. coli* isolates has been increasing since 2011, comparing with the percentage of *K. pneumoniae* isolates, which in 2011 was higher than *E. coli* but has been significantly decreasing in the last three years (Table 1).

Table 1: Percentage of *K. pneumoniae* and *E. coli* isolates among total of isolates in each year between 2011 and 2014.

	Clinical isolates selected (n)	*% of K. pneumoniae*	*% of E. coli*
2011	272	35.7	29.8
2012	384	19	48.4
2013	540	20.9	51.4
2014	524	15.1	46.8

Bacterial Identification, Susceptibility Testing and ESBL Production

In the timeframe studied, *E. coli* was always the most frequent microorganism, followed *K. pneumoniae* (data not shown). As shown in Figure 1, between 2011 and 2013 there was a reduction in the percentage of ESBL-producers. In 2011, 82.5% of *K. pneumoniae* isolates were ESBL-producers, however in 2013, this percentage decreases to 39.0%. *E. coli* ESBL-producers have also progressively diminished between 2011 and 2013, ranging between 44.4% and 13.4%, respectively. In 2014, however, there was a slight increase in ESBL-producers, to 44.1% in *K. pneumoniae* and 18.0% in *E. coli* isolates.

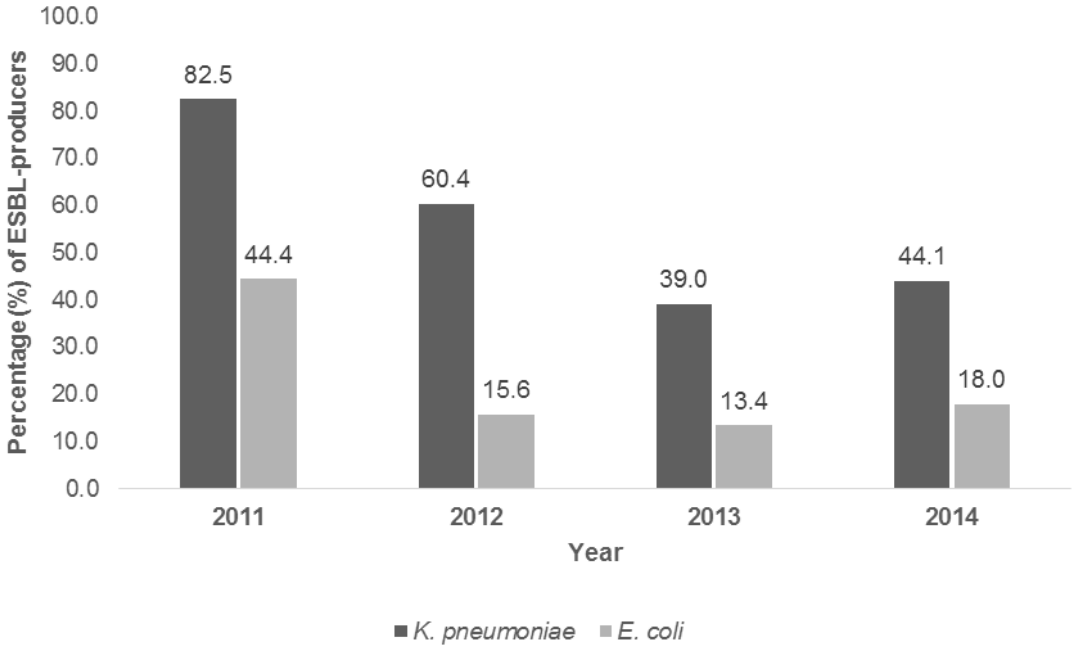

Figure 1: Percentage of *E. coli* and *K. pneumoniae* ESBL producers between 2011 and 2014.

Discussion

In recent years several studies have been conducted that focus on etiology of community acquired UTIs. The rationale behind the study conducted took into account several factors related to the incidence and severity of UTIs in elderly population. The results show, unequivocally, that *E. coli* is the most common bacteria in this type of infections [11-18]. In Aveiro, Portugal, a surveillance study carried out between 2000 and 2009, with 18797 samples from outpatients, the results show that *E. coli* was responsible for 64.5% of cases, compared with only 6.0% of *S. aureus*, 4.7% *P. mirabilis*, 4.3% *Klebsiella* spp., 3.6% *E. faecalis* and 2.4% *P. aeruginosa* [16]. In this study it is still possible to realize that individuals with 65 years or older are responsible for 38.6% of the total of UTIs, with higher prevalence in women, in all age groups. Regarding the percentage of isolates of the elderly, the trend is a slightly different from the global study, being more identical to the present work [16]. In 2007, Carlos Correia et al. [15] published a study on the etiology of UTIs in the hospital unit of Bragança, Portugal, with the results of microbiological positive urine tests in 428 outpatients. As in the present study, *E. coli* was the most frequent microorganism followed by *Klebsiella* spp.

ESBL-producing strains are a motive of concern to all health professionals worldwide. In Europe, infections caused by these bacteria have drastically raised since the discovery of ESBL enzymes, in 1970 decade [19]. Initially, TEM and SHV ESBLs were the most frequent types of these enzymes, and were isolated in *K. pneumoniae* and inpatients, leading to nosocomial outbreaks. However, nowadays, these enzymes are also find in *E. coli*, with a quickly spread of CTX-M type and around the community [19]. Among European countries, Portugal exhibits the highest rates of antibiotic resistance, in contrast with the Nordic countries [20]. Moreover, according to two studies conducted in 2002 [20] and 2008 [21], β-lactam antibiotics are the most used in our country, reinforcing the importance of monitoring rates of ESBL production, since these enzymes provide resistance to these classes of antibiotics.

In Portugal, a study conducted between March 2004 and March 2006, including *E. coli* from 9 Portuguese hospitals shows that 65,7% of *E. coli* produces CTX-M-type ESBLs with high prevalence of these isolates in community-acquired infections (56%), UTIs (76%) and patients older than 60 years (76%) [22]. In Hospital Infante D. Pedro, Aveiro, Portugal, between 2008 and 2010, there were analyzed 15843 isolates and the authors showed that there were 222 *E. coli* ESBL+ and 263 *K. pneumoniae* ESBL+, isolated mostly from urine, blood and bronchial secretions [23].

In 1999, in Spain, a group of investigators [24] studied antimicrobial susceptibility profile of isolates collected from patients with community-acquired UTIs. The results showed that, from the 2798 different strains, only 7 *E. coli* strains were ESBL-producers [24]. However, in the same country, about one decade later, this number raised to 6% (n=162) in 2009 and 7% (n=210) in 2010 [25]. Despite these works include both outpatients and inpatients, making harder to know how are these ESBL-producing strains disseminated in community, other study clearly shows that, in Spain, ESBL-producing *E. coli* are strongly disseminated in community [26]. Investigators conclude that in 1994 there were 0.20% of ESBL-producing *E. coli* in UTIs from outpatients and in 2004 this value was higher than 5.5% [26].

The results of the present study show that *K. pneumoniae* is the strain with the highest percentage of ESBL-producers. In fact, *E. coli* and *Klebsiella* spp. are the *Enterobacteriaceae* with more resistance phenotypes due to ESBL production. These data also show that depending on geographic location, ESBL are more disseminated in one species instead of others, showing the importance of local epidemiology studies to monitor and prevent unmeasured spread.

Conclusions

In the last three years E. coli was the organism causing higher number of UTI. However, the percentage of *K. pneumoniae* ESBL-producers was always higher than *E. coli*. High numbers on ESBL-producing isolates, detected in UTI patients over 65 are the main motive of concern, since these are recurrent visitors of hospitals and frequently living in nursing homes, which makes them potential carriers of multirresistant strains. Despite the percentage of ESBL-producers has been significantly reduced in the last years, the values are still high, being a motive of concern especially considering the elderly population. The results that are included in this work are of utmost importance, since they reinforce the need to adopt strict policies for the control of antibiotics administration in order to stop the spread of these isolates. Moreover, this study provides a realistic panorama of the dissemination of β-lactamases in our region. Additionally, it helps to highlight the importance of β-lactamases screening in order to prevent treatment failure. In the future, molecular biology studies are crucial to characterize these enzymes.

Funding

Sónia Ferreira was funded by a fellowship (SFRH/BPD/81509/2011) from Fundação para a Ciência e a Tecnologia de Portugal.

Part of this work was presented at the 24th European Conference of Clinical Microbiology and Infectious Diseases, Barcelona, Spain, 2014 and 25th European Conference of Clinical Microbiology and Infectious Diseases, Copenhagen, Denmak, 2015.

References

1. Mazzulli, T., 2002. Resistance Trends in Urinary Tract Pathogens and Impact on Management. The Journal of Urology, 168(4, Supplement), pp.1720–1722.
2. Nicolle, L.E., 2002. Epidemiology of urinary tract infections. Clinical Microbiology Newsletter, 24(18), pp.135–140.
3. Sheerin, N.S., 2011. Urinary tract infection. Medicine, 39(7), pp.384–389.
4. Marques, L.P.J. et al., 2012. Epidemiological and clinical aspects of urinary tract infection in community-dwelling elderly women. The Brazilian Journal of Infectious Diseases, 16(5), pp.436–441.
5. Numa, H. & Kazuya, T., 2012. Clinical study of bacterial pathogens which cause urinary tract infections in the elderly. Nishinihon Journal of Urology, 74(6), pp.334–339.
6. Nicolle, L.E. et al., 2005. Infectious Diseases Society of America guidelines for the diagnosis and treatment of

asymptomatic bacteriuria in adults. Clinical Infectious Diseases, pp.643–654.
7. Gavazzi, G. et al., 2013. Diagnostic criteria for urinary tract infection in hospitalized elderly patients over 75 years of age: A multicenter cross-sectional study. Médecine et maladies infectieuses, 43(5), pp.189–194.
8. Gonen, I. et al., 2013. Clinical and laboratory evaluation of urinary tract infections in elderly population. Acta Medica, 29, p.853.
9. Rupp, M.E. & Fey, P.D., 2003. Extended spectrum β-lactamase (ESBL)-producing Enterobacteriaceae. Drugs, 63(4), pp.353–365.
10. CLSI, 2010. CLSI Standards for antimicrobial susceptibility testing. Clinical and Laboratory Standard Institute (CLSI), Performance standards for antimicrobial susceptibility testing.
11. Akoachere, J.-F.T.K. et al., 2012. Etiologic profile and antimicrobial susceptibility of community-acquired urinary tract infection in two Cameroonian towns. BMC Research notes, 5(1), p.219.
12. Bahadin, J., Teo, S.S.H. & Mathew, S., 2011. Aetiology of community-acquired urinary tract infection and antimicrobial susceptibility patterns of uropathogens isolated. Singapore medical journal, 52(6), pp.415–420.
13. Al Benwan, K., Al Sweih, N. & Rotimi, V.O., 2010. Etiology and Antibiotic Susceptibility Patterns of Community- and Hospital-Acquired Urinary Tract Infections in a General Hospital in Kuwait. Medical Principles and Practice, 19(6), pp.440–446.
14. Coppo, E. et al., 2012. Local survelance study on etiology of community-and hospital-acquired urinary tract infections (UTI) and antimicrobial susceptibility of uropathogens. Microbiologia Medica, 27(1).
15. Correia, C. et al., 2007. Etiologia das infecções do tracto urinário e sua susceptibilidade aos antimicrobianos. Acta Médica Portuguesa, 20(6), pp.543–550.
16. Linhares, I. et al., 2013. Frequency and antimicrobial resistance patterns of bacteria implicated in community urinary tract infections: a ten-year surveillance study (2000-2009). BMC Infectious Diseases, 13(1), p.19.
17. Magliano, E. et al., 2012. Gender and age-dependent etiology of community-acquired urinary tract infections. The Scientific World Journal, 2012.
18. Rajesh, K.R., Mathavi, S. & Priyadarsini, R.I., 2010. Prevalence of antimicrobial resistance in uropathogens and determining empirical therapy for urinary tract infections. International Journal, 1(5), p.260.
19. Coque, T.M., Baquero, F. & Canton, R., 2008. Increasing prevalence of ESBL-producing Enterobacteriaceae in Europe. Euro surveillance: bulletin européen sur les maladies transmissibles= European communicable disease bulletin, 13(47).
20. Goossens, H. et al., 2005. Outpatient antibiotic use in Europe and association with resistance: a cross-national database study. The Lancet, 365(9459), pp.579–587.
21. van de Sande-Bruinsma, N. et al., 2008. Antimicrobial drug use and resistance in Europe. Emerging infectious diseases, 14(11), p.1722.
22. Mendonça, N. et al., 2007. Spread of extended-spectrum β-lactamase CTX-M-producing *Escherichia coli* clinical isolates in community and nosocomial environments in Portugal. Antimicrobial agents and chemotherapy, 51(6), pp.1946–1955.
23. Ferreira, S. et al., 2011. Detection of ESBL-producing Enterobacteriaceae during 2008-2010 in Aveiro, Portugal. Clinical Microbiology and Infection, 17(s4), pp.S108–S668.
24. Daza, R., Gutiérrez, J. & Piédrola, G., 2001. Antibiotic susceptibility of bacterial strains isolated from patients with community-acquired urinary tract infections. International journal of antimicrobial agents, 18(3), pp.211–215.
25. Briongos-Figuero, L.S. et al., 2012. Epidemiology, risk factors and comorbidity for urinary tract infections caused by extended-spectrum beta-lactamase (ESBL)-producing enterobacteria. International journal of clinical practice, 66(9), pp.891–896.
26. Ena, J. et al., 2006. Epidemiology of urinary tract infections caused by extended-spectrum beta-lactamase-producing< i> *Escherichia coli*</i>. Urology, 68(6), pp.1169–1174.