Post-surgical wound infections involving Enterobacteriaceae with reduced susceptibility to β-lactams intwo Portuguese hospitals

Rúben Fernandes, Cristina Prudêncio

ABSTRACT

The post-surgical period is often critical for infection acquisition. The combination of patient injury and environmental exposure through breached skin add risk to pre-existing conditions such as drug or depressed immunity. Several factors such as the period of hospital staying after surgery, base disease, age, immune system condition, hygiene policies, careless prophylactic drug administration and physical conditions of the healthcare centre may contribute to the acquisition of a nosocomial infection. A purulent wound can become complicated whenever antimicrobial therapy becomes compromised. In this pilot study, we analysed Enterobacteriaceae strains, the most significant gram-negative rods that may occur in post-surgical skin and soft tissue infections (SSTI) presenting reduced β-lactam susceptibility and those presenting extended-spectrum β-lactamases (ESBL). There is little information in our country regarding the relationship between β-lactam susceptibility, ESBL and development of resistant strains of microorganisms in SSTI. Our main results indicate Escherichia coli and Klebsiella spp. are among the most frequent enterobacteria (46% and 30% respectively) with ESBL production in 72% of Enterobacteriaceae isolates from SSTI. Moreover, coinfection occurred extensively, mainly with Pseudomonas aeruginosa and Methicillin-resistant Staphylococcus aureus (18% and 13%, respectively). These results suggest future research to explore if and how these associations are involved in the development of antibiotic resistance.

Keywords: Enterobacteriaceae • Extended-spectrum β-lactamases • Nosocomial infections • Post-surgical infections • Skin and soft tissue infections

Key Points

- when the skin is compromised infection may occur
- several microorganisms were found among post-surgical wounds infections
- we analysed the infectious gram-negative rods found in post-surgical wounds for the presence of extended-spectrum β-lactamases (ESBLs)
- ESBLs are plasmid-encoded enzymes that confer resistance to penicillins, cefalosporins (up to fourth generation), cephamycins and aztreonam
- ESBLs are encoded to plasmids that may also confer resis- tance to aminoglycosides and quinolones
- among the enterobacteria iso- lates we have found 70% of ESBL-producers in two Por- tuguese hospitals with different genotypic and phenotypic pro- files
- the post-surgical wounds were also coinfected with Pseu- domonas aeruginosa (18% inci- dence) and Methicillin-resistant Staphylococcus aureus (MRSA) (13% incidence)
- if further research identifies a causal relationship between ESBL production and the devel- opment of antibiotic resistance in microorganisms, these find- ings may help provide the basis for nationwide strategies to pre- vent drug resistance

INTRODUCTION

Wound is any physical damage to the body that exposes subcutaneous tissue (1). When the skin, the primary barrier between the environ- ment and the human body, is compromised, infection may occur (2). Damage to the skin tis- sue is because of any physical trauma or injury that may be accidental or intentional. Med- ical procedures often induce skin continuity loss in smaller (intravenous medical devices)

or greater extension (surgery). Hospitals are although important environments that may lead to the development of an infection. Although not intentionally induced, hospital-acquired wounds can also be because of the local ischemia caused by the pressure of the body on the bed surface, referred as decubitus or pressure ulcers. When such wounds become infected, they are often colonised by multiple bacterial species. Many studies refer staphylococci members as among the most prevalent agents found in skin and soft tissue infections (SSTI) along with enterococci, streptococci and gram-negative members (3). The prevalence of multidrug resistance (MDR) continues to increase among many pathogens largely because of overuse and misuse of antimicrobial agents (1). Such use not only adds to the cost of medical care, but also needlessly exposes the patient to potential toxicity and risks that promote the development and spread of antimicrobial resistance in healthcare facilities (4).

Antimicrobial resistance is a major contemporary issue (5). Mechanisms underlying the development of drug resistance are complex and varied. One example is the antimicrobial target modification exhibited by *Staphylococcus aureus*. Penicillin-binding proteins (PBP), the target for *β*-lactams, may be altered by mutation in *S. aureus*. This type of mutation are on the etiological course for the development of penicillin and other *β*-lactams resistance (6,7) such as methicillin-resistant *S. aureus* (MRSA). Resistance to the *β*-lactams may also occur because of the acquisition of enzymatic machinery such as *β*-lactamases, especially the ESBL (6). Little epidemiological studies have focused on the involvement of enterobacteria causing SSTI. In this study, the antimicrobial susceptibility of enterobacteria as etiological agents in the SSTI, the prevalence of ESBL among these pathogens and the most common coinfections associated in the SSTI in post-surgery complicated wounds in Portugal for a period of 2 years are described. The *β*-lactamases (*bla*) genes, bla_{TEM}, bla_{SHV} and bla_{CTX-M} were screened by means of polymerase chain reaction (PCR) and sequencing methods; furthermore, it was also used a molecular fingerprinting technique designed for Enterobacteriaceae members using the enterobacterial repetitive intragenic consensus (ERIC) sequences (8) in order to understand/establish genetic relations.

MATERIALS AND METHODS

Bacterial strains, identification and susceptibility

All isolates had been provided from two Portuguese Clinical Pathology Laboratories; all patients with positive culture results of Enterobacteriaceae from surgical site infections and that include of the following findings: (a) purulent incisional drainage; (b) positive results of culture of aseptically obtained fluid or tissue from the superficial wound; (c) local signs and symptoms of pain or tenderness, swelling, and erythema, with the incision opened by the surgeon; strains were collected by swab and transported in the same day to the laboratory during the period comprised from September 2007 and August 2009; bacterial identification and preliminary antimicrobial susceptibility were determined by a microdilution method using automated Vitek II (bioMe´rieux, Marcy-l'Etoile, France) system and double checked with the disc-diffusion methods according to the Clinical and Laboratory Standards Institute (CLSI; formerly the National Committee of Clinical Laboratory Standards) guidelines (9,10) for minimum inhibitory concentration (MIC) determination. ESBL production was assessed with two Epsilometer test (E-test, AB Biodisk, Solna, Sweden) strips and confirmed as positive whenever a ≥ threefold-dilution difference between ceftazidime and ceftazidime/clavulanic acid or/and ≥ threefold-dilution difference between cefotaxime and cefotaxime/clavulanic MICs according to the manufacturer instructions.

Conjugation experiments

Transmissibility of resistance has been tested by matting clinical isolates to F^- strains of azide-resistant *E. coli* J53 Azi^R on trypticase soy broth (TSB) according to (11).

Analytical IEF

Crude preparations of *β*-lactamases from clinical strains transconjugants were obtained by sonicating the cells in phosphate buffer, pH 7·0 (12). Then, 20 µl of crude protein concentrated extract was added to 50 µl of

nitrocefin (OXOID, Hampshire, UK) solution (50 mg/l in 1% glycine plus 50% glycerol). Samples converted from yellow to dark pink in 30 to 60 seconds; 10 µl of each sample were run in precast isoelectric focusing (IEF) minigels, pH 3–10 (Bio-Rad, Milano, Italy), in a Mini-Protean II unit (Bio-Rad, Milano, Italy) according to the manufacturer instructions.

Genetic molecular characterisation of *bla* genes and genetic profiling

PCR primers were designed for bla_{TEM} (Fw-ataaaattcttgaagacgaaa and Rv-cagttaccaatgcttaatca) (13), bla_{SHV} (Fw-gccgggttattcttatttgtc and Rv-gctctttccgatgccgccgccagtca) (14), bla_{CTX-M1} (Fw-atggttaaaaaatcactgcg and Rv-ttacaaaccgtcggtgac) (15), bla_{CTX-M2} (Fw-atgatgactcagagcattcg and Rv-ttattgcatcagaaaccgtg) (16) bla_{CTX-M9} (Fw-gtgacaaagagagtgcaacgg and Rv-atgattctcgccgctgaagcc) (17) and bla_{CTX-M8} and $bla_{CTX-M25}$ which share the same reverse primer (Rv-aacccacgatgtgggtagc) but have different forward primers (Fw8-tcgcgttaagcggatgatgc and Fw25-gcacgatgacattcggg) (18), and conditions were performed as described. PCR amplicons were run in 1·2% agarose (Bioron GmbH, Ludwigshafen, Germany) and bands were cut off from the gel and purified for subcloning. The recovered bands from agarose were subcloned in TOPO TA CloningTM kit (Invitrogen Corporation, San Diego, CA) for further sequencing. The nucleotide sequences of both ends of the insert were determined with M13 sequencing primers specific for the cloning vector (19). Regarding molecular fingerprinting it was performed with PCR reaction according the description of Versalovic *et al*. (8) using the primers ERIC1R-atgttagctcctggggattcac and ERIC2-aagtaagtgactggggtgagcg. ERIC profiles were analysed with software, FPQuest□version 4·5, Fingerprinting II (Bio-Rad Laboratories, Hercules, CA, US).

RESULTS AND DISCUSSION

Ninety-seven enterobacteriae isolates harvested during the 2-year period expressed resistance ($MIC_{50} \geq 16$ µg/ml) to at least to second generation cephalosporins. *Escherichia coli* was the most representative member ($n = 44$; 46%) followed by *Klebsiella pneumoniae* ($n = 28$; 29%) and then by *Enterobacter aerogenes* ($n = 7$; 7%), *Morganella morganii* ($n = 7$; 7%), *Enterobacter cloacae* ($n = 5$; 4%), *Serratia marcescens* ($n = 2$; 2%), *Citrobacter freundii* ($n = 1$; 1%), *Klebsiella oxytoca* ($n = 1$; 1%), *Proteus vulgaris* ($n = 1$; 1%) and *Serratia liquefaciens* ($n = 1$; 1%). These findings are in agreement with most of the studies that described that among post-surgical procedures, there is an increased risk to acquire a nosocomial infection and, that among the gram-negative rods, *E. coli*, *Klebsiella* spp. and *Enterobacter* spp. are the most frequent (3,20,21).

MIC tests show that SSTI isolates were mostly resistant β-lactams. Concerning penicillins, they were 98% resistant to ampicillin and 89% resistant to amoxicillin/clavunate combination. Strains were also 100% resistant to all first and second generation cephalorporins, including cefalotin, cefazolin, and both sodium-cefuroxime and axetil-cefuroxime, and presented high resistance levels to third and fourth generation cephalosporins. So, to ceftazidime, SSTI isolates show 97% of resistance, 88% to cefotaxime and 70% to the fourth generation cefepime. Nevertheless, regarding to carbapenems, these isolates present a general susceptibility to carbapenems, 100% to both imipenem and meropenem. The antimicrobial susceptibility pattern is variable among the aminoglycosides as SSTI isolates are 98% susceptible to amikacin and 60% resistant to gentamicin. In what concerns to quinolones, they are 70% resistant to ciprofloxacin. Finally, as regards sulfonamides such as the trimethoprim-sulphametoxazole 70% of the strains in the study presented a susceptibility pattern.

As reported in previous Portuguese (22–25), Spanish (26,27) and other European (28) studies, *E. coli* and *K. pneumonia* are the species where ESBL is the most frequently identified. In the present study from the 97 isolates 70 (72%) carried an ESBL. Again, *E. coli* was the organism presenting ESBL more often (43/44; 98%) and then *K. pneumoniae* (24/28; 86%). *E. aerogenes* and *K. oxytoca* ($n = 2$ and $n = 1$, respectively) also produced ESBL.

Regarding ESBL enzymes, the TEM type was the most frequent (29/70; 41%) followed by the CTX-M type (23/70; 33%) and finally the SHV type (18/70; 26%). Among TEM enzymes it was found the TEM-24, TEM-52, TEM-10 and TEM-116 varieties (20%, 14%, 6% and 1%, respectively). CTX-M type is a heterogeneous group constituted by five phylogenetic branches (29). In this study it

was found representatives from two branches, the CTX-M-1 and CTX-M-9. Members of the CTX-M-1 group included the CTX-M-15 and CTX-M-1 enzymes (13% and 3%, respectively). CTX-M-9 group was represented by CTX-M-9 and CTX-M-15 enzymes (10% and 7% respectively). Finally in what concerns to the SHV enzymes it was found the SHV-5 and SHV-12 (14% and 12%, respectively).

Coinfections were also relatively common among patients from which these isolates were recovered. Among the *E. coli* and *K. pneumoniae* isolates from SSTI it was found other 91 microorganisms coinfecting the wounds (Figure 1). The most frequent coinfection microorganism was *Pseudomonas aeruginosa* (17/97; 18%) followed by *S. aureus* (15/97; 15%), *E. coli* (13/97; 13%), *Klebsiella* spp. (11/97; 11%), *Enterobacter* spp. (10/97; 10%) and *Enterococcus faecalis* (7/97; 7%).

From staphylococci members coinfecting post-surgical wounds 87% (13/15) were MRSA and only 13% (2/15) were methicillin susceptible. A total of 46% (6/13) of *E. coli* isolates coinfecting post-surgical wounds were also ESBL positive but 54% (7/13) did not carried any β-lactamase. Similarly, in what concerns to *Klebsiella* spp. coinfecting post-operative wounds 45% (5/11) harbours another ESBL, whereas 55% (6/11) does not.

In a single long-term patient it was possible to isolate five ESBL-producing *E. coli* and *K. pneumoniae* from different infection episodes. The patient, a 51-year-old male, first developed a urinary-tract infection (UTI) in April 2005 caused by two distinct ESBL-producing *E. coli*; one of them harbouring a TEM-10 enzyme and the other a CTX-M-15. Later in May of the same year, the same patient developed a second UTI, and this time caused by an SHV-2 producing *K. pneumoniae*. One month later, in July, a pressure ulcer developed possibly as a result of friction, pressure or sheer and became contaminated with a SHV-5 producing *K. pneumoniae*. Finally, on September of the same year, this patient experienced another UTI with the same SHV-5 producing *K. pneumoniae* (genetic similarity ≥90%) found first in the purulent wound (Figure 2).

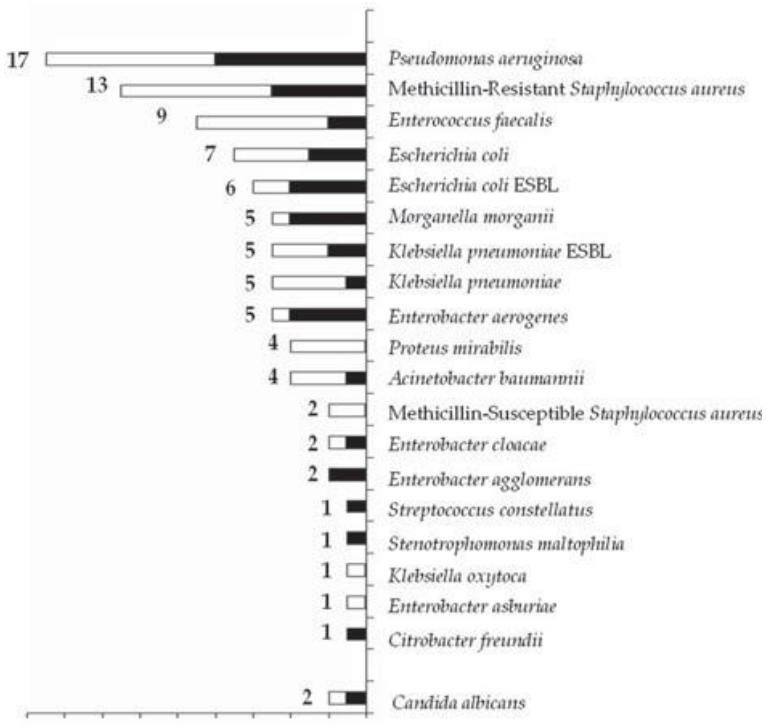

Figure 1. Occurrence of bacteria and yeast (Candida albicans) coinfecting skin and soft tissue infections (SSTI) caused by extended-spectrum β-lactamases (ESBL)-producing Escherichia coli and ESBL-producing Klebsiella pneumoniae (white bars). The diagram shows the number (left side) of microorganism (right side) that occurred simultaneously with ESBL-producing E. coli (black bars) and with ESBL-producing K. pneumoniae (white bars).

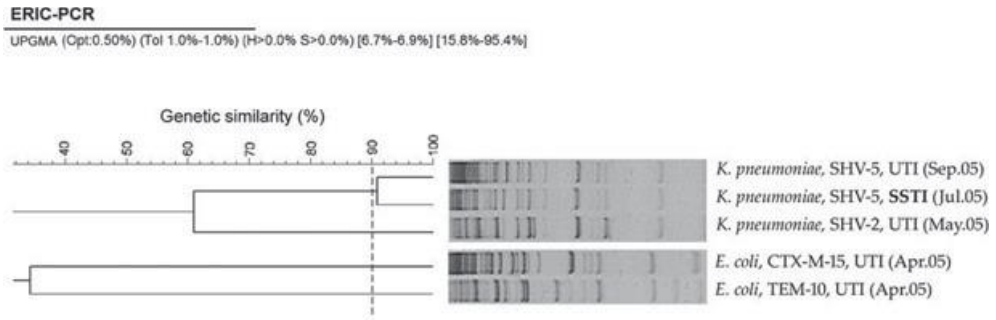

Figure 2. Enterobacterial repetitive intragenic consensus (ERIC) fingerprinting profiles of extended-spectrum β-lactamases (ESBL) producing Escherichia coli and Klebsiella pneumoniae isolated from a single patient from April to September of 2005. UTI stands for urinary-tract infection. SSTI stands for skin and soft tissue infections. Dendograms were generated by UPGMA analysis of the agarose gels using the FPQuest version 4.1.5 (Bio-Rad).

In another long-term patient with an oncological issue, from another hospital, experienced a rarely described situation, an injury caused by radiotherapy. This patient, a 55-year-old female, presented two SSTI episodes. The first one in August 2005 was caused by a CTX-M-14 producing *E. coli*. A few weeks later in September after apparent recovery the infection reappeared and became complicated with *P. aeruginosa* along the same CTX-M-14 producing *E. coli* (genetic similarity ≥90%; data not shown) that was present in the first episode in August.

Reduced susceptibility to formerly effective antibiotics is increasing worldwide among the SSTI causing agents (3). This is most certainly as a result of overuse, misuse and abuse of antimicrobial agents' sensu lato. Examples of abusive antimicrobial usage include the exaggerated prophylactic measures, self-medication or even use of commercial product with antiseptic/antimicrobial agents and also the use for non human purposes such as in livestock production (5,30,31). Centers for Disease Control and Prevention (CDC) and policy makers are very committed to the implementation of educational strategies not only for health professional (32,33) but also for general population.

In general, this pilot study in only two hospitals point to an important issue, that is the emergence of ESBL among gram-negative rods involved in nosocomial wounds in our country. These findings extend beyond the original purpose, of this study, suggesting exploration of interactions between ESBL and development of antibiotic resistance in *P. aeruginosa* and gram-positive bacteria such streptococci, enterococci and staphylococci with special attention to MRSA (34,35).

In conclusion, the emergence of ESBL among gram-negative rods and possibly related antibiotic-resistant strains of other pathogens (35) merits further research and epidemiologic study as well as immediate development of policies monitor drug delivery in hospital and ambulatory pharmacies and the implementation of public health defence strategies towards health promotion and drug resistance prevention.

REFERENCES

1 Wilson MA. Skin and soft-tissue infections: impact of resistant gram-positive bacteria. Am J Surg 2003;186 (Suppl):35–41.

2 Horan TC, Gaynes RP, Martone WJ, Jarvis WR, Emori TG. CDC definitions of nosocomial surgical site infections. Infect Control Hosp Epidemiol 1992;13:606–8.

3 Moet GJ, Jones RN, Biedenbach DJ, Stilwell MG, Fritsche TR. Contemporary causes of skin and soft tissue infections in North America, Latin America, and Europe: Report from the SENTRY Antimicrobial Surveillance Program (1998–2004). Diagn Microbiol Infect Dis 2006;57:7–13.

4 Martone WJ, Nichols RL. Recognition, prevention, surveillance, and management of surgical site infections: introduction to the problem and symposium overview. Clin Infect Dis 2001;33 Suppl 2:67–8.

5 Department of Food Safety, Zoonoses and Foodborne Diseases. Critically Important Antimicrobials for Human Medicine: Categorization for the development of risk management strategies to contain antimicrobial resistance due to non-human antimicrobial use. In: Report of the 2nd WHO Expert Meeting Copenhagen, World Health Organization, 2007.

6 Bloomfield SF, Cookson B, Falkiner F, Griffith C, Cleary V. Methicillin-resistant *Staphylococcus aureus*, *Clostridium difficile*, and extended-spectrum β-lactamase-producing *Escherichia coli* in the community: assessing the problem and controlling the spread. Am J Infect Control 2007;35:86–8.

7 Shukla SK. Community-associated methicillin-resistant *Staphylococcus aureus* and its emerging virulence. Clin Med Res 2005;3:57–60.

8 Versalovic J, Koeuth T, Lupski JR. Distribution of repetitive DNA sequences in Eubacteria and application to fingerprinting of bacterial genomes. Nucleic Acids Res 1991;19:6823–31.

9 CLSI (Clinical and Laboratory Standards Institute). Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically, 6th edn. Wayne, PA: Clinical and Laboratory Standards Institute, 2003:M7–A6.

10 CLSI. Performance standard for antimicrobial susceptibility testing. Wayne, PA: Clinical and Laboratory Standards Institute, 2005:M100–S15.

11 Álvarez M, Tran JH, Chow N, Jacoby GA. Epidemiology of conjugative plasmid-mediated AmpC β-lactamases in the United States. Antimicrob Agents Chemother 2004;48:533–7.

12 Bonfiglio G, Laksai Y, Franchino L, Amicosante G, Nicoletti G. Mechanisms of β-lactam resistance amongst *Pseudomonas aeruginosa* isolated in an Italian survey. J Antimicrob Chemother 1998;42:697–702.

13 Mabilat C, Goussard S. PCR detection and identification of gene for extended-spectrum β-lactamases. In: Persing DH, Smith TF, Tenover FC, White TC, editors. Diagnostic molecular microbiology: principles and applications. Washington: American Society for Microbiology Press, 1993:553–9.

14 Nüesch-Inderbinen MT, Hächler H, Kayser FH. Detection of genes coding for extended-spectrum SHV β-lactamases in clinical isolates by molecular genetics method and comparison with E-test. Eur J Clin Microbiol Infect Dis 1996;15:398–402.

15 Batchelor M, Hopkins K, Threlfall EJ, Clifton-Hadley FA, Stallwood AD, Davies RH, Liebana E. *bla*(CTX-M) genes in clinical *Salmonella* isolates recovered from humans in England and Wales from 1992 to 2003. Antimicrob Agents Chemother 2005;49:1319–22.

16 Steward CD, Rasheed JK, Hubert SK, Biddle JW, Raney PM, Anderson GJ, Williams PP, Brittain KL, Oliver A, McGowan JE Jr, Tenover FC. Characterization of clinical isolates of *Klebsiella pneumoniae* from 19 laboratories using the National Committee for Clinical Laboratory Standards extended-spectrum β-lactamase detection methods. J Clin Microbiol 2001;39:2864–72.

17 Sabaté M, Tarragó R, Navarro F, Miró E, Vergés C, Barbé J, Prats G. Cloning and sequence of the gene encoding a novel cefotaxime-hydrolyzing β-lactamase (CTX-M-9) from *Escherichia coli* in Spain. Antimicrob Agents Chemother 2000;44:1970–73.

18 Woodford N, Fagan EJ, Ellington MJ. Multiplex PCR for rapid detection of genes encoding CTX-M extended-spectrum β-lactamases. J Antimicrob Chemother 2006;57:154–5.

19 Naiemi NA, Duim B, Savelkoul PMH, Spanjaard L, Jonge E, Bart A, Vandenbroucke-Grauls CM, Jong MD. Widespread transfer of resistance genes between bacterial species in an intensive care unit: implications for hospital epidemiology. J Clin Microbiol 2005;43:4862–4.

20 Chaudhary SB, Vives MJ, Basra SK, Reiter MF. Postoperative spinal wound infections and postprocedural diskitis. J Spinal Cord Med 2007;30:441–51.

21 Giacometti A, Cirioni O, Schimizzi AM, Del Prete MS, Barchiesi F, D'Errico MM, Petrelli E, Scalise G. Epidemiology and microbiology of surgical wound infections. J Clin Microbiol 2000;38:918–22.

22 Costa D, Poeta P, Saénz Y, Coelho AC, Matos M, Vinué L, Rodrigues J, Torres C. Prevalence of antimicrobial resistance and resistance genes in faecal *Escherichia coli* isolates recovered from healthy pets. Vet Microbiol 2008;127:97–105.

23 Fernandes R, Gestoso A, Freitas JM, Santos P, Prudêncio C. Emergence of high resistance to fourth generation cephalosporins among clinical isolates of *Enterobacteriaceae* producing extended-spectrum β-lactamases isolated in the north of Portugal. Int J Antimicrob Agents 2008;70:93–5.

24 Mendonça N, Leitão J, Manageiro V, Ferreira E, Canica M. Spread of extended-spectrum β-lactamase CTX-M-producing *Escherichia coli* clinical isolates in community and nosocomial environments in Portugal. Antimicrob Agents Chemother 2007;51:1946–55.

25 Machado E, Coque TM, Cantón R, Baquero F, Sousa JC, Peixe L, Portuguese Resistance Study Group. Dissemination in Portugal of CTX-M-15-, OXA-1-, and TEM-1-producing *Enterobacteriaceae* strains containing the aac(6')-Ib-cr gene, which encodes an aminoglycoside- and fluoroquinolone-modifying enzyme. Antimicrob Agents Chemother 2006;50:3220–1.

26 Hernández JR, Martínez-Martínez L, Cantón R, Coque TM, Pascual A. Nationwide study of *Escherichia coli* and *K. pneumoniae* producing extended-spectrum β-lactamases in Spain. Antimicrob Agents Chemother 2005;49:2122–5.

27 Miro E, Mirelis B, Navarro F, Rivera A, Mesa RJ, Roig, MC, Gomez L, Coll P. Surveillance of extended-spectrum β-lactamases from clinical samples and faecal carriers in Barcelona, Spain. J Antimicrob Chemother 2005;56:1152–5.

28 Livermore DM, Canton R, Gniadkowski M, Nordmann P, Rossolini GM, Arlet G, Ayala J, Coque TM, Kern-Zdanowicz I, Luzzaro F, Poirel L, Woodford N. CTX-M: changing the face of ESBLs in Europe. J Antimicrob Chemother 2007;59:165–174.

29 Bonnet R. Growing group of extended-spectrum β-lactamases: the CTX-M enzymes. Antimicrob Agents Chemother 2004;48:1–14.

30 Aiello AE, Marshall B, Levy SB, Della-Latta P, Lin SX, Larson E. Antibacterial cleaning products and drug resistance. Emerg Infect Dis 2005;11:1565–70.
31 Grigoryan L, Burgerhof JG, Degener JE, Deschepper R, Lundborg CS, Monnet DL, Scicluna EA, Birkin J, Haaijer-Ruskamp FM, SAR consortium. Attitudes, beliefs and knowledge concerning antibiotic use and self-medication: a comparative European study. Pharmacoepidemiol Drug Saf 2007;16:1234–43.
32 Siegel JD, Rhinehart E, Jackson M, Chiarello L; Healthcare Infection Control Practices Advisory Committee. Management of Multidrug-Resistant Organisms in Healthcare Settings. Am J Infect Control 2007;35 (Suppl 2):165–93.
33 Larson EL, Quiros D, Giblin T, Lin S. Relationship of antimicrobial control policies and hospital characteristics to antimicrobial resistance rates. Am J Crit Care 2007;16:110–9.
34 MacAdam H, Zaoutis TE, Gasink LB, Bilker WB, Lautenbach E. Investigating the association between antibiotic use and antibiotic resistance: impact of different methods of categorising prior antibiotic use. Int J Antimicrob Agents 2006;28:325–32.
35 Mroczkowski P, Lauf H, Lippert H, Kö nig W, Meyer F. Microbial spectrum in surgical infections based on a microbiological routine monitoring over the 10-year period from 1995 to 2004. Zentralbl Chir 2009;134:226–30. Article in German.