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Prevalence of AmpC and other β-lactamases in enterobacteria at a large urban university hospital in Brazil

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Abstract

Production of extended-spectrum β-lactamases (ESBLs) has been reported in virtually all species of Enterobacteriaceae, which greatly complicates the therapy for infections caused by these organisms. However, the frequency of isolates producing AmpC β-lactamases, especially plasmid-mediated AmpC (pAmpC), is largely unknown. These β-lactamases confer resistance to extended-spectrum cephalosporins and aztreonam, a multidrug-resistant (MDR) profile. The aim of the present study was to determine the occurrence of ESBL and pAmpC β-lactamases in a hospital where MDR enterobacterial isolates recently emerged. A total of 123 consecutive enterobacterial isolates obtained from 112 patients at a university hospital in Rio de Janeiro, Brazil, during March to June 2001 were included in the study. ESBL was detected by the addition of clavulanate to cephalosporin containing disks and by double diffusion. AmpC production was evaluated by a modified tridimensional test and a modified Hodge test. The presence of plasmid-mediated *ampC* β-lactamase genes was evaluated by multiplex polymerase chain reaction. Sixty-five (53%) of 123 enterobacterial isolates were MDR obtained from 56 patients. ESBL production was detected in 35 isolates; 5 clonal *Escherichia coli* isolates exhibited high levels of chromosomal AmpC and ESBL production. However, no isolates contained pAmpC genes. Infection or colonization by MDR enterobacteria was not associated with any predominant resistant clones. A large proportion of hospital infections caused by ESBL-producing enterobacteria identified during the study period were due to sporadic infections rather than undetected outbreaks. This observation emphasizes the need to improve our detection methods for ESBL- and AmpC-producing organisms in hospitals where extended-spectrum cephalosporins are in wide use.

Keywords: Multidrug resistance; Enterobacteria; AmpC β-lactamase; ESBL; Three-dimensional test; Hodge test

1. Introduction

Infections by enterobacterial isolates resistant to extended-spectrum cephalosporins or aztreonam have become a serious problem worldwide (Winokur et al., 2001; Bell et al., 2002). Increased prevalence of multidrug-resistant (MDR) enterobacteria is often due to intense prescription of 3rd-generation cephalosporins or quinolones in communities and hospitals, and dissemination of these organisms by inappropriate hygienic practices (Cohen, 1992; Davin-Regli et al., 1997; Hobson et al., 1996; Holmberg et al., 1987; Meyer et al., 1993; Rice et al., 1990). In addition, MDR strains of enteric pathogens can emerge from animal reservoirs due to selection by antimicrobial agents used as growth promoters (Ramchandani et al., 2005).

Nosocomial outbreaks of MDR infections in hospitals have led to endemic occurrence of these infections, with

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dissemination of resistance genes, plasmids, and strains of a variety of bacterial species (Ben Redjeb et al., 1988; Bradford, 2001; Philippon et al., 1989; Winokur et al., 2001). MDR enterobacteria often produce extended-spectrum β-lactamases (ESBLs) or overexpress AmpC β-lactamases (AmpC) (Thomson, 2001). However, many clinical microbiologists are unaware of plasmid-encoded AmpC because its phenotypic detection is difficult, and these β-lactamases can be misidentified as ESBLs (Hanson, 2003). There is confusion about the importance of such resistance mechanisms, optimal test methods, and appropriate reporting conventions. Failure to detect these β-lactamases has contributed to their uncontrolled spread and occasional therapeutic failures. The Clinical and Laboratory Standards Institute (CLSI) recommends ESBL screening and confirmation only for *Escherichia coli* isolates, which is an organism with constitutive or minimal AmpC chromosomal expression, and *Klebsiella oxytoca*, *Klebsiella pneumoniae*, and *Proteus mirabilis*, which have no chromosomally encoded AmpC (CLSI/National Committee for Clinical Laboratory Standards [NCCLS], 2005). No standards have been published to date for the other enterobacterial species. In addition, there are no CLSI recommendations for detecting plasmid-mediated AmpC (pAmpC) in any species.

Perez-Pérez and Hanson (2002) developed a multiplex polymerase chain reaction (PCR) assay for the detection of plasmid-encoded *ampC* genes that proved useful as a rapid screening tool to distinguish cefoxitin-resistant non-AmpC producers from cefoxitin-resistant AmpC producers. In addition, this PCR-based method can distinguish hyperproducing chromosomal AmpC *E. coli* isolates from *E. coli* isolates encoding an "imported" plasmid *ampC* gene.

Hospital Universitário Clementino Fraga Filho (HUCFF) is a large (490-bed) tertiary care university hospital, in the city of Rio de Janeiro, Brazil. In 2000, the Committee for Control of Healthcare-Associated Infections at HUCFF detected a 5-fold increase in the number of MDR enterobacterial infections from 35 patients, in 1998, to 172 patients, in 2000. The present study was designed to provide a better understanding of the microbiologic factors associated with this observation at HUCFF. Our main study objective was to determine the occurrence of ESBL and pAmpC in association with the emergence of MDR enterobacteria and whether this sudden increase in the number of MDR enterobacterial infections was due to an outbreak of limited clonal groups of ESBL/AmpC-expressing strains or an increase of distinct strains that were selected by the widespread use of extended-spectrum cephalosporins in the hospital.

2. Materials and methods

2.1. Study design

The study period was from March to June 2001. Patients were retrospectively selected by review of records at the microbiology laboratory and the patient hospital database. A total of 56 patients with MDR enterobacteria and 56 with non-MDR enterobacteria were included in the study. The institution's ethics committee approved the study.

2.2. Microbiologic methods

2.2.1. Study population and bacterial isolates

A total of 128 enterobacterial isolates were obtained from 117 patients consecutively admitted to HUCFF between March and June 2001. Patients included in the study were those who had the bacterial isolate confirmed as an Enterobacteriaceae species and obtained at least 72 h after admission. Only 1 isolate of each bacterial species was selected per patient, preferably, 1 obtained from a normally sterile site. Five isolates from 5 (7.5%) patients were excluded: 2 were from patients whose records were not found and 3 were from patients with more than 1 isolate of the same bacterial species. Therefore, 123 enterobacterial isolates obtained from 112 patients infected (90, 80.4%) or colonized (22, 19.6%) by these agents were included in the study.

Bacterial species were identified by the Vitek system GNI card (bioMérieux, Marcy l'Etoile, France) and conventional biochemical tests (Farmer, 2003; Winn et al., 2006). Antimicrobial susceptibility was evaluated by the disk diffusion method (CLSI/NCCLS, 2000) for the following agents: amikacin, aztreonam, cefepime, cefotaxime, ceftazidime, cefoxitin, cephalothin, ciprofloxacin, gentamicin, imipenem, piperacillin–tazobactam, and trimethoprim–sulfamethoxazole. The category of intermediate susceptibility was analyzed together with the resistant strains.

2.2.2. Tests for β-lactamase production

ESBL screening was based on disk diffusion results for aztreonam, ceftazidime, and cefotaxime and confirmed by standard ceftazidime and cefotaxime disks combined with clavulanic acid (10 μg) (CLSI/NCCLS, 2005). In addition, we evaluated all isolates by the double-disk diffusion method with disks containing cefepime, cefotaxime, ceftazidime, and aztreonam placed 25 mm apart (center to center) to a disk containing a β-lactamase inhibitor (amoxicillin–clavulanic acid) (Jarlier et al., 1988).

Ceftazidime-resistant and intermediate isolates were also evaluated for metallo-β-lactamase production by a disk approximation test with disks containing ceftazidime and 2-mercaptopropionic acid (Arakawa et al., 2000).

AmpC production was evaluated for isolates belonging to species with no chromosomally encoded AmpC (*K. pneumoniae* and *P. mirabilis*) or with constitutive or minimal AmpC chromosomal expression (*E. coli*). AmpC detection methods included the Hodge test and the tridimensional test modified from Yong et al. (2002) and Coudron et al. (2000), respectively. The indicator strains were *E. coli* ATCC 25922 and *E. coli* ATCC 35218. To test *P. mirabilis* isolates, we used MacConkey agar plates to suppress swarming (including those of unlysed cells in the