Title: ISOELECTRIC FOCUSING FOR DETECTION OF AmpC β-LACTAMASE IN *ENTEROBACTERIACEAE*

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Abstract:

β-lactamase production is the most common mechanism of resistance in *Enterobacteriaceae*. AmpC-β-lactamase is classified in molecular class C by Ambler, and functional group 1 by Bush-Jacoby-Medeiros. This type of enzyme confers resistance to penicillins, cephalosporins, especially to cephamycins and is resistant to inhibition by common beta-lactamase inhibitors as clavulanic acid. Dissemination of these AmpC producing *Enterobacteriaceae* isolates in hospital environments is quite frequent and is closely related to therapeutic failure situations in admitted patients. Isoelectric focusing (IEF) is an analytical and preparative technique allowing discrimination of amphoteric substances, as proteins, according to their isoelectric points (pI). This technique is widely used in modern day proteomics and allows the detection of bacterial enzymes. This study aimed to confirm, by IEF, the AmpC-β-lactamase production in enterobacteria isolated from clinical samples obtained from a hospital in northern Portugal, in 2009. A total of 116 *Enterobacteriaceae* clinical isolates were tested by disk diffusion with beta-lactams and beta-lactamase inhibitors including inhibitors of AmpC-β-lactamase, cloxacillin. Eighteen isolates previously characterized as AmpC producers were randomly selected and biochemically identified by ID32GN (Biomérieux) as *Klebsiella pneumoniae* (77,8%)*, Escherichia coli* (11,0%)*, Enterobacter agglomerans* (5,6%)*,* and *Proteus mirabilis* (5,6%). After preliminary evaluation of the enzymatic extraction of bacterial growth under cefoxitin and cefotaxime pressure, these extracts were submitted to IEF to pI identification and classification of enzyme. AmpC-type β-lactamases have pIs between 7.6 (DHA-1) e 9,4 (LAT-1). It was possible to observe a single band corresponding to pI approximately 9,0 in 44,4% (9/18) of crude extracts (*K. pneumoniae* e *E. coli*). This technique is quite delicate and is necessary a good quantity of the enzyme for its detection. Thus, even after the optimization of extract production it was not possible to determine the presence of the enzyme in some isolates. In the future, PCR (Polymerase Chain Reaction) technique will be performed to detect the AmpC-β-lactamase genes using the primers AmpC, ACCM, CITM, CMY, EBCM, in order to elucidate the findings in IEF and phenotypic test.

Keywords: Enterobacteria, antimicrobial resistance, isoeletric point

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