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Phenotypic and Molecular Study of Carbapenemase-Producing Enterobacteriaceae in a Regional Hospital in Northern Morocco

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INTRODUCTION:

The interest shown in recent years for bacterial infections caused by multidrug-resistant bacteria (MDRB), in particular carbapenemase-producing enterobacteria (CPE), by the pharmaceutical companies in charge of therapy is still current. In fact, these infections are of clinical importance because they constitute a major public health risk due to the difficulty of their treatment. Many authors have been interested in evaluating infections of carbapenemase-producing bacteria under various aspects at the epidemiological, clinical or even therapeutic level in order to fight against them. To our knowledge, few studies report the epidemiological and molecular aspects of these infections in Morocco. Our work aims to establish the prevalence and study the strains of carbapenemase-producing enterobacteria (CPE) found in patients at the Mohamed V regional hospital in Tangier in northern Morocco.

MATERIALS AND METHODS

Study framework: This was a 12-month prospective study, carried out between January and December 2015, of 2178 bacteriological examinations carried out in the microbiology laboratory of the Mohamed V regional hospital in Tangier. The bacterial strains were isolated from urine, blood, pus, bone samples, sputum, pleural fluid, and peritoneal fluid, obtained from in patients hospitalized at the regional hospital in Tangier in the North of Morocco.

Enterobacteriaceae identification: The bacterial culture was grown on cysteine lactose electrolyte deficient (CLED) agar and eosin methylene blue (EMB) media, and the identification of the Enterobacteriaceae was carried out using the usual techniques such as Gram staining, oxidase and Api 20E gallery (BioMérieux-France) following the recommendations of the Clinical and Laboratory Standards Institute CLSI [1].

Antibiotics tested: The antibiotic sensitivity study was carried out using the KirbyBauer method on Muller-Hinton agar medium according to CLSI recommendations [1]. The eighteen antibiotics tested were: Amoxicillin / clavulanic acid (25/10 30 µg), Ceftaxidine (30 µg), Ceftazidime (30 µg), Cefixime (10 µg), Ceftriaxone (30 µg), Colistine (50 µg), Cefotaxime (10 µg), 10 mg), norfloxacin (10 µg), trimethoprim / sulfamethoxazole (1 µg), fosfomycin (50 µg), gentamycin (10 µg), piperacillin / tazobactam (25 / 23.75 µg), trimethoprim (5 µg). A cloxacillin disc (5 µg) was also used for the hyperproduction of cephalosporinases with loss of porine [1,2]. The E. coli ATCC 25922 strain was used as internal quality control.

Phenotypic characterization of carbapenemase-producing enterobacteria

The detection of carbapenemase-producing bacteria was carried out using the Kirby-Bauer disc diffusion method using 10 µg ertapenem discs, 10 µg of meperenem and 10 µg of imipenem, on Muller-Hinton agar. Any strain with reduced susceptibility to Ertapenem.

Modified Hodge test (MHT): a MacFarland 0.5 turbidity suspension of the E. coli E. coli ATCC 25922 strain was prepared and inoculated on Muller Hinton Agar medium. The medium was dried and a disc of ertapenem (10 µg) or meropenem (10 µg) was placed in the center of the test area. Using a straight swab, the test strain was distributed from the center edge to the center and this was repeated with each of the test strains plus the control strains (positive and negative) in different directions. The samples were incubated overnight at 35 ° C +/- 2 ° C. The results were read after incubation; if the bacterial growth in the form of a flood occurred at the intersection of the zone of inhibition of E. coli 25922 with the line of the test strain, then the test was considered to be positive, otherwise the test was considered negative for the production of carbapenemases.

Phenotypic inhibition tests: In short, they are based on the inhibitory properties of boronic acid or clavulanic acid with respect to carbapenemases of class A, and cloxacillin with respect to cephalosporinases. Using a cloxacillin disk helps to differentiate the strains which have carbapenem sensitivity from those which exhibit resistance by production of low-level carbapenemases, while the detection of metallo-betalactamase production was carried out by the disk test combined with two imipenem discs (10 µg), one of which contained 10 µg of anhydrous 0.1 M (292 µg) EDTA (Sigma Chemicals, St. Louis, MO). The disks were placed at an interval of 25 mm on a Muller-Hinton agar box, an increase in the inhibition diameter of the zone of > 4 mm around the imipenem-EDTA disk compared to that of the imipenem disc alone was considered positive for the production of metallo-beta-lactamase.

Detection of ESBL: The detection of extended spectrum beta-lactamases (ESBL) is based on the study of the synergy between amoxicillin + clavulanic acid (AMC) discs and third generation cephalosporins C3G, ceftazidime, a cefepime C4G disc and a Monobactam disc. aztreonam (Figure 1). Figure 1: Image showing the synergy between the AMC disc in the center and the C3G discs (CTX and CAZ), the C4G disc (FEP) and the aztreonam (ATM). Search for genes coding for carbapenemases DNA extraction: The extraction of chromosomal DNA was carried out by boiling from a bacterial culture in stationary phase (16-24 h) on a solid medium at 37 ° C. Two colonies were suspended in 100 µl RNase and DNase distilled water, then vortexed for 10 seconds. The preparation was placed in a water bath at 100 ° C for 10 minutes and then stored at -20 ° C until

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use. The samples were centrifuged at 19,000 xg for 5 min. 2 µl of extracted DNA were used as a PCR template, while the extraction of plasmid DNA was carried out using a commercial kit (Bioline Isolate plasmid Mini Kit Cat No. Bio- 52026) according to the manufacturer's instructions.

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