Research Article

Prevalence of Metallo- β -Lactamases Producing *Acinetobacter baumannii* in a Moroccan Hospital

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Objective. To determine the prevalence of metallo beta-lactamases (MBL) among carbapenem resistant strains of *Acinetobacter baumannii* in our hospital. **Methodology.** During a period of 12 months (January–December 2010), 47 isolates of *Acinetobacter baumannii* were collected from different clinical specimens of in-patients. Antimicrobial susceptibility was determined and interpreted using the disk diffusion method according to the Antibiogram Committee of the French Society for Microbiology guidelines. Imipenem nonsusceptible isolates were further screened for production of MBL. **Results.** All *Acinetobacter baumannii* isolates were resistant to ticarcillin, ticarcilline/clavulanate, piperacillin, piperacillin/tazobactam, gentamicin, tobramycin, and ciprofloxacin, except an isolate that was sensitive to ceftazidime and cefepime. In addition to that, amikacin and trimethoprim/sulfamethoxazole were, respectively, sensitive by 59.5% and 53%. Among 57.4% (27/47) imipenem non-susceptible isolates of *Acinetobacter baumannii*, 74% (20/27) were found to be MBL producers. **Conclusion.** Although the rate of imipenem non-susceptible isolates of *Acinetobacter baumannii* seems to remain stable in 2005 (57%) and 2010 (57.5%), the prevalence of MBL producer strain is increasing (38% in 2005 versus 75% in 2010). The findings strongly suggest that there is a need to track the detection of MBL producers; moreover, a judicious use of carbapenems is necessary to prevent further spread of these organisms.

1. Introduction

Acinetobacter baumannii is a typical nosocomial pathogen causing infections and a high mortality often among patients hospitalized in the Intensive Care Unit. *Acinetobacter baumannii* is intrinsically less susceptible to antibiotics than Enterobacteriaceae is; moreover, it has propensity to acquire resistance. The resistance of *Acinetobacter baumannii* to carbapenem is now a major worldwide issue [1–4]. A mechanism of this resistance is being characterized by the production of a specific enzyme called metallo beta-lactamases (MBL). These enzymes belong to Ambler class B β -lactamases based on their amino acid sequence homology and to group 3 according to the Bush classification based on their substrate profiles (imipenem hydrolysis.) These enzymes are inhibited by ethylene diamine tetra-acetic acid (EDTA) [1]. The rapid detection of MBL positive isolates is necessary to control

infection and to prevent their dissemination. PCR method was initially of simple use in detecting MBL-producing isolates but became more difficult with the increased number of types of MBLs; moreover, it is expensive for daily routine application in developing countries' laboratories as it is the case in Morocco. In 2010, the aim of this study was first to determine the prevalence of MBL among carbapenem resistant strains of *Acinetobacter baumannii* in our hospital, and second to compare this rate to the one found in 2005 [5].

2. Methodology

This study was conducted in the specialty hospital of Rabat, a 322 bedded tertiary care teaching hospital including Intensive Care, Neurology, Neurosurgery, Otolaryngology, and Ophthalmology Departments. Between January and

December 2010, 47 nonduplicate *Acinetobacter baumannii* were isolated and identified based on a battery of biochemical tests of which the galleries 20 NE API (BioMerieux SA, Marcy-l'Etoile, France). These were taken from various clinical specimens of in-patients. Antimicrobial susceptibility of all isolate was determined using the disk diffusion method according to the AntibioGram Committee of the French Society for Microbiology (CA-SFM), guidelines [4]. The panel of antimicrobial agents tested were as follows: ticarcillin (75 µg), ticarcillin/clavulanate (75/10 µg), piperacillin (75 µg), piperacillin/tazobactam (75/10 µg), ceftazidim (30 µg), cefepim (30 µg), imipenem (10 µg) gentamicin (15 µg), amikacin (30 µg), tobramycin (10 µg), ciprofloxacin (5 µg), trimethoprim/sulfaméthoxazole (1,25/23,75 µg), and colistin (50 µg). The source of the Mueller-Hinton agar and the antibiotics discs is the same as both of them belong to the Oxoid Ltd Company. Intermediately susceptible strains were accepted as resistant. *Pseudomonas aeruginosa* ATCC 27853 was used as the control strain for susceptibility testing.

3. Detection of MBL Production

All strains of *Acinetobacter baumannii* non-susceptible to imipenem (diameter < 24 mm) were screened for MBL production by a phenotypic method as described by Yong et al. [4]. In this test, organisms were inoculated in plates of Mueller-Hinton agar, as recommended by CA-SFM [6]. A solution was prepared by dissolving 186.1 g of disodium EDTA.2H₂O in 1,000 mL of distilled water and adjusting it to pH 8.0 by using NaOH. The mixture was sterilized by autoclaving. Two 10 µg imipenem disks were placed on the plate, and 750 mg of a 0.5 M EDTA solution was added to one of them. From 18 to 24 hours of incubation in the air at 37°C, the inhibition zones with imipenem-EDTA disks were ≤14 mm for the MBL-negative isolates, while they were ≥17 mm for the MBL-positive isolates. All the MBL positive isolates were repeatedly checked for reproducibility.

4. Results

A total of 47 *Acinetobacter baumannii* isolates were cultured from hospitalized patients; 39 (82%) were found in the intensive care unit, 6 (13%) from the Neurosurgery unit, and 2 (5%) from the Neurology. The isolates were obtained from different clinical specimen, including respiratory samples (69%), urine (22%), surgical site infection (5%), and cerebrospinal fluid (4%).

All isolates of *Acinetobacter baumannii* tested in this study were resistant to ticarcillin, ticarcilline/clavulanate, piperacillin, piperacillin/tazobactam, gentamicin, tobramycin, and ciprofloxacin. Only one strain (2%) was susceptible to ceftazidim and cefepim. Amikacin and trimethoprim/sulfamethoxazole were, respectively, susceptible by 59.5% (28/47) and 53% (25/47). All isolates were sensitive to colistin. A total of 57.4% (27/47) were not sensitive to imipenem; among them 74% (20/27) were found to be MBL producers.

5. Discussion

The present study revealed a high proportion of imipenem resistance among *Acinetobacter baumannii* isolates in our hospital. Although this rate seems to remain stable by 57% and 57.5%, respectively, in 2005 and 2010. Over all, this rate of resistance was higher than those reported from other Moroccan studies (23,8% [6] and 42,6% [7]). Within the last five years, the increasing number of carbapenem resistant *Acinetobacter baumannii* is of major importance in the context of resistance to β -Lactams. Indeed, between 1998 and 2004, this rate of resistance in Europe, North America, South America, and Asia ranged between 0% and 40% [8]. But in most studies after 2005, the rate of resistance was greater than 50%. The higher rates of resistance were found in Iran (63%) [2], Italy (62,5%) [9], China (55,6%) [10], Turkey (53,7%) [11], and Korea (51%) [12]. The source of *Acinetobacter baumannii* in our hospital was and still is in the intensive care unit (76% in 2005 versus 82% in 2010) especially from respiratory samples being taken from patients mechanically ventilated (62% for 2005 and 69% for 2010). The same distribution was reported by Peymani et al. [2] and Lee et al. [13]. This suggested that the invasive devices such as tracheal tubes are important reservoirs involved in *Acinetobacter baumannii*'s transmission.

In 1988, Japan reported the first plasmid-mediated MBL (IMP-1) in *Pseudomonas aeruginosa* [14]. Later this enzyme and its variants were detected in different Gram negative bacilli in different countries. In our present study, 74% of imipenem nonsensitive strains of *Acinetobacter baumannii* were MBL producers. This proportion was higher than the one found in 2005 in our institution (38% in 2005 versus 75% in 2010) and higher than those reported in other studies such as in Korea 14,2% [13], India 14,8% [15], and Iran 49% [2]. However, in two recent studies made in Pakistan by Kaleem et al. [16] and by Irfan et al. [17], the frequency of MBL *Acinetobacter baumannii* is higher by 84% and 96%, respectively. This emergence is a serious epidemiological risk for at least two reasons. First of all, the MBL does not confer resistance to carbapenem only, but to all β -Lactams and other classes of antibiotics such as aminoglycoside and fluoroquinolone. Second of all, the genes encoding these enzymes spread easily on plasmids, by that causing nosocomial infections and outbreaks with a mortality rates range from 25% to 75% [1]. These results indicate that the available choices for appropriate treatments for infection, caused by *Acinetobacter baumannii*, are currently limited. In vitro, studies reveal that tigecyclin, and colistin are the only antibacterial agents with consistent activity against MBL-producing strains [1]. Random controlled trials are required in order to evaluate the available therapeutic regimens, including treatment combinations.

6. Conclusion

The prevalence of MBL *Acinetobacter baumannii* is possibly increasing because of clonal and horizontal dissemination of resistance in our hospital. Respiratory and urine samples collected from Intensive Care Unit patients were found to be

the main sources of MBL-producing isolates. Early detection and infection control practices are the best defenses against these organisms; therefore, systematic surveillance to detect MBL producers is necessary. Last, but not least, a judicious use of carbapenems is essential to prevent the spread of these organisms.

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