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A Silent Invader –Acinetobacter: Picture of a Peripheral Medical College and Hospital of Eastern India

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Abstract

Background: Carbapenem resistant *Acinetobacter baumannii*, especially the metallo beta lactamase (MBL) positive ones, are fast coming up the list of pathogens causing nosocomial infections. Thus it should be a present day concern of all tertiary care hospital to detect the presence of MBL positive *Acinetobacter baumannii*.
Aims: To determine the presence and extent of MBL positive *Acinetobacter baumannii* group.

Methods and Material: 66 isolates of *Acinetobacter baumannii* group were found, and subjected to disc diffusion testing against various antibiotics. Imipenem resistant isolates (5) were selected for MBL detection by zone enhancement with EDTA impregnated imipenem discs.

Results: 5 (7.5%) were carbapenem resistant, and 1 (1.5%)was MBL producer. None of the isolates showed resistance to Amikacin.

Conclusions: MBL producing *Acinetobacter baumannii* group is present in this peripheral institution, although in low prevalence. However, to keep it in check, regular detection of this bacteria and judicious use of antibiotics to which it is still susceptible is mandatory.

Introduction

Hospitalized patients, worldwide, remain virtually submerged in a sea of potential pathogens. Many of these pathogens are multidrug resistant as well. Among these silent invaders of hospital environment is a non-fermenter with a metallic gun– Metallo beta lactamase (MBL) positive *Acinetobacter baumannii*.

With the introduction of carbapenems into the therapeutic scene, came the blissful feeling that the ultimate arsenal against beta lactam resistant gram-negative bacilli was finally at hand. With the turn of the century, however, came the bitter awareness gram-negative bacilli have started producing 2 types of carbapenem hydrolyzing enzymes: serine enzymes possessing a serine moiety at the active site (Ambler class A or D), and Metallo beta-lactamases (MBLs, belonging to Ambler class B), requiring divalent cations, usually zinc, as metal cofactors for enzyme activity [1].

These enzymes can hydrolyze not only carbapenems but many β-lactams as well [2]. The genes responsible for production of MBLs are VIM and IMP. Most of these genes are found as gene cassettes in integrons. These genes lie on a plasmid, and hence can be horizontally transferred to other, till now innocent bacteria easily, efficiently and rapidly [3].

Not only *Acinetobacter baumannii* has slowly emerged to be a chief pathogen of nosocomial infection, it has been seen that the community isolates are lacking in multidrug resistance – hence strengthening the belief that battle against carbapenemase positive isolates should start at the hospital [4].

We, the microbiologists of a peripheral Medical College of eastern India, took up the thread from here to find out how much of our territory has already been captured by this silent invader with a license to kill – MBL.

Material and methods

Twelve hundred and eighty four samples were collected during two years period from January 2009 to December 2010, in the department of Microbiology. The samples processed were: urine, pus / wound swab, sputum, and blood. Samples were cultured on MacConkey agar and Sheep Blood agar. Identification was done by biochemical test and a total of 66 isolates of *Acinetobacter baumannii* group were found and the isolates were stored at -20^0 C [5]. Antibiotic sensitivity was done on Mueller Hinton agar with commercially available Discs (Hi-media, Mumbai) by Kirby-Bauer method following CLSI guidelines.

The antibiotics incorporated were amikacin (30 μg), gentamicin (10 μg), tobramycin(10 μg), ceftazidime (30 μg), ceftriaxone (30μg), cefepime (30μg), cefoperazone(7.5μg), ciprofloxacin (5μg), gatifloxacin (5μg), piperacillin-tazobctum (100/10 μg),and imipenem (10μg).*Pseudomonas aeruginosa* reference strain ATCC27853 was used to check potency of the discs.

Among the various recommended techniques for phenotypic determination of MBL, like Double disc synergy test using imipenem and EDTA or ceftazidime and EDTA discs, EDTA impregnated Imipenem discs, E-test strip and microdilution technique for determining MIC of Imipenem, we took up the EDTA impregnated Imipenem disc potentiation test [6,7].

We used zone enhancement with EDTA impregnated imipenem discs. Test organisms were inoculated on the MHA plates as recommended by CLSI. A 0.5M EDTA solution was prepared by dissolving 186.1 gm of Disodium EDTA.2H_2O in 1000 ml of distilled water and its pH was adjusted to 8.0. This solution was sterilized by autoclaving. Then two 10μg imipenem discs were placed on test organism inoculated MHA and 5μl EDTA was added to one imipenem disc. After 16 hours of incubation at 35^{0}c, the zones of inhibition around both the discs were measured. The strains with enhanced zones around EDTA impregnated imipenem discs were identified as MBL producing *Acinetobacter baumannii* group.

Results

A total of 66 isolates of *Acinetobacter baumannii* group were found -17 were from 277 blood cultures, 27 were from 629 urine cultures, 16 were from 215 wound/pus, and 6 were from 163 sputum cultures. Of these 66 isolates of, 5(7.5%) showed resistance to imipenem. Among these, 1(1.5%) was seen to be MBL producer. None of the isolates were resistant to amikacin, and showed good susceptibility to gentamicin, tobramycin, and piperacillin-tazobactum.

TABLE I. Antibiotic sensitivity of *Acinetobacter baumannii* group.

		Numbers (%)	
ANTIBIOTICS	R	IS	S
Amikacin	0(0)		66(100)
Gentamycin	8 (12.1)		58 (87.8)
Tobramycin	7 (10.6)		59(89.3)
Ceftazidime	57 (86.3)		9 (13.6)
Ceftriaxone	55 (83.3)	2 (3)	9(13.6)
Cefepime	49 (74.2)		27(40.9)
Cefoperazone	61 (92.4)		5 (7.5)
Ciprofloxacin	65(98.4)		1 (1.5)
Gatifloxacin	17 (25.7)	1 (1.5)	48 (72.7)
Piperacillin-Tazobactum	10(15.1)		56 (84.8)
Imipenem	5(7.5)		61(92.4)

Discussion

In our study we found a low prevalence (1.5%) of MBL producing *Acinetobacter baumannii* group. Interestingly, we found that all the isolates, whether MBL positive or negative, showed susceptibility to amikacin. There are studies indicating that some older antimicrobials like polymixin and colistin, long kept in the shelf due to side effects, are acting well against multidrug resistant *Acinetobacter* [8, 9]. But there are no as yet found data on amikacin alone showing good results. On the contrary, reports indicating an increased trend toward amino glycoside resistance among *Acinetobacter* species are plenty [10].Although our study was done mainly to detect the presence of MBL positive *A. baumannii* group in our Medical College, this susceptibility pattern to aminoglycosides, especially to amikacin, unfolded to our pleasant surprise.

Out of the 66 isolates, 20 were from patients attending the outdoor clinics, and we considered these isolates as community acquired. None of these isolates showed resistance to imipenem.

There are reports showing failure of phenotypic methods to detect MBL positive isolates, but we had no means to avail the molecular methods in our resource restricted setup [11].

The sensitivity pattern shown by the isolates with a high resistance to third generation cephalosporins but low resistance to piperacillin-tazobactum indicated that they could be extended spectrum beta lactamase (ESBL) producing, and ESBL positive *Acinetobacter* is well documented in literature [12].

In our study, the *Acinetobacter baumannii* group isolates showed a disparity between the sensitivity patterns towards ciprofloxacin (98.4% resistant) and gatifloxacin (25.7% resistant). We attributed this difference to the fact that ciprofloxacin is available 'over the counter' from the medicine shops, as well as are freely distributed from several government hospitals in our country. Gatifloxacin, although available over the counter, is costlier, and hence not affordable by many, and is not distributed free of cost from government hospitals.

Lastly, in the Government hospitals like ours, situated in the periphery and catering mostly to village people who are economically downtrodden, the physician always has to keep in mind the cost of the medicine , if it is not supplied free of cost from the hospital. Under such circumstances, prescribing an antibiotic by the physician depends on the affordability by the patient. More often than not, the antibiotic which is on the cheaper side is selected from the sensitivity report, and is used as so called 'first line'.

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