

# Biofilm detection amongst Extended Spectrum Beta Lactamase (ESBL) and Metallo Beta Lactamase (MBL) producing clinical isolates of *Acinetobacter baumannii*

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## INTRODUCTION

*Acinetobacter baumannii* has emerged as one of the most troublesome pathogen responsible for various infections especially in the intensive care units, accounting for almost 10% of nosocomial infections. Over the recent years *Acinetobacter baumannii* has become a red alert pathogen as a result of their profundity in developing multidrug (MDR) and pan drug resistance (PDR).<sup>1</sup> Moreover, they have remarkable ability to acquire resistant determinant genes encoding for enzymes like extended spectrum beta lactamase (ESBL) and metallo beta lactamase (MBL), which make it resistant to extended spectrum cephalosporins and carbapenems. Patients infected with XDR or MDR strains act as reservoir of resistant organism and can further spread these lethal strains to community and health care worker.<sup>2</sup>

Acting in synergy with this emerging resistance profile is the ability of *Acinetobacter baumannii* to form biofilm. A biofilm is “a functional consortium of microorganisms organized within an extensive exopolymeric matrix.”<sup>3</sup> Organisms that form biofilm are more resistant to antimicrobial agents, as the concentrations of antimicrobials required to achieve bactericidal activity against biofilm forming organisms are three- to four-fold higher than for those bacteria which do not produce biofilm.<sup>4</sup> Biofilms are responsible not only for resistance to antibiotics, but also for transferring the resistance plasmids to other organisms. Thus, the ability of the organism to form biofilm enhances its survival properties, persistence on various inanimate objects and in further spreading of resistance to other microorganisms.<sup>5</sup> This study was done to know the association of various resistance mechanisms like ESBL and MBL production and biofilm production in *Acinetobacter baumannii* isolated from the lower respiratory tract infections of the patients admitted to ICU.

## METHOD AND MATERIALS

The present prospective observational study was conducted in the Microbiology department of a teaching tertiary care hospital during 6 months period from January to July 2016. Transtracheal or bronchoscopic aspirates collected aseptically from 55 patients of all age and sex groups were included in study. All the specimens received were immediately plated on the blood agar and Mac-Conkey agar by semi-quantitative method and incubated aerobically overnight at 37°C. Organisms were identified as commensal or pathogen as per protocol. Tracheal aspirates showing less than 10<sup>5</sup> cfu/ml and bronchial secretions with less than 10<sup>4</sup> colony forming unit (cfu/ml) by semi quantitative culture were regarded as commensal or contaminant.<sup>6</sup> All the consecutive, nonduplicate, non fermenting, gram negative cocobacilli, non motile, oxidase negative, catalase positive organisms were further speciated along with antimicrobial susceptibility testing with BD Phoenix automated system (Becton Dickinson, USA). Strains were further evaluated for ESBL production as per CLSI confirmatory tests and MBL production by combined disc test as explained below.

### ESBL detection:

Ceftazidime discs (30µg) alone and in combination with clavulanate (10 µg) were placed on lawn culture of the organism on Mueller Hinton agar plate and incubated for 24 hours at 37°C. A difference of >5mm between the zone diameters of both discs was taken as positive confirmation of ESBL production.<sup>7</sup>

### MBL detection:

Imipenem disc alone and in combination with EDTA were placed on lawn culture of the organism on Mueller Hinton agar plate and incubated for 24 hours at 37°C. A difference of more than 7 mm was considered positive for MBL production.<sup>8</sup>

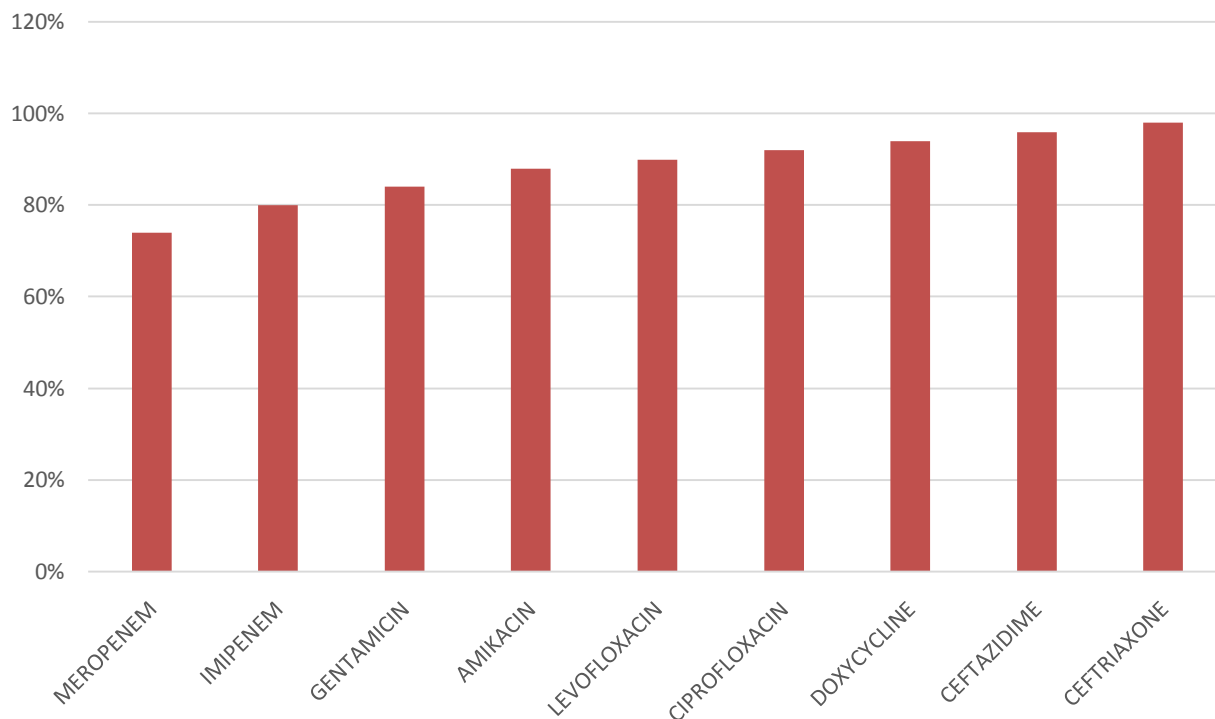
### Biofilm detection

Biofilm detection was done using the Microtitre tissue culture plate (MTCP) method as described by Christensen et al.<sup>9</sup> Overnight cultures of the isolate from nutrient agar plate was inoculated into Brain heart infusion broth with added 2% sucrose. A 1:100 dilution was prepared and 200 µl of this dilution was loaded into 96 wells flat bottom microtitre plate and the plates were incubated for 24 hrs at 37°C in aerobic conditions. The wells were then decanted and washed 4 times with PBS. It was then fixed with 2% sodium acetate for 15-30 minutes and then the wells were stained with crystal violet for 20 minutes. Optical density (OD) of each well was measured at 570 nm using an automated ELISA plate reader. Results were interpreted as follows:

Cutoff OD>0.29 = Strong biofilm producer; OD 0.12-0.24= Moderate biofilm production ; OD<0.12= Negative biofilm production.<sup>9</sup>

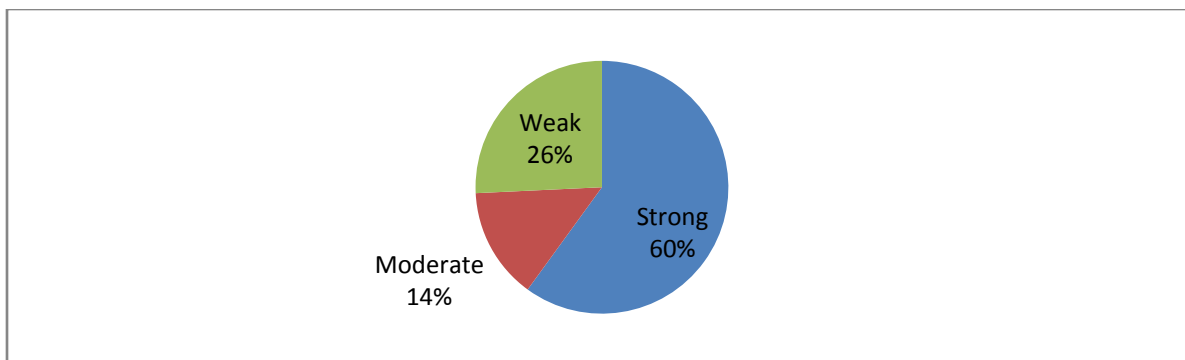
## RESULTS

During the study period a total of 35 *Acinetobacter baumannii* were recovered from tracheal or bronchial aspirates. The ratio of male to female was 3:1. The mean age of the patients was 46 years. *Acinetobacter baumannii* was found to be maximum sensitive to meropenem (26%), followed by imipenem (20%). A high level of drug resistance (76-98%) to all the other routinely used antibiotics was seen ( Fig.1).



**Fig 1: Antibiotic resistance profile of *Acinetobacter baumannii* isolated from lower respiratory tract infections**

Out of 35 isolates biofilm production was seen in 26 (74.28%) isolates. Out of 26 biofilm forming isolates, 21 (60%) were strong biofilm producers and 5 (14.25%) were moderate biofilm producers (Fig: 2). Out of all 35 isolates, ESBL production was seen in 25 (71.4%) and MBL production was seen in 22 (62.8%) isolates. Of 25 ESBL producers and of 22 MBL producers, strong biofilm formation was seen in 19 (76%) and 21 (95.45%) isolates respectively (Table 1).



**Fig 2: Grading of Biofilm Production**

**Table 1: Correlation of ESBL and MBL production with grading of biofilm production**

Resistance enzyme	Number	Strong Biofilm production
ESBL	25	19 (76%)
MBL	22	21 (95.45%)
Total	47	40 (85.11%)

## DISCUSSION

During the last decade, antimicrobial resistant strains of *Acinetobacter baumannii* have increased exponentially posing a major public health dilemma. Cephalosporins and carbapenems are amongst the most potent antibiotics currently available, but resistance to both the groups of antibiotics have emerged.<sup>10</sup> In our study *A. baumannii* was found to be maximum sensitive to carbapenems and highly resistant to routinely available antimicrobials. Our study correlates with Tripathi et al and Sinha et al who have also observed maximum sensitivity to imipenem and meropenem respectively and a higher resistance to all other available routinely used drugs.<sup>11,12</sup> Increasing antimicrobial resistance leaves few therapeutic options for multidrug-resistant (MDR) *Acinetobacter* infection. The problem is further worsened by the acquisition of drug resistance enzymes like ESBL and MBL by these organisms and knowledge about the prevalence of these enzymes possessed by the organisms is an essential guide towards appropriate antibiotic treatment. These organisms are not easily detected by the routine antimicrobial susceptibility tests leading to falsely reporting a resistant organism as a susceptible one. Hence it becomes essential to screen all *Acinetobacter* isolates for the production of ESBL or MBL enzymes. In our study, ESBL production was seen in 25 (71.4%) isolates and MBL production was seen in 22 (62.8%) isolates. Variable results have been observed by different studies done across India and abroad. Tripathi et al demonstrated ESBL production in 29.9% isolates and MBL production in as high as 86.9% isolates whereas Yong et al observed 54.63% isolates to be ESBL producers and 50% isolates to be MBL producers.<sup>11,13</sup>

Further exacerbating the problem of multidrug resistance is the formation of biofilm by *Acinetobacter* species. Gara et al have demonstrated that clinical MDR isolates of *Acinetobacter* have this profound ability to form significant amounts of biofilms which are highly resistant to human immune system as well as to different antimicrobial agents available.<sup>14</sup> This high colonising capacity of *Acinetobacter*, along with its resistance to multiple drugs, helps in the organism's survival and further dissemination in the hospital setting. In our study, biofilm formation was seen in 26 (74.28%) isolates, out of which, 21 (60%) were strong biofilm producers and 5 (14.25%) were moderate biofilm producers. Different studies have demonstrated variable results of biofilm formation by *Acinetobacter* isolates ranging from 50%-65%.<sup>4,15,16</sup> A remarkable observation of the study was that of the 25 ESBL producers and 22 MBL producers, strong biofilm formation was seen in 19 (76%) and 21 (95.45%) isolates respectively. This observation shows strong correlation between ESBL, MBL production and biofilm formation. Our study was supported by Gurung et al who demonstrated 73% of the biofilm forming *Acinetobacter* isolates to be ESBL and MBL producers.<sup>4</sup> The importance of biofilm, ESBL and MBL producing *A. baumannii* in infection can be a valuable guide to clinicians for judicious use of antibiotic.

## CONCLUSION

We conclude that there is a high prevalence of ESBL, MBL and biofilm producing *A.baumannii* in our ICU. Knowledge regarding these organisms could help us in formulating hospital antibiotic policy as well as better patient outcome.

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