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Original Article

Incidence of ESBL, AmpC ß-lactamases and Metallo ß-lactamase producing *Acinetobacter baumannii*in intensive care units of a tertiary care hospital from north India

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ABSTRACT

Background: *Acinetobacter baumannii* is an emerging cause of nosocomial infections. These infections are difficult to control and treat because of the increasing antimicrobial resistance worldwide. **Objective:** To study the incidence of ESBLs, AmpC ß-lactamase and metallo ß-lactamase producing *Acinetobacter baumannii***. Material and methods:** The isolates of *Acinetobacter baumannii* obtained from all samples received in the department of microbiology over a period of one year were further characterized as of ESBLs, AmpC ß-lactamase and metallo ß-lactamase produuers**. Results:** Out of the 380 *A.baumanni* isolates, 373 (98%) were probable ESBL producers and 37(9.7%) were confirmed ESBL producers. 96.3%(366) of the isolates, were probable AmpC producers, whereas only 180 (47.3%) isolates were confirmed AmpC producers. Further, 292 (76.8%) isolates were probable MBL producers and very few, 72(19%) could be confirmed as MBL producers**. Conclusion:** The high prevalence of resistance in Acinetobacter isolates emphasizes the need for early detection so that it can help in providing appropriate antimicrobial therapy and also to combat the nosocomial infections.

Keywords: ESBL, Ampc ß-lactamases,metallo ß-lactamase, *Acinetobacter baumannii*, ICU

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INTRODUCTION

Acinetobacter baumannii has emerged as a major cause of healthcare associated infections, most of which occur in critically ill patients admitted in intensive care units.[1] Treatment of *Acinetobacter* infections has conventionally involved the use of ß-lactams, aminoglycosides, and quinolones. However, the increased use of these antibiotics has resulted in a widespread emergence of antibiotic resistant strains.[2]The various mechanisms responsible for drug resistance in *Acinetobacter baumannii* includes production of enzymes inactivating antibiotics, reduced entry of antibiotic into the target site of bacteria with porin loss &efflux mechanisms and alteration of the target or cellular functions due to mutations.[3]

In the recent years, carbapenam being the drug of choice for the treatment of infections caused by drug resistant *Acinetobacter species,* carbapenem resistant *A. baumannii* has emerged as a potential threat and it is usually resistant to almost all antimicrobial classes except colistin and tigecycline.[4,5]The most important mechanism of carbapenem resistance in *A. baumannii* is the production of ß-lactamases, which hydrolyze the carbapenems. These hydrolyzing enzymes include Extended Spectrum β-lactamases(ESBLs),metallo-β-lactamases(MBLs) and AmpC β-lactamases.

Keeping this in mind, present study was undertaken to study incidence of ESBLs, AmpC ß-lactamases and metallo ß-lactamase producing *Acinetobacter baumannii* in intensive care units of a tertiary care hospital from north India

MATERIALS AND METHODS

A total of 380 isolates of *Acinetobacter baumannii* from various clinical samples (Blood, Urine, pus, sputum, endotracheal secretions, bronchoalveolar lavage, body fluids etc.) received in the Department of Microbiology, during the study period (March 2014-Feb2015) were included in the study. These isolates were identified as per standard protocols and were further screened and confirmed for the production of ESBL, AmpC and MBL.

Detection of ESBLS*:*

Screening for ESBLs: All the isolates were screened for the production of ESBLs using ceftazidime Disc (30µg).An inhibition zone of <22mm indicated resistance to ceftazidime and probable ESBL producer.

*Double Disc Diffusion Test (DDDT) for confirmation of ESBL:*A lawn culture of test organism was done on MHA. The discs of ceftazidime alone (30µg) and in combination with clavulanic acid (10µg) were applied on the plate. The discs were placed in such a way that the centre to centre distance between the discs was 30 mm. The MHA plate was incubated at 35°C for 24 hours as per CLSI guidelines.[6]An expansion of zone of inhibition ≥ 5 mm around the combination disc was considered a positive result.(Photo-1)

Detection of AmpC*ß *lactamases: .[7]

*Screening for AmpC:*All the isolates were screened for the production of AmpC using cefoxitin Disc (30ug). An inhibition zone of <18mm indicates resistance to cefoxitin and probable AmpC producer.

*AmpC Disc Test for confirmation of AmpC*ß*-lactamase production***:**A lawn culture of *E.coli* ATCC 25922, using a culture suspension adjusted to 0.5 Mcfarland, was done on a Mueller Hinton agar plate. A cefoxitin disc (30 µg) was placed on the surface of the agar. AmpC disk was moistened with 20 µl of sterile saline & inoculated with few colonies of test organism. This disk was then placed besides the cefoxitin disc (almost touching) with the inoculated side facing downwards. The MHA plate was incubated at 35°C for 24 hours. Flattening or indentation of cefoxitin inhibition zone was considered as an AmpCproducer.(Photo-2)

Detection of MBL: .[6]

Screening forMBL: Theisolates were screened for the production of MBLusing ertapenemdisc[6]. An inhibition zone of <22 mm indicated resistance to ertapenem and probable MBL producer.

*Modified Hodge Test (MHT)[6] for confirmation of MBL production:*A lawn culture of *E.coli* ATCC 25922 was done on MHA plate and ertapenem disc was placed on the plate. Test organism was inoculated in a straight line, out from the edge of the disc. The plate was incubated at 35°C for 24 hrs. Enhancement of growth around the test organism,was considered as MBL production.(Photo-3)

RESULTS

A total of 380 isolates of *A. baumannii* were obtained from various clinical samples received during the study period.Out of the total *Acinetobacter baumannii* isolates, 373 (98%) were probable ESBL producers and 37 (9.7%) were confirmed ESBL producers. 96.3% (366) of the isolates, were probable AmpC producers, whereas only180 (47.3%) isolates were confirmed AmpC producers. Further, 292 (76.8%) isolates were probable MBL producers and very few, 72(19%) could be confirmed as MBL producers. (Table

1&Figure-1). Co-production of ESBL &AmpC was seen in 1.3% of the isolates. Whereas, simultaneous production ESBL & MBL was observed in 1.5% of the isolates.On the other hand, AmpC& MBL co–production was seen in 2.8% of the *Acinetobacter baumannii* isolates. Simultaneous production of all the three types of lactamases was seen in only 0.2% of the isolates (Figure 2).

Figure1:Percent distribution of probable and confirmed ESBL/AmpC/MBL producing *Acinetobacter baumannii* isolates (n=380)

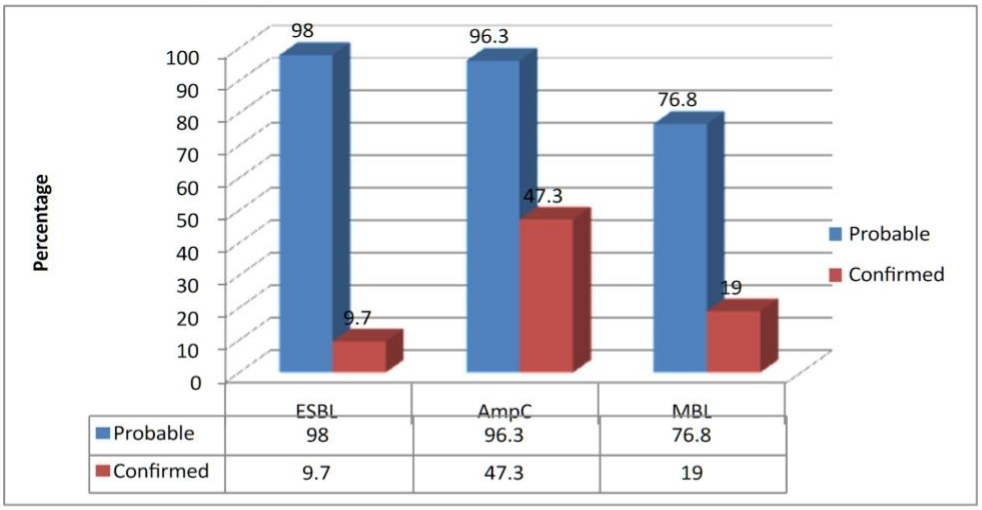
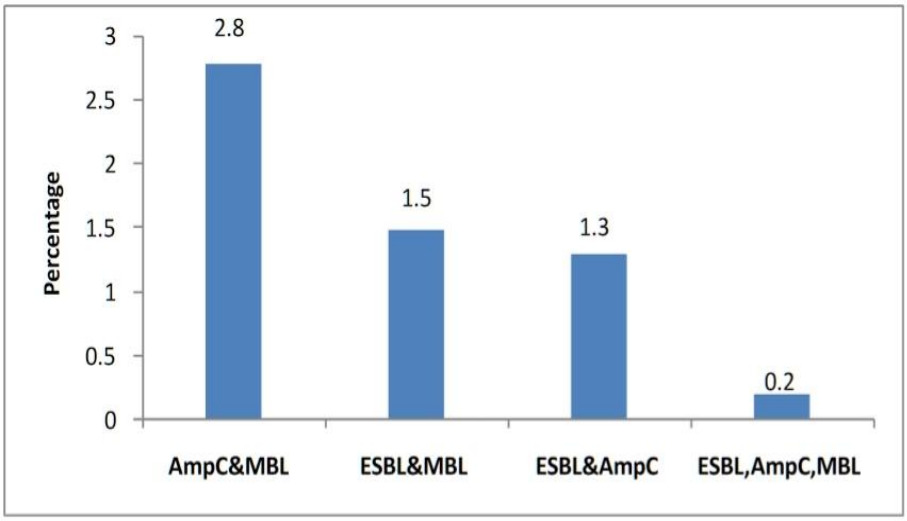


Figure 2: Distribution of ESBL, AmpC and MBL coproduction in *Acinetobacter baumannii* isolates (n=380)



DISCUSSION

A.baumannii is an effective human colonizer in the hospital. Combination of its environmental flexibility and presence of multiple resistance determinants makes it a successful nosocomial pathogen. Nosocomial infections tend to occur more frequently in immunocompromised individuals. The epidemiological, clinical, prognostic and therapeutic characteristics of *A.baumannii* isolated from infected patients have been studied widely in the last decade. The most alarming problems encountered during this period are the organism's ability to accumulate diverse mechanisms of resistance and the emergence of strains that are resistant to all commercially available antibiotics. These resistant organisms may lead to therapeutic dead ends if not detected earlier.

In the present study, a total of 380 *Acinetobacter baumanni* isolates were characterised into ESBL, Amp C and MBL producers. ESBL production was seen to be in 9.7% of the *Acinetobacter baumannii* isolates. This is comparable to the study done by Goel*et al* in tertiary care hospital of Karnataka. 40

isolates of *Acinetobacter* species were tested for beta lactamase production and a prevalence rate of ESBL production among the Acinetobacter isolates was found to be 17.9%. [8] Another study done by Parviz*et al* showed 27 isolates of Acinetobacter out of 126 (21%) as ESBL producers.[9] Two different studies on *Acinetobacter baumannii* in Turkey and Korea showed comparatively higher incidence of ESBL production.[10,11] In another study performed in Saudi Arabia, it was reported that 8.1% of *A. baumanni* strains isolated from burn patients were ESBL producers.[12]

The important beta lactamases which have been identified in *Acinetobacter baumannii* include metallo beta lactamases, and AmpC beta lactamases. In present study, we used an AmpC disc test which is an easier, reliable and rapid method of detection of isolates that harbour beta lactamase enzyme. AmpC production was seen in 47.3% of the isolates of *A. baumannii* which is similar to study done at Karnataka that showed 43.5% of the *Acinetobacter* species as AmpCproducers.[8] The present study is also comparable to a study done by Kumar et al in Andhra Pradesh in which nearly 82% of the Acinetobacter isolates were AmpC producers .[13] .Our data is also comparable to the study done in Ghaziabad, U.P. India which showed 52.9% of Acinetobacter isolates to be AmpC producers.[14]
MBL production was observed in 19% of the*Acinetobacter baumannii*isolates in the present study, while a similar study performed in France, showed 10% MBL production in *A. baumannii*.[15] Another study by Kaleem*et al* has reported MBL production among 37% of the *Acinetobacter baumannii*isolates.[16] A study done by Goel*et al* in a tertiary care hospital of Karnataka, has shown that 48.7% of the Acinetobacter isolates were MBL producers[8] . Our data is also comparable to the study done from Ghaziabad, U.P. India which showed 26.4% of Acinetobacter isolates to be MBL producers.[14]

It is apparent that various different mechanisms exist for production of multiple β lacatamase especially in high risk area such as ICUs where newer β lactamas are routinely prescribed. Though a high level of resistance has been shown by the Acinetobacter isolates against carbapenems but these are still the choice of drugs which should be kept in reserve. The marked increase in AmpC along with ESBL and MBL has left us with a few alternatives in combating serious infections. The high prevalence of these organisms in the ICUs emphasizes the need for an early detection of the β lactamases producing organisms by simple screening methods, which can help in providing an appropriate antimicrobial therapy and in avoiding the development and the dissemination of these multidrug resistant strains. The need of the hour is that every health care institution must develop its own antimicrobial stewardship program which is based on the local epidemiological data and international guideline, to optimize the antimicrobial use among the hospitalized patients, to improve the patient outcomes, to ensure a cost effective therapy and to reduce the adverse consequences of the antimicrobial use. Preventive measures like a continuous surveillance of the ICUs and a strict implementation of infection control practices can go a long way in containing the menace of drug resistance in the health care settings.

Photo-1: Double disc diffusion test

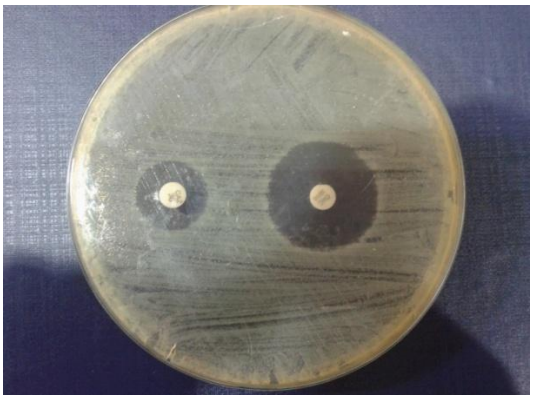


Photo-2: Amp C disc Test

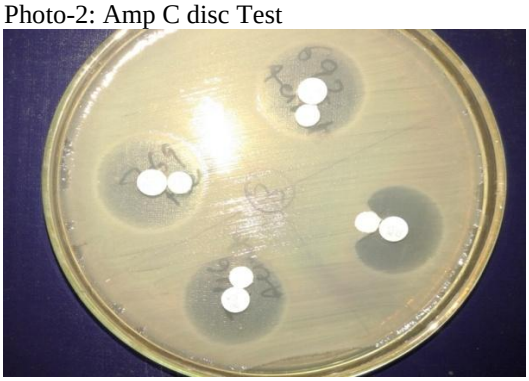
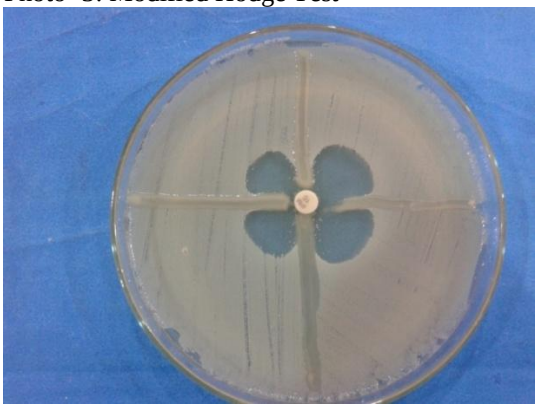


Photo- 3: Modified Hodge Test



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