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Reserach Article

INCIDENCE OF ESBL, AMPCß-LACTAMASE PRODUCING, MULTI DRUG RESISTANT AND EXTENSIVELY DRUG RESISTANT *ACINETOBACTER* SPP. IN INTENSIVE CARE UNITS OF A TERTIARY CARE HOSPITAL FROM NORTH INDIA

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A R T I C L E I N F O

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A B S T R A C T

Background: *Acinetobacter spp* 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 ESBL, AmpC, beta lactamases, MDR, XDR, and PDR *Acinetobacter spp.* **Material and methods:** The isolates of *Acinetobacter spp.* obtained from Blood and body fluid samples received in the department of microbiology over a period of one year were characterized on basis of their sensitivity to various drugs and were further categorized as ESBL or Amp C producers and also MDR,XDR and PDR **Results:** Out of the 44 *Acinetobacter* isolates, 38(86.4%) were probable ESBL producers and 3(7.8%) were confirmed ESBL producers. 38(86.4%) of the isolates, were probable AmpC producers, and 20(45.5%) of them were confirmed AmpC 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.

INTRODUCTION

Emergence of resistance to multiple antimicrobial agents in pathogenic bacteria has become a significant public health threat as there are fewer, or even sometimes no, effective antimicrobial agents available for infections caused by these bacteria.The problem of increasing antimicrobial resistance is even more threatening when considering the very limited number of new antimicrobial agents that are in development [1-2]

The combination of its environmental resilience and its wide range of resistance determinants renders it a successful nosocomial pathogen.[3] MDR *Acinetobacter. spp*. infections tend to occur in immunosuppressed patients, in patients with serious underlying diseases, and in those subjected to invasive procedures and treated with broad-spectrum antibiotics. Thus, infections due to *Acinetobacter*. spp. are frequently found in intensive care units (ICUs). [4]

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.[5]

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The various mechanisms responsible for drug resistance in *Acinetobacter spp.* includes production of enzymes inactivating antibiotics, reduced entry of antibiotic into the target site of bacteria with porin loss &efflux mechanisms and a*lteration of the target or cellular functions due to mutations*.[6]

In the recent years, carbapenam being the drug of choice for the treatment of infections caused by drug resistant *Acinetobacter species,* carbapenem resistant *A. spp.* has emerged as a potential threat and it is usually resistant to almost all antimicrobial classes except colistin and tigecycline.[7,8] The most important mechanism of carbapenem resistance in *A. spp.* 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 and AmpCß lactamase producing *Acinetobacter spp.* in intensive care units of a tertiary care hospital from north India

MATERIALS AND METHODS

A total of 44 isolates of *Acinetobacter spp* from Blood/Body fluid samples 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, their Antibiotic susceptibility testing was done and were further screened and confirmed for the production of

ESBL and AmpC beta lactamases. These isolates were then characterized as MDR, XDR and PDR organisms

Antibiotic Susceptibility testing

Antibiotic susceptibility testing was done by Kirby Bauer's disc diffusion method on Mueller Hinton Agar (MHA) as per CLSI guidelines.[9] The antimicrobial disc applied were gentamicin (10µg), amikacin (30µg), cefotaxime (30µg), ceftazidime (30µg), ciprofloxacin (5µg), cotrimoxazole (1.25/23.75µg), piperacillin-tazobactam (100µg/10µg), cefoperazone-sulbactam (75µg/30µg), imipenem (10µg), meropenem (10 µg), For the quality control *E.coli* ATCC 25922 was used as control organism as per CLSI recommendation.[9]

The zones of inhibition were measured after 24 hours of incubation and compared with the Performance Standards of Antimicrobial Disc Susceptibility Test provided by CLSI.

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.[9]An expansion of zone of inhibition ≥ 5 mm around the combination disc was considered a positive result.(Photo-1)

Detection of AmpC ß lactamases:[10]

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 AmpC producer.(Photo-2)

Categorization Into MDR, XDR, PDR.[11]

MDR: non-susceptible to ≥1 agent in ≥3 antimicrobial categories.
XDR: non-susceptible to ≥1 agent in all but ≤2 categories.
PDR: non-susceptible to all antimicrobial agents

RESULTS

A total of 44 isolates of *Acinetobacter spp* were obtained from Blood/Body fluid samples received during the study period. Out of the 44 isolates, nearly 86% were resistant to aminoglycosides and cephalosporins, 84% of the isolates were resistant to fluroquinolones, carbapenems, β lactam and β lactam inhibitor combinations. 38(86.4%) of the isolates were probable ESBL producers and 3(7.8%) were confirmed ESBL producers. 38(86.4%) of the isolates, were probable AmpC producers, and 20(45.5%) of them were confirmed AmpC producers. (Figure-1)

38(86.4%) of the isolates were Multi drug resistant (MDR) and Extensively drug resistant (XDR).None of the isolates was Pan Drug Resistant (PDR)

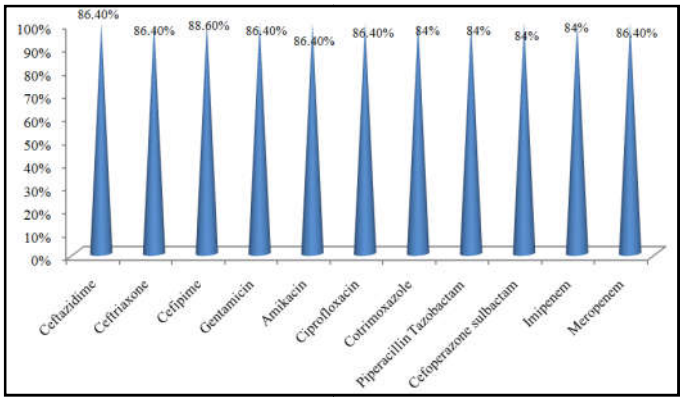

Figure 1 Antimicrobial resistance pattern of *Acinetobacter spp* (n=44)

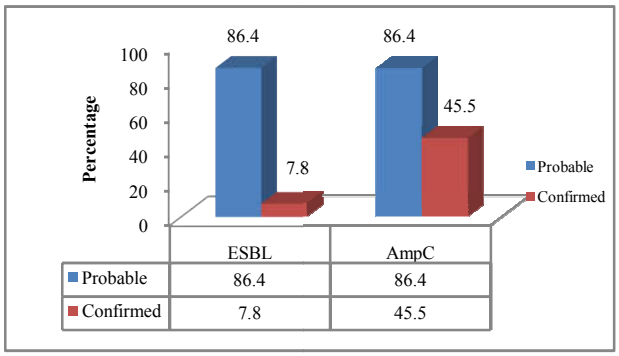

Figure 2 Percent distribution of probable and confirmed ESBL/AmpC producing *Acinetobacter* isolates (n=44)

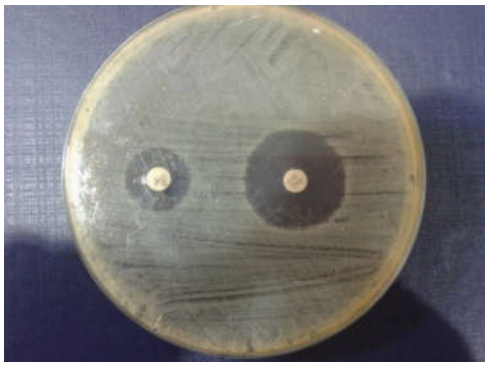

Photo 1 Double disc diffusion test

Photo 2 Amp C disc Test

Acinetobacter spp. 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.spp.* 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.

Antibiotic resistance in *Acinetobacter* is increasing at an alarming rate leading to increased morbidity, mortality and treatment costs in ICU settings as revealed by surveillance studies.[12] Multidrug resistance limits therapeutic options and thus worsen the serious infections caused by non fermentive Gram negative bacilli. The emergence of antibiotic resistant strains poses a significant problem both in community as well as hospital practice in deciding empiric therapy. It is therefore important to monitor the changing trends in bacterial infections and their antimicrobial susceptibility patterns. Studies like the present one, help in establishing the etiological agents and deciding empiric therapy from time to time. Our study showed that 86.4% of the *Acinetobacter* isolates were multidrug resistant and extensively drug resistant. A study done by Dent *et al*, also showed that out of 247 *Acinetobacter spp.* isolates, 72% were multidrug resistant and 58% of the isolates were highly resistant to imipenem, amikacin and ampicillin-sulbactam.[13]

Acinetobacter spp. have become increasingly resistant to almost all antimicrobial agents that are currently available, including the aminoglycosides , broad spectrum beta lactams, and cephalosporins. In the present study also, high level of resistance towards third generation cephalosporins, fluoroqinolones and aminoglycosides(86.4%), was seen. Our findings were similar to findings of a study done on 152 isolates of *Acinetobacter spp,* where resistance to two or more antibiotics was seen in 69.2% of the isolates. [14] The findings of present study are also supported by other studies. [15-17] Earlier studies in India have reported lower resistance rates towards carbapenems (9.8-18.5%) in *A.spp.*.[18] however in the present study very high resistance towards carbapenem (84-86%) was seen. This clearly explains that our study brings up an important aspect of increasing resistance in *A.spp.* towards carbapenems.

In the present study, a total of 44 *Acinetobacter* isolates were characterised into ESBL and Amp C producers. ESBL production was seen to be in 7.8% of the *Acinetobacter* 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%. [19] Another study done by Parviz *et al* showed 27 isolates of Acinetobacter out of 126 (21%) as ESBL producers.[20] Two different studies on *Acinetobacter spp.* in Turkey and Korea showed comparatively higher incidence of ESBL production.[21,22] In another study performed in Saudi Arabia, it was reported that 8.1% of *A. baumanni* strains isolated from burn patients were ESBL producers.[23]

The important beta lactamases which have been identified in *Acinetobacter spp.* 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 40.5% of the isolates of *Acinetobacter species* which is similar to study done at Karnataka that showed 43.5% of the *Acinetobacter* species as AmpC producers.[19] 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 .[24]

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.[25]

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.

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