Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v29i04.3688

HIGHER PREVALENCE OF ESBL PRODUCING *ACINETOBACTER SPECIES* AT ROHILKHAND REGION IN U.P.

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Abstract

Introduction: Acinetobacter is an opportunistic pathogen that has evolved into important bacteria associated with nosocomial infections worldwide and is known to develop multidrug resistance (MDR), extensive drug resistance (XDR) and pan drug strains resistance (PDR) including the carbapenem group.

Methods: This study was a hospital cross-sectional study conducted at the Department of Microbiology, Rohilkhand Medical College Bareilly Utter Pradesh on 350 specimens of Acinetobacter species. All clinical specimens of Acinetobacter species were obtained from the microbiology laboratory of Rohilkhand medical college Bareilly. Antimicrobial susceptibility tests (AST) were performed according to the disk diffusion method of scientist Kirby Bauer and based on CLSI guidelines. Extended-spectrum β-lactamase (ESBL)-producing Acinetobacter species were identified using DDST.

Results: Out of 350 samples, 88% of the samples showed resistance to ceftazidime, 78.6% to cefepime and 89.7% to ceftriaxone. Of these, 53% of Acinetobacter isolates were phenotypically found to be ESBL producers.

Conclusion: This current study concluded an alarming level of ESBL producing Acinetobacter spp. in various clinical specimens in the Rohilkhand region. Prevalence of ESBL producing Acinetobacter spp. increasingly threatens health care settings and clinical patient outcomes worldwide and points to the overuse of antibiotics. Detection of an ESBL producer requires active surveillance. This will help prevent the spread of infection.

Keywords: Increased Prevalence, ESBL, Acinetobacter Species and Double Disc Synergy Test

1. Introduction

The species of *Acinetobacter* are Gram-negative bacteria, strictly aerobic non-fermenting, non-fastidious, non-motile, catalase –positive and oxidase negative cocco bacillary type of bacteria. These types of infections are often very difficult for the clinician to treat because of the extensive resistance of these bacteria to the major groups of antibiotics. As stated in the most recent scientific

literature, it is the second most common non-fermenting gram negative bacteria isolated from clinical samples after Pseudomonas aeruginosa and is listed by the American Society of Infectious Diseases (IDSA) reported as one of the six most dangerous microorganisms (HW Boucher et al., 2009). Its most important representative are *Acinetobacter baumannii* and other species such as *Acinetobacter lwoffii*, *Acinetobacter haemolyticus* and *Acinetobacter johnsonii* are rarely isolated from patients.

The *Acinetobacter* species bacteria are diligent pathogens venturesome found in immune compromised patients reported by (S Saha et al., 2018). However the proportion of infection with non-fermenters is less when compared to that of Enterobacteriaceae, non-fermenters are of censorious importance given the rigidness of infections they can cause and intrinsic resistance to all most antibiotics studied by (JD Perry., 2017). A side from the intrinsic property, they have a high position to acquire resistance to broad-spectrum β-lactams, amino glycosides, fluoroquinolones and the tetracyclines. N Goel et al., 2011 observed that the *Acinetobacter species* bacteria have emerged as a high infuriating pathogen for numerous hospitals especially in intensive care units universally due to their immense capacity to acquire or up-regulate antibiotic drug resistance determinants.

The *Acinetobacter spp*. bacteria have arisen as a cause of intensive care units infection. Multi resistant Acinetobacter spp. bacteria including the major species, A Agodi et al., 2006 examine the Acinetobacter baumannii is growing as real infectious threat mainly in the intensive care units. S Rungruanghiranya et al., 2005 observed that the Acinetobacter species bacteria usually increase the incidence of *Acinetobacter* infection over the past two decades. M Nguyen et al., 2021 studied major hospital-acquired infections and the treatment of patients with multidrug-resistant organisms, such as *A. baumannii*. Since carbapenem-resistant *A. baumannii* has increased globally, this microbe has substantially hurt public health. To develop new remedy and control measures for *A. baumannii*, this microbe must be well understood. The development, categorization, and biological properties of carbapenem and its usefulness in the field of critical care medicine were examined by M Nguyen and SG Joshi 2021. Extended-spectrum β-lactamase (ESBLs) enzymes is plasmid mediated and produced by gram negative bacteria that mediate a resistance to beta lactums, second and third generation of cephalosporin's, and carbapenem studied by D Rawat et al., 2010. These enzymes belong to TEM and SHV families reported by H Mehrgan et al., 2008. Being plasmid mediated, they can be easily transferred from one organism to another organism. I Czobor et al., 2014 identified that clinical types of ESBL have includes various gene like *bla* SHV, *bla* TEM, *bla* VEB, *bla* KPC, *bla* PER, *bla* BEL-1, *bla* BES-1, *bla* SFO-1, *bla* TLA and *bla* BIC that are associated with plasmids. ESBL take part in an important role in the grow of resistance against second and third generation cephalosporin such as ceftazidime, cefotaxime, cefixime, cefepime, Ceftriaxone GG Zhanel et al., 2013.The prevalence of ESBL producing *Acinetobacter* species was growing in the various region in the world due to spread of antibiotic resistance is mediated by mobile genetic element plasmid. The aim of our study was to investigate the prevalence of ESBL production among *Acinetobacter* species obtained from various clinical samples.

2. Materials and Methods:

The present current study was conducted in the microbiology department at Rohilkhand Medical College & Research Bareilly Uttar Pradesh India after taking approval from the Institutional Ethical Committee. In our study we used clinical isolate of the *Acinetobacter species* recovered successively from 350 clinical sample that include blood, pus, urine, sputum, body fluid, ET Tip and high vaginal swab samples to the department of microbiology laboratory. The all samples of study were processed for identification of clinical isolates according to standard conventional methods.

Antimicrobial Susceptibility Testing: In this study the sensitivity of different classes of antimicrobial agents was examined using disk diffusion method according to CLSI guidelines (2019). There are following antibiotics were used; Amikacin (AMK:30µg), cefepime (FEP: 30 µg), Colistin (Col:110 µg), ceftazidime (CAZ :30 µg), Levofloxacin (LEV:5 µg), Imipenem (Imp :10 µg**),** meropenem (MER: 10 µg), ciprofloxacin (CIP: 5 µg), ceftriaxone (CRO:30 µg), piperacillin-tazobactam (PTZ: 100/10 µg), Gentamycin (GEN:10µg), Cefeparazone-sulbactum (CFS:75/30µg)

Amoxicillin clavulanic acid(AMC Netilmicin(NET), Polymyxin B(PB) and Ampicillin-sulbactum(A/S) (10/10µg) Minimum Inhibitory Concentrations (MICs) were determined by E strips test by HI media Ezy MIC ™ strip (CLSI2018). We preserved all samples were at -80°C for further testing.

Screening of ESBL enzyme producing isolates: were resistance for cefepime and ceftazidime were tested for extended spectrum beta lactamase production by double disc Synergy test (DDST) method. A disc of Ceftazidime (30mcg) or Cefotaxime (30mcg) alone and ceftazidime clavulanic acid (30mcg+10mcg) or Cefotaxime clavulanic acid ((30mcg+10mcg) was placed20 mm apart, center to center on MHA agar plate and was incubated at 37°C temperature for 24hrs. A ≥ 5mm raise in diameter of the inhibition zone of ceftazidime clavulanic acid disc, when compared to ceftazidime and Cefotaxime disc alone, was clarify as phenotypic verification of ESBL production studied by JD Perry 2017.

3. Results

In over current study 350 sample were processed in that out of 350 samples of the *Acinetobacter species* in various clinical isolates were found 98.3% in which *A baumannii*,. 1.4% of *A. lwoffii*, and 0.3% were *A. Haemolyticus* showing in (figure-1).

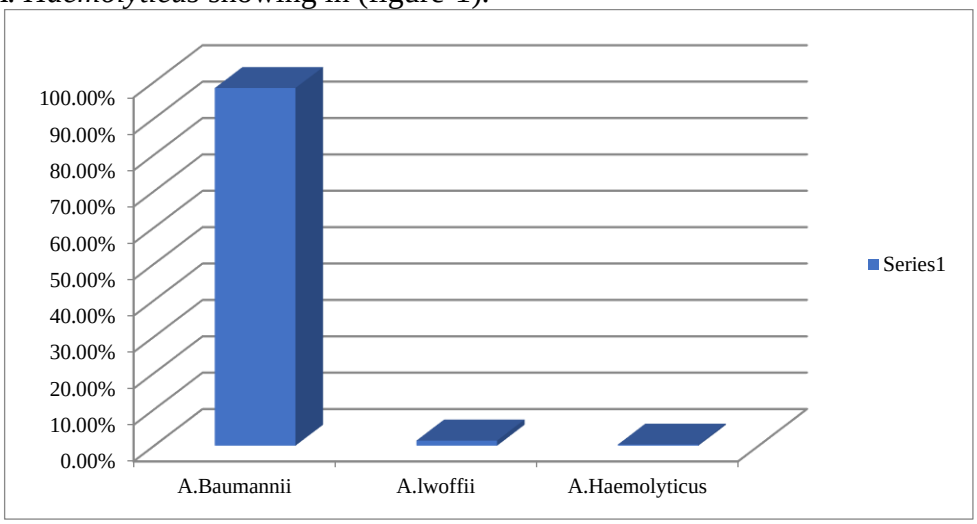


Figure- 1 Distribution of *Acinetobacter species.*

Isolation rate of the *Acinetobacter spp.* was observed higher in the pus sample, followed by blood, endotracheal tip and urine. The lowest no of isolation was found in high vaginal swab and cerebrospinal fluid.

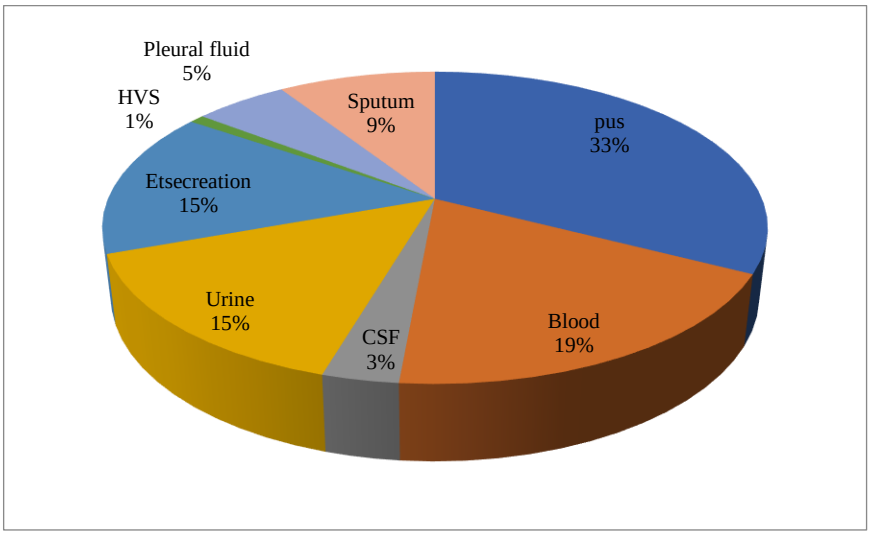


Figure-2 Sample wise distribution of *Acinetobacter species*

Acinetobacter species shows high level of resistance to most of the antibiotics tested. In our study amoxicillin clavulanic acid showed 90.9%resistance, ceftriaxone 89.7%, ceftazidime88%, 78.6% resistance were observed in cefepime and 80.9%were resistant to imipenem and 77.4% in meropenem .100% sensitivity was observed in Polymyxin B. 43.9% sensitivity was reported towards doxycycline22.6% sensitivity were observed in Meropenem .

Table 1 Antibiogram of Acinetobacter species.

Antibiotic	**Sensitive**	**Resistance**
Ampicillin sulbactum	29.2%	70.8%
Amoxycillin-clavulanic acid	9.1%	90.9%
Piperacillin -tazobactum	13.5%	86.5%
Ceftazidime	12%	88%
Cefepime	21.4%	78.6%
Ceftriaxone	10.3%	89.7%
Amikacin	20.9%	79.1%
Gentamicin	14%	86%
Cotrimaxazole	23.7%	76.3
Ciprofloxacin	26.1%	73.9%
Imipenem	19.1%	80.9%
Meropenem	22.6%	77.4%
Doxycycline	43.9%	56.1%
Polymixin B	100%	0%

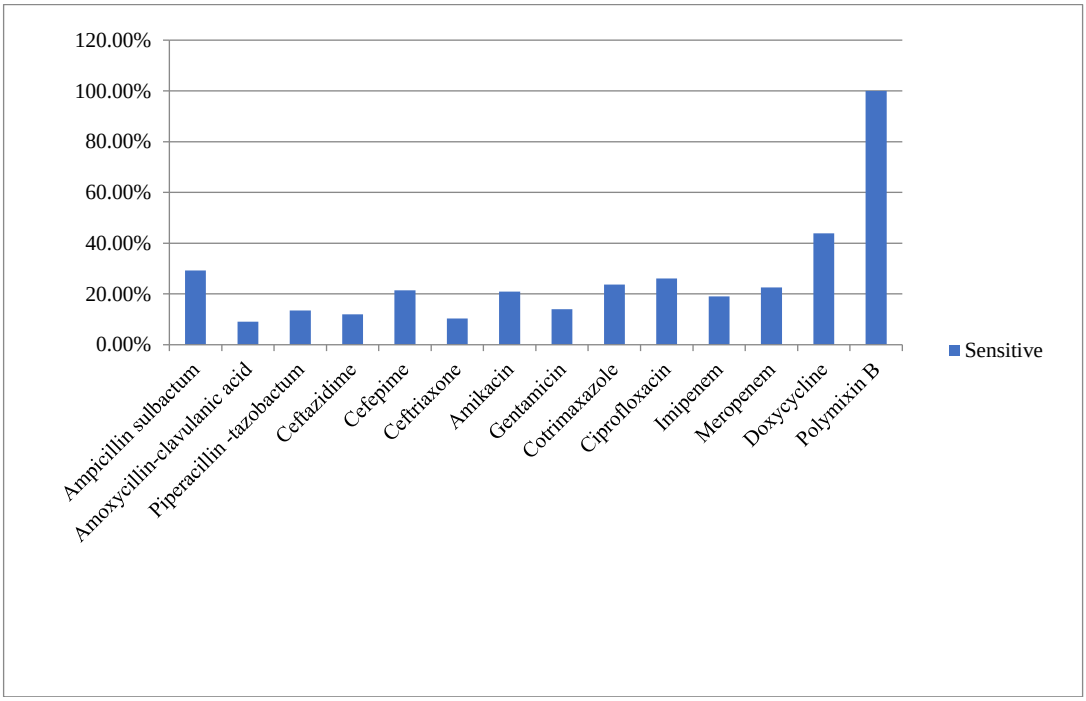
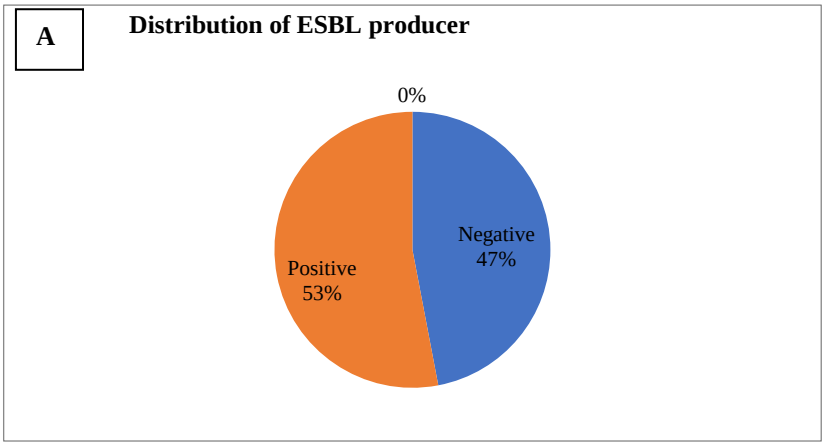


Figure 3. Antimicrobial sensitivity pattern of *Acinetobacter species*.

All the ceftazidime resistance isolates of *Acinetobacter spp*. were confirmed by double disc synergy test for ESBL production. Among these isolates 53%isolates were found ESBL positive by double disc synergy test as shown in table -2.

Table-2 Distribution of Extended spectrum beta lactamase producing strain by double disc synergy test.

(DDST) Double disc synergy test		
Result	**Frequency**	**Percentage**
Negative	**118**	**47%**
Positive	**133**	**53%**



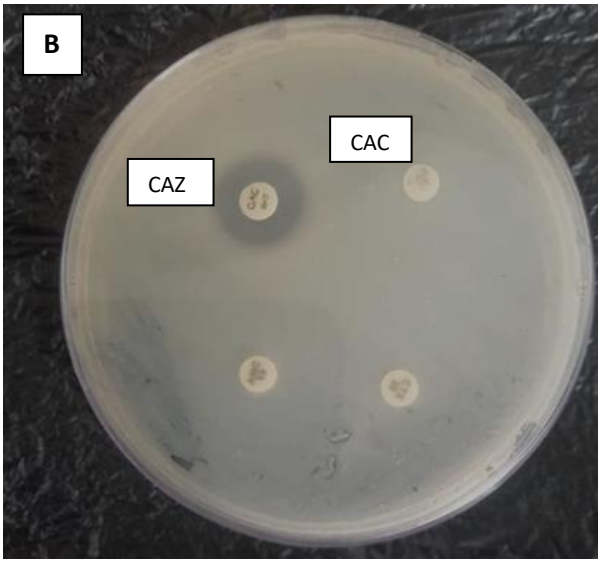


Figure- 4 (A) Distribution of ESBL producer and **(B)** Double disk synergy test (DDST) for detection of ESBL by using ceftazidime, ceftazidime -clavulanic acid disc.

3. Discussion

There are many bacterial diseases, such as *Acinetobacter species*, have arisen in recent years as antibiotic-resistant bacteria have spread rapidly. Many researchers are striving to uncover the components that contribute to virulence and examine antibiotic resistance, even though lack a basic biological understanding of the processes. Members of the subfamily *Acinetobacter* are everywhere, free living, small aerobic Gram negative coco-bacilli that prefer moist types of environment and can be simply gained from soil, water, food and sewage reported by Gerner-Smidt 1995. Up to 25% of healthy ambulatory adults show cutaneous colonization and are the most common Gram negative bacilli carried on the skin of hospital workers observed G Mandell et al., 2000. The Acinetobacter species bacteria are more commonly considered to be opportunistic microbes and of recent study have been reported by KJ Towner 1997 and I Levi, E Rubinstein 1996 to identify cause of a number of outbreaks of nosocomial infections in the hospitalized patients like septicemia, pneumonia, wound sepsis, endocarditis, meningitis and urinary tract infection. In this study isolation rate of Acinetobacter spp. was higher in pus sample

(33%), followed by blood (19%), ET tip (15.1%) urine (14.9%), sputum (9.1%) and pleural fluid (5.4%) cerebrospinal fluid (3.1%). Similarly another study was conducted by Teimoor et al., 2022. reported that among 100 isolates 18% isolates were from pus sample, 29% isolates from blood sample, 23% ET Tube, 15% from sputum, and 9 % isolates found in urine samples.one another study was done by Faten M Elabd etal 2015 observed in 108 Isolates that 31.5% isolates were tracheal aspirates, 22.2% isolates were from wound swab sample, 24.1% isolates were from sputum, 11.1% were urine and 5.6% isolate were blood sample. One another study was done by Ramoul A etal in 2013 they reported that 63.15% isolates of A. Baumannii were from respiratory tract infection,7.14% isolates were from blood stream infection, 16.66% isolates were collected from urinary tract infection and 50% isolates were collected from superficial pyogenic infection.

In our present study out of 350 isolates rate of the *Acinetobacter baumannii (*98.3%) was higher followed by A. *lwoffii* (1.4%) and A. Haemolyticus (.3%). Amarjeet kour et al., 2014 identified that out of 964 samples, 94.7% isolates were A. baumannii 4.7% were A. lwoffii and 3% were A. Haemolyticus. K. Prashant et al., (2004) studied isolation of Acb complex in 71%, A. lwoffii in 20.3%, A. johnsonii 1.6%, A. Haemolyticus 3.38%, A. junii 1.6% and DNA group 1.6%.In our current study we observed that 90.9% isolates resistant from Amoxycillin-clavulanic acid, ceftazidime 88%, ceftriaxone 89.7%, 86.5% Piperacillin tazobactum, gentamicin86%, Cotrimaxazole86%,imipenem80.9%meropenem77.4%doxycycline56.1% .carbapenem resistance higher than those reported in previous studies in Saudi Arabia.(Faten A Elabd etal2015).similar study was done by Teimoor Roshan Ravan etal in Maharashtra in 100 isolates of Acinetobacter species in which higher resistance develop in ceftriaxone(100%), Meropenem 84% resistance Imipenem 84%, Cefeparazone sulbactum 69% ,Ciprofloxacin 85% . Colistin showed 100% sensitive in this study. Carbapenem is most effective drug of choice for the treatment of *A. baumannii* infection but over use of carbapenem has developed resistance and increased prevalence of metello beta lactamase producing bacteria.

We found in our study comparison out of 350 Acinetobacter isolate 251 isolates were examined for ESBL production in which 53% were positive for ESBL similar study done by Choudhry et al., 2014. They observed that 72.2% *A. baumannii* was ESBL positive. Kour et al., 2018 had reported that ESBL production to be 27.5% which is lesser as compare to present study. Reza Ranjba et al., 2018 reported that the frequency of ESBL positive test was 19%.similar study was done by sumitra kumari etal in Bikaner, Rajasthan. They observed 30% isolates of Acinetobacter spp. Were ESBL producer.

4. Conclusion

The prevalence of ESBL producing strain was found increasing in Rohilkhand region of Utter Pradesh population in India. This study documents an alarming level of ESBL producing *Acinetobacter spp*. in the several clinical samples and increasing to threat the health care setting and clinical outcome of patients worldwide and indicates over use of antibiotics due to lack of knowledge. Detection of ESBL producer requires active observation. It will help to preventing the spread of infection. The double disc synergy test (DDST) is very easy and effective method that can be used in lab for routine test for monitoring of ESBL producing strain.

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