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Original research article

Multidrug-Resistant Acinetobacter: Detection of ESBL and MBL at a Tertiary Care Hospital in Bihar

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Abstract

Background:- Ability to develop multiple drugs resistance and biofilm formation have made Acinetobacter species an important hospital-acquired pathogen and a challenge to their effective management.

Objective:- Through this study we can isolate different Acinetobacter sps. and study their antimicrobial susceptibility patterns. Isolated resistant Acinetobacter was further analyzed for the detection of Extended-spectrum β-lactamases (ESBLs), Metallo β-lactamases (MBLs), Carbapenemase production.

Materials and Methods:- Various clinical specimens which were submitted to the Department of Microbiology, Patna Medical College & Hospital, Patna, Bihar were studied for antibiot ic susceptibility testing, detection of ESBL and MBL production by standard microbiologic a l methods.

Results:- The pre-dominant Acinetobacter species isolated was A. calcoaceticus-bauma nnii Complex (Acb complex) 167 (52.1). Among those, all A. species 127 (44.7%) were multidr ug resistant (MDR). In which 12 (4.22%) were ESBL producers and 36 (12.8%) Carbapenemases producers. The majority of A. species were resistant to cefotaxime 72.6% and cefepime 78.4%.

Conclusion:- Drug-resistant Acinetobacter formed a substantial proportion of this hospital's samples. This situation warranted stringent surveillance and adherence to infection prevention and control practices.

Keywords:- Acinetobacter, ESBL, MBL, Carbapenemase, MDR.

Introduction

Acinetobacter, a widely distributed, saprophytic bacteria in nature, has established itself as one of the most common nosocomial pathogen [1,2]. Although different species of Acinetobacter are the potential to cause infection, 80% of infections are caused by Acinetobacter *baumannii*. Ease of survival even in adverse environments, ability to form biofilms on surfaces, and possession of many genes for antimicrobial resistance have made this bacterium an important pathogen. The potential ability of the bacterium to form biofilms in certain instances, indeed, provides a potential explanation for outstanding antibiotic resistance and survival properties in the harsh environment of hospitals, particularly in the intensive care setting [3–5]. Over the

past few decades, its clinical importance had increased due to its ability to receive antimicrobial resistance factors [6,7] through the transfer of plasmid or transposons that contained antimicrobial resistant genes, particularly in a hospital setting where usage of antibiotics are huge, leading to selective pressure [8,9]. Multidrug resistant (MDR) Acinetobacter species are defined as isolates resistant to the major three classes of antimicrob ia l agents - all penicillins and cephalosporins (including inhibitor combinations), fluoroquinolo nes and aminoglycosides [7–11]. These strains are implicated in a variety of life-threate ning infections such as ventilator-associated pneumonia (VAP), urinary tract infectio ns, bloodstream infections, surgical site infections and infections associated with medical devices, occurring especially in patients of intensive care units. Moreover, a significant correlation between biofilm formation and multidrug resistance has been attributed to the threat imposed by Acinetobacter to the current antibiotic era [8, 9, 12]. Diagnosis of multidrug-resistant Acinetobacter infectionis a great challenge owing to the distribution of various species in relation to the type of infection, their antimicrobial profile, and biofilm- forming phenotype. Hence, from effective management and infection control perspectives, it is crucial to minimize the risk associated with Acinetobacter infection in a healthcare setting.
This study was conducted to characterize the clinical Acinetobacter isolates with special reference to the detection of antimicrobial resistance.

MATERIALS AND METHODS

Various clinical specimens submitted to the Department of Microbiology, Patna Medical College and Hospital, Patna, Bihar were included in the study. This study was conducted from 01st September 2021 to 31st August 2022.

1.1. Identification of Acinetobacter Species- Direct microscopic examination of Gram-stained smear of all samples except blood were performed. Inoculation of samples onto appropriate culture media, incubation, and detection of growth after the recommended duration was carried out by standard microbiological techniques [13]. On blood agar suspected smooth, opaque colonies corresponding to non-lactose fermenting colonies on MacConkey and on CLED agar plates were presumed as Acinetobacter and processed further. Species identification of the genus Acinetobacter was carried out by several biochemical tests which included triple sugar iron (TSI) fermentation test, oxidase, indole, motility, urease, and arginine hydrolysis [14,15].

1.2. Antimicrobial Susceptibility Test- An antibiotic sensitivity test was conducted on Mueller Hinton agar (MHA) by the Kirby Bauer disc diffusion method recommended by the Clinic a l and Laboratory Standard Institute (CLSI) guidelines [13]. Escherichia *coli* ATCC 25922 and Pseudomonas *aeruginosa* ATCC 27853 were used as control and tested along with the test strain. Antimicrobial drugs tested were piperacillin (100 µg), ceftazidime (30 µg), ceftriaxo ne (30 µg), cefotaxime (30 µg), cefepime (30 µg), ciprofloxacin (05 µg), imipenem (10 µg), amikacin (30 µg), and ampicilllin/sulbactum (10/10 µg). Resistances to at least one antimicrobial agent in $\underline{>}$03 antimicrobial classes were considered as multidrug resistance (MDR) [13].

1.3. Detection of Extended Spectrum-β-Lactamase (ESBL) Phenotype- According to the CLSI guidelines, probable ESBL-producing isolate had a zone of inhibition for ceftazidime (30 µg) $\underline{\leq}$22mm and cefotaxime (30 µg) $\underline{\leq}$27mm [13]. In order to confirm ESBL production, ceftazidime (30 µg) and ceftazidime + clavulanate (30/10 µg) discs were placed in

Acinetobacter culture. Zones of inhibition were compared with the ceftazidime and cefotaxime discs alone and compared with the combined ceftazidime + clavulanate disc. An enhanced zone of the diameter of ≥05mm in combination with clavulanate was confirmed isolates as ESBL [13].

1.4. Detection of Metallo-β-Lactamase Enzyme (MBL) Phenotype-

1.4.1. Combined Disc Diffusion Test- A combined disc diffusion test was employed to determine the MBL-producing phenotype in Acinetobacter. On the MHA plate lawn culture of Acinetobacter, imipenem disc (10 µg) and imipenem disc with 10 µl of 0.5M EDTA were applied 20mm apart from center to center. The zone of inhibition of >07mm around the imipenem-EDTA disc compared to the imipenem disc alone classified the isolate as an MBL producer [16].

Carbapenemase Production Test- Phenotypic detection of carbapenemase-producing MDR Acinetobacter was determined by a Modified Hodge Test (MHT) [13]. First of all, an overnight broth culture of Escherichia *coli* ATCC 25922 was adjusted to 0.5 McFarland standards and spread on the dried surface of Mueller Hinton agar (MHA) plate by sterile cotton swab. After transitory drying, a 10µg imipenem (IMP) disc was placed at the center of the plate, and tested strains were streaked from the center to the periphery of the plate in four different directions. Following overnight incubation at 37°C, Carbapenemase-positive isolates showed the distorted zone of inhibition, and a "clove leaf pattern" was observed due to Carbapenemase production by isolates [13]

1.4.2. Statistical Analysis- Data were entered in MS Excel 2013 worksheet and statistica l analysis were carried out by using *R* package version 0.55 [17]. The principle component analysis among the several factors such as MDR, MBL were carried out by using the "prcomp" function of the *R* stat package, correlation, and visualization of the plot were demonstrated by the ggbiplot package [18].

Results

Among 284 isolates of Acinetobacter, 148 (52.1%) were Acinetobacter *calcoaceticus-baumannii complex (Acb complex)* followed by 68 (23.9%) *A. lwoffii*, 34 (11.9%) *A. haemolyticus*, 22 (7.7%) *A. radioresistens,* and 12 (4.4%) *A. junii.* Amongst those differe nt specimens analyzed, *Acb complex* was the predominant species (Table 1). In this study, 34.8% of the samples were obtained from the medical ward, 25% from ICU, 04.9% from OPD, 14.8% from surgery and pediatrics, 07% from gynecology, 13.5% of emergency, NICU, and orthopedic department. *Acb complex* was predominant in ICU (69.7%).

The resistance percentages of Acinetobacter in the descending order of frequency were cefepime 78.4% cefotaxime 72.6%, ceftriaxone 72%, ceftazidime 71%, ceftazidime + clavulanic acid 68%, piperacillin 65%, ampicillin + sulbactam 42%, amikacin 39%, ciprofloxacin 36%, and imipenem 32.2%. *Acb complex* was found to have the highest drug- resistant phenotypes to analyze antibiotics with 65.3% being resistant to imipenem. For the Acb complex, cefotaxime was the antibiotic with the highest resistance frequency (91%), as for A. hemolyticus, it was 20 isolates out of 22 (90.9%). More than 60% of A. lwoffii and A. junii isolates were sensible to the investigated antimicrobials (Table 2). Acinetobacter isolates from ICU were more resistant to the antibiotics than those from other wards. Among 284 isolates, 127 (44.7%) were MDR. Most of MDR were from patients in ICU 58.3% followed by OPD 48.2%, Ward 26%, and Emergency 22.0%. *Acb complex* had the highest rate of MDR phenotype as shown in Table 3.

Table 1:

Specimen type	Acb complex	*A. lwoffii*	*A. haemolyticus*	*A. radioresistens*	*A. junii*
Urine	27	10	5	0	1
Pus	30	8	7	7	3
Endotracheal aspirate	55	39	17	6	2
Blood	36	11	5	9	6
Total= 284	148	68	34	22	12

Table 2:

Antibiotics	Acb complex (a= 148)	*A.lwoffii* (a= 68)	*A.haemolyticus* (a= 34)	*A.radioresistens* (A= 22)	*A.junii* (a= 12)
Piperacillin (65%)	125 (85%)	40 (59%)	23 (70%)	10 (49%)	7 (62%)
Ampicillin+ sulbactam (42%)	76 (52%)	29 (43%)	13(40%)	4 (20%)	6 (50%)
Ceftazidime+ clavulanic acid (68%)	124 (84%)	47 (70%)	18 (53%)	6 (29%)	7(62%)
Ceftazidime (71%)	100 (68%)	42 (62%)	19(55%)	13(60%)	5 (41%)
Cefepime (78.4%)	93 (63%)	37 (55%)	16 (48%)	7 (35%)	8 (70%)
Cefotaxime (72.6%)	134 (91%)	44(65%)	30(90.9%)	13 (62%)	7 (62%)
Ceftriaxone (72%)	103 (70%)	48(72%)	23(70%)	14 (68%)	8 (70%)
Imipenem (32.2%)	96 (65.3%)	28 (41%)	10(32%)	8 (40%)	6 (50%
Amikacin (39%)	50 (34%)	13(20%)	4 (12%)	6(30%)	7 (62%)
Ciprofloxacin (36%)	44 (30%)	17 (25%)	6 (20%)	9(42%)	7 (62%)

Table 3:

Characteristic of isolates (n= no of isolates)	Acb complex (a= 148)	*A.lwoffii* (a= 68)	*A.haemolyticus* (a= 34)	*A.radioresistens* (a= 22)	*A.junii* (a= 12)
MDR (n= 127)	100	12	9	5	1
ESBL(n= 12)	6	2	1	2	1
Carbapenemase (n= 36)	19	9	4	2	2
MBL (n=60)	35	17	5	3	0

Discussion

Acinetobacter is one of the notorious nosocomial pathogen and its tendency to develop resistance against antimicrobial drugs is an important rationale for infection control at Health care facility. Among five Acinetobacter species, Acb (Acinetobactercalcoaceticus - A. baumannii) complex was one of the most predominating species (52.1%) in this study, which was comparable to the findings of other studies [15]. It suggests Acb complex has more survival rate even in an unfavourable environment and causes hospital acquired infect io n. About 20% of isolates were obtained from ICU which is similar to findings reported in the previous study from Nepal [19]. This indicates that ICU could be the most important location for the colonization and survival of Acinetobacter in at hospital environment [5]. ICU patients usually require a prolonged hospital stay, need repeated invasive procedures and utilizes various devices for life support, and frequently receives treatment with broad-spectrum antimicrobials. Most of the sample isolates were of the cases of sepsis from the ICU. Previous antimicrobial therapy, medical devices and prolonged hospitalization are the known risk factors for bacterimia in such patients [20]. Resistance to cefepime and cefotaxime were detected in 78.4% and 72.6% of isolates respectively, followed by ceftriaxone (72%), ceftazidime (71%), and piperacillin 65%. It was found that the isolates resistance to amikacin was 39% and to ciprofloxacin 36% which were consistent with other reports [20, 21]. This indicates that Acinetobacter species have intrins ic and/or easily acquired mechanisms of resistance against many of the available antimicrob ia l agents making this pathogen one of the most significant microbial challenges for the current period.

Although carbapenem was the first-line drug against Acinetobacter infection in the late 1990s, carbapenem resistant strains are increasingly reported worldwide [10]. Among the ICU isolates, 58.3% were sensitive to ampicillin/sulbactam and imipenem. The finding of higher imipenem resistance poses a concern. In this study, 127 (44.7%) isolates were determined as multidrug resistant (MDR), in which it was found that all species were MDR strains. Acinetobacter appeared to have the propensity to develop antibiotic resistance rapidly, as a consequence of prolonged antibiot ic exposure. Hence, the increasing trend of Acinetobacter MDR strains were reported globally [22]. In this study, 201 (71%) of the strains were ceftazidime resistant, and 12 (4.22%) of them demonstrated ESBL production by double disc synergy test which disagree with other reports [22, 23]. Since the antimicrobial susceptibility pattern could be variable depending on several factors, the surveillance studies have a crucial role in deciding the therapy against Acinetobacter infection [15]. In this study among MDR isolates, 12.8% had demonstrated Carbapenemase production by the MHT method. There is high sensitivity but low specific ity rate of combined disc test for detection of MBL production, whereas, results of MBL production by a phenotypic method may increase the false positive rate of detection [24]. The data from this study demonstrated that Acinetobacter species were resistant to many of the available antimicrobial agents, making those nosocomial pathogens as one of the most significant microbial challenges to have the control in future.

Conclusion

The clinical isolates of Acinetobacter in this setting were multidrug-resistant MBL producers. These isolates have been proven to cause nosocomial infection in healthcare settings and are challenging to treat. Therefore, a consolidated effort by all healthcare providers by strict implementation of infection prevention and control activities, early diagnosis, and antibiot ic stewardship are recommended to reduce the burden of antimicrobial resistance on patients and health facilities.

Conflicts of interest

The authors declare that they have no conflicts of interest regarding the publication of this paper.

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