Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(4s), 802-805 | ISSN:2251-6727

Phenotypic Detection of Drug Resistance among *Acinetobacter Species* Isolated from ICUs in a Tertiary Care Hospital

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Keywords:-
Acinetobacter species, ESBL, CarbaNP test, Amp C test.

ABSTRACT

Introduction: The spread of *Acinetobacter* infections and its drug resistance become emergence in past few years, resistant to majority groups of antibiotics is a great concern. *Acinetobacter species* are frequently associated with healthcare associated infections. Treatment and management of drug-resistant *Acinetobacter* infections is a big challenge for clinicians and microbiologists.

Methodology: A total 50 *Acinetobacter* species were collected and isolated from ICU patients were included in this study. The isolates were subjected for Antibiotic sensitivity testing and phenotypic detection of drug resistance (ESBL, CarbaNP test, Amp C test).

Results: Among 50 isolates 47 were *Acinetobacter baumanii*, 2 were *Acintobacter lwofii*, 1 *Acinetobacter calcaucatius*. In this study 7(14%) of the isolates were ESBL producers and 19(38%) of the isolates showed carbapenemase production, 14(28%) of the isolates showed Amp C production.

Conclusion: *Acinetobacter* infections are often associated with hospital acquired infections especially in critical care units. Speciation, In-vitro susceptibility testing and detection of drug resistance plays a crucial role to choose an appropriate antibiotic for treatment and management of case.

INTRODUCTION

Acinetobacter baumannii emerged has a medically important pathogen because of the increasing number of infections produced by this organism, over the preceding three decades and global spread of strains with resistance to multiple antibiotic classes. (1)

Acinetobacter baumannii is non-fermentative, gram negative, non-motile, oxidase negative bacillus, whose natural reservoir still remains to be determined (2). Since the 1970s, the spread of multidrug – resistant (MDR) *Acinetobacter* strains among critically ill, hospitalized patients, and subsequent epidemics, have become an increasing cause of concern. Reports of community acquired *Acinetobacter* infections have also increased over the past decade. (3)

Acinetobacter baumannii can causes serious healthcare- associated infection and the incidence is increasing, many strains now resistant to multiple antibiotic classes. (4) *Acinetobacter baumannii* is known to be "intrinsically insensitive" to most beta-lactams, particularly cephalosporins. (5)

MDR *Acinetobacter baumannii* infection tends to occur in immunosuppressed patients, in patients with serious underlined diseases and in those subjected to impassive procedures and treated with broad spectrum antibiotics. Thus, infection due to *Acinetobacter baumannii* are frequently found in ICUs, where they are implicated as the cause of VAP, UTIs and bacteremia. (6) Management of multidrug resistance *Acinetobacter species*. Infection is a great challenge for physicians and clinical microbiologists. (7)

Therefore, the present work compares the phenotypic methods of ESBL detection test, screening of Amp C disc test, CarbaNP test, from clinical isolates to evaluate the findings and to demonstrate which one more suitable.

MATERIALS & METHODS

Acinetobacter species isolated in the laboratory from the patients admitted in ICUs were collected and antibiotic susceptibility testing is done as per CLSI guidelines and phenotypic ESBL detection will be done by using Ceftazidime (300mg) and Ceftazidime clavulanic acid (30mg/10mg) discs by standard disc diffusion method and the zone size is calculated. A>5mm increase in zone diameter for antimicrobial agent tested in combination with clavulanic acid and its zone when tested alone is considered to be an ESBL producer.

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JCHR (2023) 13(4s), 802-805 | ISSN:2251-6727

Screening test for Amp C Disc test was done by using standard protocol, a lawn culture of ATCC *E. coli* 252922 will be done and 30mg Cefoxitin disc will be kept on agar surface and a blank disc will be moistened with sterile saline and will be inoculated with few colonies of test strain. The inoculated disc will be then placed beside cefoxitin disc almost touching it. The plates were incubated at overnight at 35^0C and a flattening or indentation of cefoxitin disc inhibition zone in the vicinity of the disc with test strain will be interpreted as positive for production of Amp C producer.

CarbaNP Test will be performed following the protocol recommended by CLSI (CNPt-CLSI)[8]. A loopful of bacterial growth was suspended in Eppendorf tube contain 20ml of Tris-hcl lysis buffer and mixed by using a vortex device. This lysate was mixed with 100ml of an aqueous indicator solution consisting of 0.05% phenol red with 0.1mmol/liter ZnSO4, previously adjusted to pH7.8 and 6mg/ml imipenem or 12mg/ml imipenem-cystatin injectable form (equivalent to 6mg of imipenem standard powder) (reaction tube) and , as a control tube the phenol red solution without antibiotic and tubes will be incubated at 35°C and monitored throughout 2hr for color change from red to orange/ yellow in the antibiotic containing tube , which will be interpreted as a positive result.

RESULTS

The current study was conducted in department of microbiology for a period 6 months. A total of 50 *Acinetobacter species* were included in this study. Out of 50 isolates collected in the hospital from ICUs. 32 were collected from male and 18 were from female. Of 50 isolates in that ET were 33(66%), Pus7(8%), Urine 2(4%), CSF1(2%), Sputum 3(6%). Among 50 isolates *Aci.baumannii* were 47(94%), *Aci.lwoffi* were 2(4%) and *Aci.calcauticus* were 1(2%) species are isolated **(Table 1)**. The *Acinetobacter* isolates are then subjected to antibiotic susceptibility testing as per CLSI guidelines and then tested for drugs resistance such as ESBL, Amp C and Carbapenemases. Of 50 isolates sensitivity profile revealed 64% of the isolates showed sensitive to Gentamycin followed by 58% were sensitive to Tigecycline, 56% were sensitive to Imipenem, 54% were sensitive to Meropenem shown in **(Table.2**). Among 50 *Acinetobacter* isolates Out of 50 isolates 7(14%) found ESBL producers and 43(86%) are Non ESBL producers. CarbaNP test was done for 50 isolates in which, 19 (38%) of the isolates were showed positive and 31 (62%) isolates were negative. Amp C test was also performed to all the isolates in which 14(28%) isolates showed Amp C production positive and 36 (72%) were showed negative **(Table 3).**

Table 1: showing distribution of different species

No. of isolates	*Aci.baumannii*	*Aci.lwoffi*	*Aci.calcauticus*
50	47(94%)	2(4%)	1(2%)

Pie chart 1 showing sample wise distribution

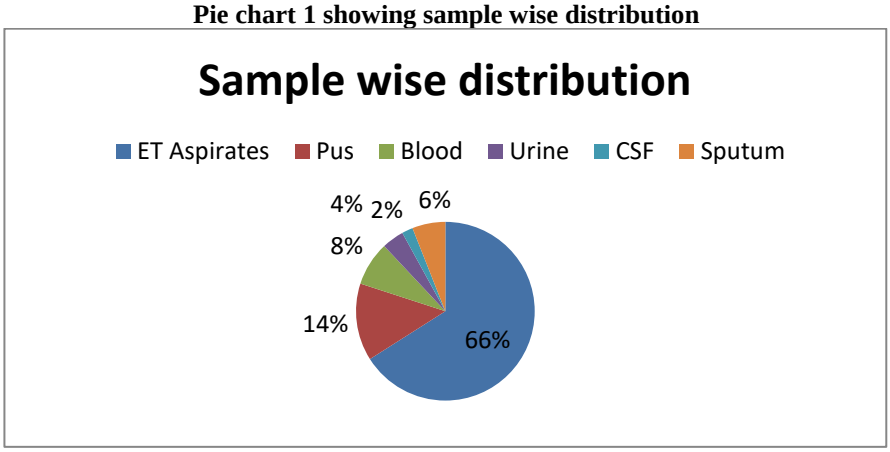

Table 2: Showing antibiotic susceptibility profile of Acinetobacter species

Antibiotics	Sensitive %	Resistant
Cefeperazone/sulbactam	24 (48%)	26 (52%)
Ceftriaxone	5 (10%)	45 (90%)
Imipenem	28 (56%)	22 (44%)
Meropenem	27 (54%)	23 (46%)
Tigecycline	29 (58%)	21 (42%)

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JCHR (2023) 13(4s), 802-805 | ISSN:2251-6727

Ciprofloxacin	23 (46%)	27 (54%)
Cotrimoxazole	26 (52%)	24 (48%)
Cefepime	21 (42%)	29(58%)
Gentamycin	32 (64%)	18(36%)
Piperacillin/Tazobactam	24 (48%)	26(52%)
Ceftazidime	19 (38%)	31 (62%)
Amikacin	24 (48%)	26 (52%)

Graph 1 showing the AST profile of Acinetobacter isolates

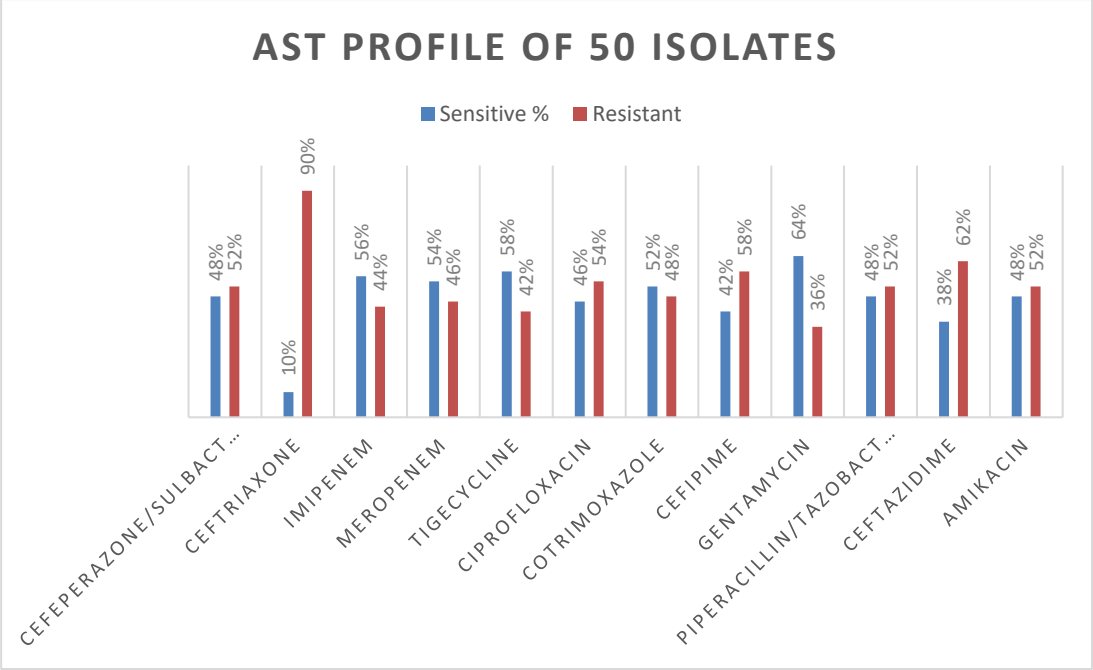

Table 3: showing phenotypic detection of various drug resistance of 50 isolates

Phenotypic detection of Drug Resistance	Number/Percentage
ESBL	7(14%)
Non-ESBL	43(86%)
CarbaNP test Positive	19 (38%)
CarbaNP test Negative	31 (62%)
Amp C test Positive	14 (28%)
Amp C test Negative	36 (72%)

DISCUSSION

Nosocomial outbreaks infection by *Acinetobacter* species have been reported worldwide and most isolates of this bacterium are resistance to many different classes of antibiotics usually used in patient treatments.

Present study showed that the most useful antibiotics for infections caused by *Acinetobacter species* were Gentamycin, Tigecycline followed by imipenem by Disc diffusion method. Ceftriaxone, Cefepime and Ciprofloxacin showed high resistance in our study. Similar sensitivity patterns were reported by Vika Manchanda Sanchaita et.al in their study[4]. In the present study *Acinetobacter baum*annii showed high resistance to beta-lactam antibiotics.

In our study revealed 19 isolates were carbapenemase producers. Similar findings were reported by E. Kumar, K. usha, et.al[12]. The prevalence of multidrug resistance strains expressing ESBL, Amp C, and CarbaNP test are increasing all around the world. The ESBL enzyme ability to hydrolyze 3rd generation cephalosporin's and efficiently inhibited by clavulanic acid. In our study 14% of the isolates are ESBL producers. Amp C beta-lactamases are performed in *Acinetobacter species*. Although the current guidelines do not describe any method for detection of isolates producing Amp C beta-lactamases, we have followed a simple and easy method to detect this enzyme i.e., Amp C disc test. In this study

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28% of the isolates have shown Amp C beta lactamases production[12].

CONCLUSION:

Acinetobacter are the "superbugs" of the modern hospital environment causing significant proportion of infections in specific patient populations, especially in critically-ill patients in the ICU's. The high rate of antibiotic resistance in our isolates reemphasizes the essential need for applying of the new strategies for the prevention and control of MDR infection caused by *Acinetobacter species* Also, epidemiological information help to design better programs for infection control in different hospitals. *Acinetobacter species* showed a very high level of carbapenemases production, Amp C production and beta lactamase mediated resistance mechanisms as part of MDR.

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