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STUDY ON ANTIBIOGRAM OF PSEUDOMONAS AERUGINOSA AND ACINETOBACTER BAUMANNII IN THE ICU SAMPLES FROM TERTIARY CARE HOSPITAL ON VITEK 2 COMPACT SYSTEM

Sangeeta Kumari
Post Graduate Student,
Dept. of Microbiology Santosh Medical college and Hospital Ghaziabad UP (INDIA)

Dr. Ashutosh Rawat
Professor & HOD
Dept. of Microbiology Santosh Medical college and Hospital Ghaziabad UP (INDIA)

DR. Ritu Jain
Associate Professor
Dept. of Microbiology Santosh Medical college and Hospital Ghaziabad UP (INDIA)

Abstract: **Introduction: Acinetobacter and Pseudomonas aeruginosaonce considered as an opportunistic pathogen has recently emerged as an important nosocomial pathogen world over, mostly involving patients with impaired host defences. Critically ill patients acquire an infection during their stay in Intensive Care Unit (ICU) and the frequency of these infections varies considerably in different populations and clinical setting. The purpose of this study was to know Antimicrobial sensitivity pattern of A.baumannii, P.aeruginosafrom various clinical samples collected from patients admitted in ICU at SANTOSH HOSPITAL GHAZIABAD over a period of one year from June 2022 to June 2023.**

Material and methods: A total of (17) A.baumannii, (3)P. aeruginosa were obtained from 90 GNB samples (22.22%). Antimicrobial susceptibility testing of all A.baumannii, P.aeruginosaisolates was done using VITEK 2 COMPACT SYSTEM AUTOMATED as per recommendations of Clinical Laboratory Standards Institute (CLSI).

Results: Maximum number of GNB were isolated from Blood (50%), Pus (24.44%), Endotracheal secretions and sputum (15.55%), Urine (4.44%). samples-tracheal aspirate (4.44%), followed by BAL (1.11%), All A.baumannii isolates were resistant to ceftazidime and cefepime. Higher level of resistance was also recorded for piperacillin/tazobactam (100%) gentamicin and amikacin (94.12%), ciprofloxacin (88.24%), ampicillin (100%). Resistance towards imipenem was recorded as (100%) and meropenem as (94.12%). Minimum resistance was shown towards Minocycline (47.06%) and Tigecycline (64.71%) P. aeruginosa Minimum resistance was shown toward Cefoperazone/Sulbactam (33.33) amikacin (66.67%) and ceftazidime (66.67%), also Piperacilline /Tazobactam (66.67%) all other drugs was most of resistance.

Conclusion: A.baumannii, P. aeruginosa is emerging as a predominant healthcare associated multidrug resistant pathogen, especially in the ICU's. The findings of this study will help our clinicians to apply prescribesuitable antibiotics for treatment of patients admitted in ICU

Keywords: **Pseudomonas aeruginosa, Acinetobacter baumannii, Antibiogram, VITEK 2 COMPACT**

I. INTRODUCTION-

The VITEK system originated in the 1970s as an automated system for identification and AST and has evolved today into the VITEK 2 system, which automatically performs all of the steps required for identification and AST after a primary inoculum has been prepared and standardized.1In over the past years, a variety of automated systems for the identification and antimicrobial susceptibility testing (AST) of microorganisms has been developed based on automated interpretation of the results of biochemical tests or using microdilution trays following overnight incubation and photometric determination of growth.2,3,4,5. Advances in

technology that may provide rapid bacterial identification and AST are now recognized as having both clinical and financial benefits.6

This system allows kinetic analysis by reading each test every 15 min. The optical system combines multichannel fluorimeter and photometer readings to record fluorescence, turbidity, and colorimetric signals. The purpose of this study was to evaluate the VITEK 2 system (software version VT2-R01.02) for identification and AST or microorganisms Because of the increased incidence of diseases caused by these microorganisms and the emergence of resistance to several antimicrobial agents 7,8,9,10,11, rapid and accurate identification as well as MIC evaluation for these pathogens has become increasingly important.

Taxonomically, they are diverse group of aerobic, Nonsporing bacteria that either do not utilize carbohydrates as a source of energy or degrade them through metabolic pathway than fermenting or utilizing it oxidatively.12

They have been isolated from soil, water and medical devices as well. NFGNB can exist as normal commensal.13 Previous studies reported up to 15% NFGNB isolation rate from clinical specimens.14Non-fermenting gram-negative bacilli (NFGNB) such as Pseudomonas spp. And Acinetobacter spp. are most frequently encountered pathogens in the health-care environment.15,16 Involvement of other species in causing human infections are very rare.17

A.boumanii, P.aeruginos is responsible for an increasing number of cases of blood stream infection, urinary tract infection, and ventilator-associated pneumonia. Additionally, it is reported as a cause of outbreaks worldwide, especially in personnel involved in military operations in Iraq and Afghanistan.18

In recent years, the problem is further compounded by the emergence of resistance to antimicrobial agents which are widely used against the non-fermenters especially pseudomonas aeruginosa and Acinetobacter baumannii, making them as an important healthcare associated pathogen. Understanding the spectrum and resistance patterns may guide effective empirical antibiotic therapy, decrease treatment failure and costs. Resistance pattern of microorganisms vary widely.19

There are only few studies from India that provide identification and antimicrobial susceptibility pattern of NFGNB.20

Hence, there is a need to conduct region wise study on susceptibility patterns of various pathogens with which clinician can choose the correct empirical treatment. Therefore, the present study was undertaken to determine the prevalence of NFGNB and antibiogram of dominant pathogens.

II. EXCLUSION/INCLUSION CRITERIA-

Patients eligible were all over age group included with clinically study all type sample (Blood, Sputum, Urine, Pus, ET, BAL, Skin scrapping). Inadequate sampleNon critical patients were not included, Patients which was already on antibiotics therapy were excluded, repeated sample concerned of same patients also were excluded.

III. MATERIALS AND METHODS-

This was a prospective, observational study conducted in a tertiary care teaching hospital over a period of one year from May 2022 to May 2023. A total of 750 clinical specimens were processed in the department of clinical microbiology. 170 clinical specimens yielded the growth of microorganism out of 170 clinical sample 90 Gram negative bacilli sample isolates and out of 90 sample 20 sample Acinetobacter baumannii and Pseudomonas aeruginosa was positive.

All the clinical specimens were plated on CLED and Mac Conkey's agar and Blood agar incubated at 37°C for 48 hours before being reported as sterile. The isolates that showed non lactose fermenting colonies on Mac Conkey agar and failed to acidify the butts of triple sugar iron (TSI) agar were provisionally considered as NFGNB and they were further identified by using a standard protocol for identification.[22] The characters assessed were gram staining morphology.

A-Inoculum preparation-

Suspensions were prepared by emulsifying bacterial isolates in 0.45% saline to the equivalent of a 0.5 McFarland turbidity standard. The same suspension was used for identification and AST for the VITEK 2 system. Suspensions for the comparative identification method were made according to the manufacturer's recommendations. Identification with VITEK 2. The test panels (ID-GN) (AST-406) For GNB organism and AST for NLFGNB.

The Densi Chekturbidity meteris used to measure the turbidity and adjust it as necessary GN- 0.50–0.63.

IV. RESULTS

During the study period, 90 GNB clinical sample isolates out of 150,and 20 clinical sample (A.baumanii, P. aeruginosa) were isolated out of 90. E.coli (n=22), Klebsiella species(n=24), P. aeruginosa (n=3) was the predominant isolate, followed by Acinetobacter baumannii(n=17). Other GNB isolated were. The spectrum and clinical sources of these isolates are shown in **Table No 1**. 4to6% of GNB organisms that are non-Pathogens.

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Table No 1

Isolated strains of GNB	Percentage	T. Isolates
Escherichia coli	24.44	22
K.pneu.ssp pneumoniae	26.67	24
Enterobacter cloacae complex	2.22	2
Achromobacter denitrificans	1.11	1
Achromobacter xylosoxidan	2.22	2
A.baumannii complex	**18.89**	**17**
Acinetobacter lwoffii	1.11	1
Brevu.diminuta/vesicularis	1.11	1
Citrobacter amalonaticus	1.11	1
M.morganiissp morganii	1.11	1
Proteus mirabilis	4.44	4
Pseudomonas aeruginos	**3.33**	**3**
S.enterica ssp diarizonae	1.11	1
Sphingomonas paucimobilis	2.22	2
Stenotrophomonas maltophilia	4.44	4

Both P.aeruginosa and A.baumannii isolates represent 22.22% of all GNB isolated. Therefore, antibiotic resistance rates were performed specifically against these two bacteria.

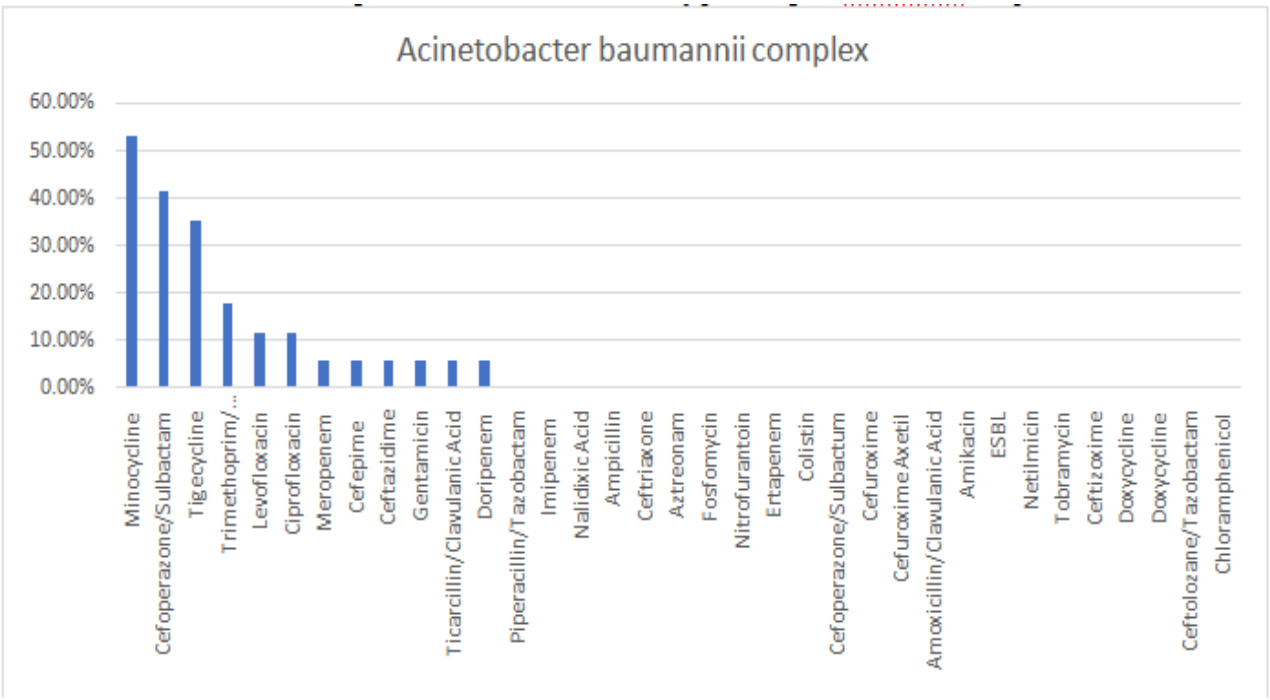

Chart no 1 Higher to lower antibiotics sensitivity percentage of A.baumannii complex

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Chart no 2 Higher to lower antibiotics sensitivity percentage of P.aeruginosa

Antibiotics sensitivity percentage of P.aeruginosa, A.baumannii complex

Pseudomonas aeruginos	
Cefoperazone/Sulbactam	66.67%
Imipenem	33.33%
Ciprofloxacin	33.33%
Cefepime	33.33%
Meropenem	33.33%
Ceftazidime	33.33%
Amikacin	33.33%
Piperacillin/Tazobactam	33.33%
Gentamicin	33.33%

Acinetobacter baumannii complex	
Minocycline	52.94%
Cefoperazone/Sulbactam	41.18%
Tigecycline	35.29%
Trimethoprim/ Sulfamethoxazole	17.65%
Levofloxacin	11.76%
Ciprofloxacin	11.76%
Meropenem	5.88%
Cefepime	5.88%
Ceftazidime	5.88%
Gentamicin	5.88%
Ticarcillin/Clavulanic Acid	5.88%
Doripenem	5.88%

V. CONCLUSION

Non fermenting Gram-negative bacilli considered to be contaminants in the past have now emerged as important major pathogenic organisms.

In the present study, highest number of the NFGNB isolates were from Blood and Pus sample, similar to the observations made by others.[23] Acinetobacter baumannii was found to be commonest non fermenter in previous studies.[24]followed by Pseudomonas aeruginosa and this is in concordance to our finding.

Majority of A. baumanii (18.88%) were isolated from respiratory specimens such as Endo Tracheal Tube, Trans Tracheal Tube, Sputum and Blood. This is comparable with the study conducted by Shanti and Shekar[26] Who reported 41.8% isolation rate of A.baumanii from respiratory specimens as dominant pathogen. A. baumannii have emerged as important pathogen in intensive care units (ICUs), and this is probably related, at least in part, to the increasingly invasive diagnostic and therapeutic procedures used in hospital ICUs in recent years.

Our study against of Previous study % data.P. aeruginosa and A. baumannii are resistant to various antimicrobials which are commonly being used to treat infections. Outer membrane impermeability, increased activity of multidrug efflux pumps, target site alterations, or enzymatic degradation could be the reason for antimicrobial resistance (e.g., aminoglycoside-modifying enzymes and ß-lactamases). Resistance to noncarbapenem ß -lactams in P. aeruginosa.[27] and A. baumannii [28] is due to excessive production of cephalosporinases.[29] P. aeruginosa presents a serious therapeutic challenge for treatment of both community-acquired and nosocomial infections, and selection of the appropriate antibiotic to initiate therapy is essential to optimizing the clinical outcome.

Even more problematic is the development of resistance during the course of therapy, a complication which has been shown to double the length of hospitalization and overall cost of patient care. [30,31]

In our study, P.aeruginosa and Acinetobacter baumanii were isolated from ocular specimens. Maximum number of GNB were isolated from Blood (50%), Pus (24.44%), Endotracheal secretions and sputum (15.55%), Urine (4.44%). samples-tracheal aspirate (4.44%), followed by BAL (1.11%), In India, second most important cause of bacterial keratitis after gram positive cocci is Pseudomonas aeruginosa.[25]

P. aeruginosa isolates in our study were highly susceptible to Cefoperazone/Sulbactam (66.76), Amikacin (33.33%), and Piperacillin/tazobactum (33.33%), ceftazidime (33.33%). P. aeruginosa showed a lower range of sensitivity against other drugs. According to the study conducted by Karlowsky, P.areginosa showed high degree of susceptibility to amikacin and piperacillin-tazobactam followed by ceftazidime. [32]

All A.baumannii isolates were lower resistant to ceftazidime and cefepime. Higher level of resistance was also recorded for piperacillin/tazobactum (100%) gentamicin and amikacin (94.12%), ciprofloxacin (88.24%), ampicillin (100%). Resistance towards imipenem was recorded as (100%) and meropenem as (94.12%). A. baumannii were highly susceptible was shown towards Minocycline (52.94%) and Tigecycline (35.29%), Cefoperazone/ Sulbactam (41.18%).

The resistance patterns of A. baumannii towards various antimicrobial agents were determined. In the present study Less susceptibility was to levofloxacin and ciprofloxacin compared to P.aeruginosa.

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