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RESEARCH ARTICLE

EMERGENCE OF IMIPENEM RESISTANT ACINETOBACTER BAUMANNII ISOLATES FROM VARIOUS CLINICAL SAMPLES IN A TERTIARY CARE HOSPITAL IN KANCHIPURAM DISTRICT.

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Abstract

Background: Acinetobacter is an important opportunistic pathogen and is a common cause of hospital acquired infections including bacteremia, pneumonia, urinary tract infection, peritonitis, etc. In the recent past carbapenems had been drugs of choice for serious infections with Acinetobacter baumannii, but imipenem resistant strains are rapidly emerging.

Objectives: The aim of the study was to determine the prevalence of imipenem resistant Acinetobacter baumannii(IRAB) isolates from various clinical samples in a tertiary care hospital.

Methods: A total of 734 samples were collected over a period of one year from November 2017 to November 2018. Bacterial isolates were identified using standard methods. Antibiotic susceptibility testing was done by Kirby Bauer disc diffusion method according to the Clinical and Laboratory Standards Institute(CLSI) guidelines.

Results: Of 734 specimens, 191 (26%) were Acinetobacter baumannii. Among the Acinetobacter baumannii isolates 93 (49%) were resistant to imipenem. Isolates were predominantly from males(56%) followed by females(44%). Maximum number of Acinetobacter baumannii were from urine samples 67(35%), followed by pus 43(23%), sputum 37(19%), miscellaneous (body fluids and others) 29(15%) and blood 16(8%). Imipenem Resistant Acinetobacter baumannii (IRAB)were from urine samples 31(33%), followed by pus 22(24%), sputum 18(19%), miscellaneous (body fluids and others) 15(16%) and blood 7(8%).

Conclusion: This study highlights the high prevalence of imipenem resistant Acinetobacter baumannii among clinical samples. The emergence of IRAB is a serious global threat to public health. Hence there is a need for strict infection control and monitoring of antimicrobial therapy to combat infections caused by IRAB.

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Introduction:-

Acinetobacter spp. are Gram negative, strictly aerobic, non-fastidious, non-fermenting encapsulated coccobacilli causing mostly hospital acquired infections. According to recent literature, Acinetobacter spp. are the second most common non-fermenting gram negative pathogen isolated from clinical samples after Pseudomonas aeruginosa.[1]

Most frequently encountered species is Acinetobacter baumannii and it is commonly associated with infections, such as bacteremia, urinary tract infection, meningitis, skin and soft tissue infections and pneumonia with high mortality rate of 30-75% in hospitalised patients.[2]

In the recent past carbapenems had been drugs of choice for serious infections with Acinetobacter baumannii, but carbapenem resistant strains are rapidly emerging as a potential threat. There are several factors leading to carbapenem resistance(CRAB) in Acinetobacter baumannii, most important being the acquisition of carbapenem hydrolysing β-lactamases. Other mechanisms include the presence of mobile genetic elements, reduced expression of outer membrane proteins, altered affinity or expression of penicillin-binding proteins and multidrug efflux pumps.[3]

However, increased resistance to carbapenem class of antibiotics has been reported worldwide. Results from studies have reported carbapenem resistance rate of A. baumannii as 40-75 per cent throughout India.[4]

The main objectives of this study is to determine the prevalence of the Acinetobacter infections from various clinical samples and to investigate imipenem resistant A. baumannii strains isolated from patients in a tertiary care hospital.

Methods:-

Bacterial Isolates

This study was conducted in the Department of Microbiology, in a tertiary care hospital in Kanchipuram district, chennai. Acinetobacter baumannii isolated from various clinical samples over a period of one year (November 2017 to November 2018). Out of 734 samples, Acinetobacter baumannii was isolated from 191 clinical samples such as urine, pus, sputum, miscellaneous (body fluids and others) and blood were collected from patients. Informed consent was obtained from the participants before starting the procedures. The samples received in the laboratory were inoculated on 5% Sheep Blood agar and Mac Conkey agar and incubated overnight aerobically at both 37◦C and 44◦C. All isolates were further processed and identified by routine microbiological and biochemical tests as per standard methodology.[5]

Bacterial identification and purification

In case of urine samples, the isolates were subjected to biochemical tests only if the colony count was significant ($>10^5$ CFU/ml). In order to ensure that all isolates were pure, they were cultured three times. To confirm that all these isolates belonged to Acinetobacter baumannii characteristic colonies (Non Lactose-fermenting, glistening, small mucoid colonies) were subjected to Gram stain (Gram negative coccobacilli), motility (non-motile) and standard biochemical reactions (Catalase, Oxidase, Oxidation- fermentation test, Indole production, Methyl Red(MR), Voges–Proskauer (VP), Citrate utilization, reaction in Triple Sugar Iron medium, Mannitol Motility test, Urease activity).[5]

Antibiotic Susceptibility of A. baumannii

After identification by phenotypic methods, antibiotic susceptibility was performed for each isolate by the Kirby Bauer disc diffusion method on Mueller-Hinton agar using 0.5 MacFarland turbidity standard and comparing zone sizes with control strain Pseudomonas aeruginosa ATCC 27853.[6] The antimicrobial agent used were carbapenem group imipenem. Carbapenem resistant was tested by using commercially available imipenem disc (10μg) by Kirby Bauer disc diffusion method. Antibiotic susceptibility results were interpreted by measuring the zone diameters produced and correlating them with the CLSI standards.[7]

Statistical analysis:-

The statistical analysis was performed using the SPSS version statistics 20. The Chi^2 (x^2) test was used to compare the percentages of Acinetobacter baumannii prevalence and imipenem resistance rates from different clinical samples. The p values < 0.05 were considered statistically significant.

Results:-

During the study period, out of 734 specimens received, 191 isolates were positive for Acinetobacter baumannii **(Figure 3)** of which 93 isolates were imipenem resistant (Figure 4) Male patients (56%)predominated over female patients(44%) as depicted in the **Table 1**.

Table 2 & **Figure 1** shows the distribution of the isolates in various clinical samples. Maximum isolates were isolated from urine samples 67(35%), followed by pus 43(23%), sputum 37(19%), miscellaneous (body fluids and others) 29(15%) and blood 16(8%).

Among all the 191 (26%) isolates of Acinetobacter baumannii, 93 (49%) were resistant to imipenem by disc diffusion method. Therefore maximum imipenem resistance was observed in urine samples 31(33%), followed by pus 22(24%) sputum 18(19%), miscellaneous (body fluids and others) 15(16%) and blood 7(8%) as revealed in the **Table 3** & **Figure 2**.

Statistical analysis:-

Data were recorded and statistical analyses were performed by SPSS version 20 software using chi-square test. Hence, P value < 0.05 was considered as the statistically significance level.

Table 1:-Gender distribution of Acinetobacter baumannii from different clinical samples
(n=191)

Gender	No of isolates	Percentage (%)
Male	107	56%
Female	84	44%
Total	191	100%

Table 2:-Isolation of Acinetobacter baumannii from various samples
n=191

Clinical samples	No of isolates	Percentage (%)
Urine	67	35%
Pus	43	23%
Sputum	37	19%
Miscellaneous (body fluids and others)	29	15%
Blood	16	8%
Total	**191**	**26%**

Table 3:- Isolation of Imipenem resistant Acinetobacter baumannii(IRAB) from various samples
n=93

Clinical samples	No. of isolates	Percentage (%)
Urine	31	33%
Pus	22	24%
Sputum	18	19%
Miscellaneous (body fluids and others)	15	16%
Blood	7	8%
Total	**93**	**49%**

Figure 1:-Distribution of A.baumannii from various clinical samples

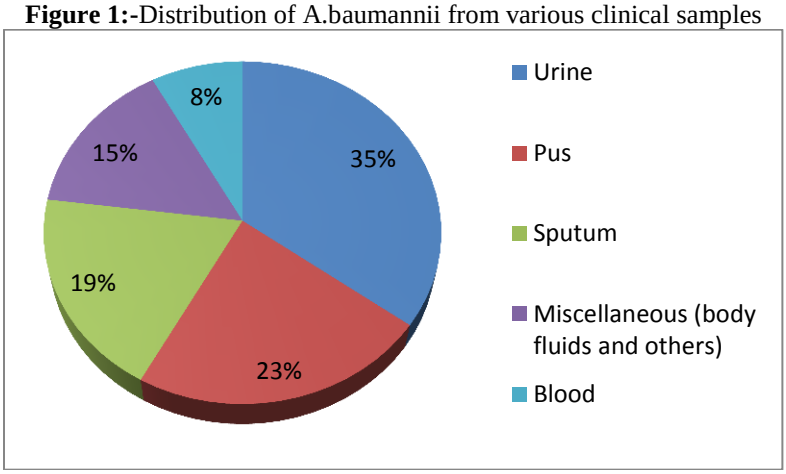


Figure 2: Distribution of IRAB from various clinical samples

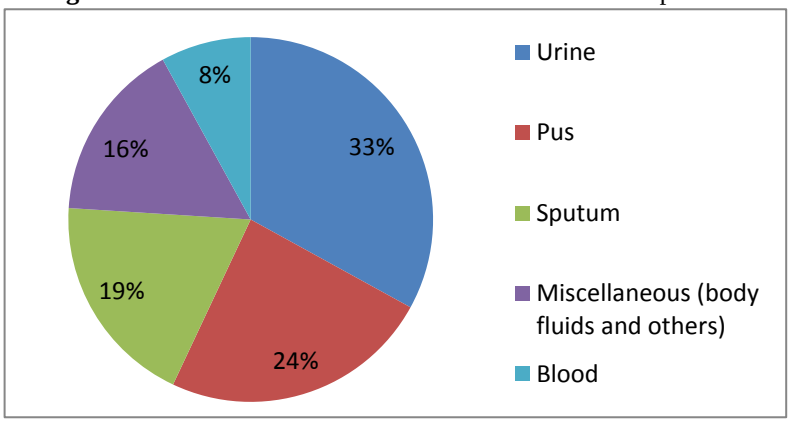
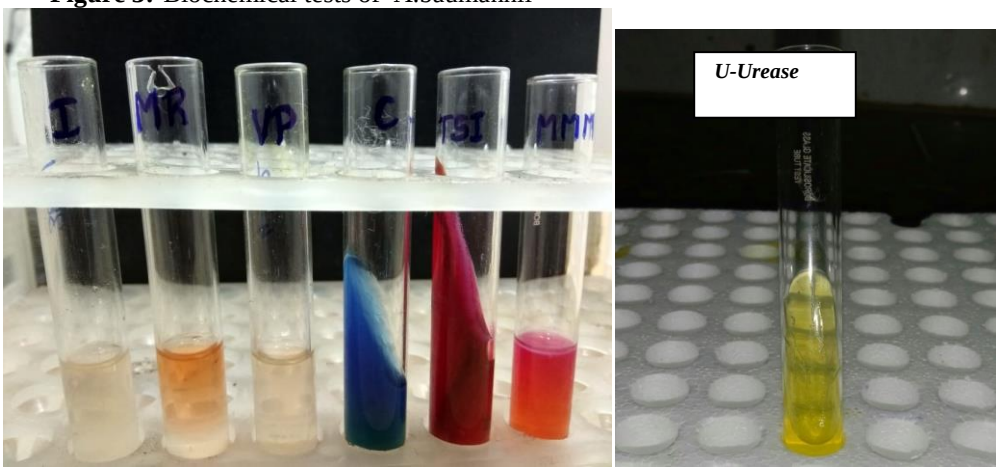
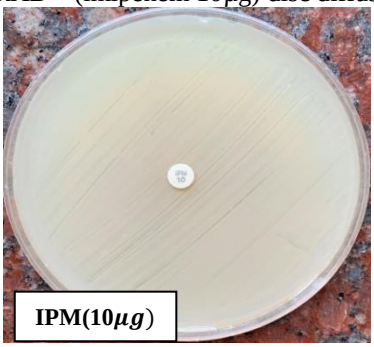


Figure 3:-Biochemical tests of A.baumannii



I-Indole, MR-Methyl Red, VP-Voges Proskauer, C-Citrate,
TSI-Triple Sugar Iron, MMM-Mannitol Motility Test

Figure 4:-IRAB – (imipenem 10µg) disc diffusion method



Discussion:-

In the recent years, A. baumannii infection has become a critical challenge to healthcare systems and has contributed to increased morbidity and mortality among patients in Tamil nadu. Control of A. baumannii infections is always difficult because A. baumannii is resistant to several antimicrobial agents, including imipenem, which remains as the drug of choice, even though frequency of IRAB isolates are reported to be on the rise worldwide. [8]

Among 191(26%) strains, 56% isolates were from males and 44% from females. In correlation with this study, Nadheema et al.,(2013)[9]showed A. baumannii isolates were recovered from 75 patients; 67 (89%) men and 8(11%) women. In contrast to this study Anuradha et al., (2015)[10] reported that Acinetobacter was isolated most commonly from females.

In the present study Acinetobacter baumannii was isolated from various clinical samples including urine, pus, sputum, miscellaneous (body fluids and others) and blood. The total percentage of distribution of Acinetobacter baumannii among the culture samples showed more distribution among urine samples (35%) followed by pus (23%), sputum (19%), miscellaneous (body fluids and others) (15%) and blood (8%). Similar prevalence was shown in a study conducted by Lone et al (2009)[11] which showed predominant distribution of Acinetobacter among urine (39.6%) followed by pus (29.5%).

Unlike this study, Vishnu Preyaet al. (2019)[2] reported prevalence of Acinetobacter isolates from pus samples (42.5 %) followed by urine (22.5%), sputum (15%), others(12.5%), blood(5%) and body fluids(2.5%). Studies by Manikal et al.(2000)[12] and Fiji E et al.,(2018)[13] were in concordance with this present study showing lower isolation rates of 17% and 12.8% from blood samples.

The present study demonstrates the presence of imipenem resistant Acinetobacter baumannii (IRAB) isolates were found to be (93)49%. This study rate is higher than those of previous studies by Anuradha et al.,(2015)[(10)] 9.5%, Gaur A et al.,(2008)[14] 23.1% and a study by Amarjeet Kaur et al.,(2014)[15] 40.3%.

This study rate is lower than that other studies where the antibiotic resistance to imipenem was 57.4% by Kabbaj et al.,(2013),[16]76.19% by Jean et al.,(2016)[17] and 100% resistance to imipenem by Vishnu Preya et al.,(2019) [2]. But these reports were not in consistent with studies by Anitha et al., (2016)[18] who showed 100% sensitive to IRAB.

Current study revealed imipenem resistant A. baumannii isolated from urine, pus, sputum, miscellaneous (body fluids and others) and blood were found to be 33%, 24%, 19%, 16% followed by 8%. **(Table 2)** In a study by Nadheema et al.,(2013), [9] the proportion of imipenem resistant A. baumannii (IRAB) isolates was found to be 77.27% from sputum specimens followed by 74.07%, 56.67%, 37.5% and 16.67% from wounds, blood, urine and burns specimens respectively, which is not in agreement with the present study.

Conclusion:-

Acinetobacter is a common threat in hospital acquired infections. In this study Acinetobacter baumannii were found to be 49% imipenem resistant. Emergence of carbapenem resistance is troublesome nowadays. It is a great challenge for physicians to treat Acinetobacter baumannii infections which is associated with high mortality, highlighting the need for strict infection control strategies.

To avoid antibiotic resistance, antibiotics should be used sensibly and alternative therapy should be considered for each hospital according to the resistance rates in the hospital…

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