

Isolation Of Acinetobacter Species From Pus Samples In A Tertiary Care Hospital

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Abstract

Introduction: Acinetobacter species are Gram Negative coccobacilli causing various nosocomial infections. The spread of multidrug resistant Acinetobacter strains among hospitalized patients became an increasing cause of concern.

Aims:

1. To isolate Acinetobacter species from pus samples received from various wards and ICU's
2. To study their antibiogram pattern.

Materials & Methods: A total of 748 pus samples received at AMC Microbiology laboratory during a period of 2 months in 2014 were included. The samples were processed for Grams stain and culture by inoculating on Blood agar & MacConkey agar and incubated overnight at 37°C. Acinetobacter species were isolated and identified by biochemical tests. The antibiotic susceptibility testing was done by Kirby-Bauer disk diffusion method and zones were interpreted as per CLSI guidelines. ESBL production was tested by double disk diffusion method using Ceftazidime and Ceftazidime clavulanate discs.

Results: Out of 748 samples 51.4% were males and 48.6% were females. Middle aged and elderly age groups were commonly affected in our study. Out of 748 samples 58(7.8%) were culture positive for Acinetobacter species. Among 58 isolates 32(55.2%) were Acinetobacter baumannii, 24(41.3%) A.lwoffii, 2(3.4%) were A.heamolyticus. Most of the isolates were sensitive to Tigecycline(91.3%) followed by Imipenem(84.4%), Piperacillin tazobactam(82.8%) and levofloxacin(81%) and resistant to Cefotaxime, Ceftazidime, Gentamycin and Amikacin. Among 58 isolates 45(77.6%) were ESBL producers.

Conclusion:

1. Acinetobacter infections became a common drug resistant threat in hospital acquired infections.
2. A combined effort of continuous surveillance and infection control protocols have to be implemented to control the increasing incidence of highly resistant Acinetobacter infections.

Keywords: Acinetobacter, Gram negative coccobacilli, Nosocomial infections, ESBL production, Tigecycline

I. Introduction

Acinetobacter species are Gram Negative coccobacilli causing various nosocomial infections. According to most recent scientific literatures, Acinetobacter species are the second most common non-fermenting Gram negative pathogens isolated from clinical samples after *Pseudomonas aeruginosa*¹. The spread of multidrug resistance Acinetobacter strains among hospitalized patients became an increasing cause of concern². Despite their low pathogenic potential Acinetobacter Species survive in the hospital environment for a long time and there by getting an opportunity to cause hospital acquired infections³.

II. Aims

1. To isolate Acinetobacter species from pus samples
2. To study their antibiogram pattern of the Isolated Organisms

III. Materials & Methodology

A total of 748 pus samples received from various wards & ICUs at AMC microbiology laboratory during the period of 2 months in 2014 were included. Isolation, identification and antibiogram of Acinetobacter spp were included in the study. Processing of other bacterial isolates were excluded. The samples were processed for Gram stain (Fig 1) & culture was done by inoculating on blood agar (Fig 4) & MacConkey agar (Fig 2 & 3) and incubated over night at 37 °C. Acinetobacter species were isolated and identified by Biochemical tests (Fig 5,6 & 7). The antibiotic susceptibility testing was done by Kirby-Bauer disk diffusion method (Fig 8) & zones were interpreted as per CLSI guidelines. ESBL production was tested by double disk diffusion method using Ceftazidime & Ceftazidime clavulanate discs⁴.

IV. Results

Out of 748 samples, 51.4% were males and 48.6% were female. Middle age and elderly age group were commonly affected in our study. Out of 748 samples 58 (7.8%) were culture positive for Acinetobacter Species. Among 58 isolates 32 isolates (55.2%) were *A. baumannii* 24 isolates (41.3%) were *A. lowffii*. 2 isolates (3.4%) were *A. hemolyticus*.

Most of the isolates were sensitive to Tigecycline (91.3%) + Imipenem (84.4%) Piperacilline + Tazobactam (82.8%), Levofloxacin (81%) and resistant to Cefotaxime, Ceftazidime, Gentamicin and Amikacin. Among 58 isolates 45 (77.6%) were ESBL producers.

TABLE 1 : Age and Gender wise distribution n= 748

Age	Male (51.4%)	Female (48.6%)
0-10 years	20	17
11-20 years	38	50
21-30 years	55	96
31-40 years	68	72
41-50 years	64	54
51-60 years	73	29
>60 years	67	45
Total	385	363

TABLE 2: Isolation of species

S.NO	Species	n=58(7.8%)
1	<i>Acinetobacter baumannii</i>	32(55.2%)
2	<i>Acinetobacter lowffii</i>	24(41.3%)
3	<i>Acinetobacter hemolyticus</i>	2(3.4%)

TABLE 3: Antibiotic susceptibility testing of Acinetobacter spp. (n = 58)

S.No	Antibiotic	Sensitivity patterns in percentage	
		Sensitive	
		Number	Percentage
1	Tigecycline	53	91.3%
2	Imipenem	49	84.4%
3	Piperacillin-Tazobactam	48	82.8%
4	Levofloxacin	47	81%
5	Amikacin	26	44.8%
6	Gentamicin	23	39.7%
7	Cefotaxime	23	39.7%
8	Ceftazidime	20	34.5%

V. Discussion

Acinetobacter Species is widely distributed and has tremendous colonizing potential hence it became very difficult to explain its significant role in the ICU⁵. *Acinetobacter baumannii* is now recognized as the species of great clinical importance⁶. In the present study 51.4 % were males and 48.6% were females which correlates with Smeeta Huidrom et al⁷ (66.1% & 33.9%) and Bhattacharya et al^{7,8} (1.46 & 1) Middle and elderly age group were more affected in our study which correlates with Smeeta Huidrom et al⁷ where as Bhattacharya et al⁸ reported the mean age 27 years. In the present study out of 748 samples 58 (7.8%) were culture positive for *Acinetobacter* Species from the samples received from various areas of the hospital. Smeeta Huidrom et al⁷ reported 12.6% and Patwardhan RB et al⁹ reported 13.2% from ICU samples. Prevalence rates of 14% and 9.6% among hospital isolates were observed by Mostofi et al¹⁰ and Joshi et al¹¹. Lower rates were reported by Rit et al¹² (4.5%) and Dash et al¹³ (3%).

Acinetobacter baumannii was isolated in 55.2%. *A. lowffii* in 41.3% and *A. hemolyticus* in 3.4% in the present study which correlates with Bhattacharya et al⁸ who reported 54%, 44% and 2% in their study and Poonam Sharma et al¹⁴ reported 41.5% of *A. baumannii* isolates from exudates and abscesses whereas Sengupta et al¹⁵ reported a lower isolation rates of 11.5% of *A. baumannii* from wounds.

Most of the isolates were sensitive to Tigecycline, Imipenem, Piperacilline Tazobactam and Levofloxacin. Multi-drug resistance (MDR) i.e., resistance to Cefotaxime, Ceftazidime, Amikacin and Ciprofloxacin is an emerging problem with *Acinetobacter* species^{16,17,18}. ESBL producers (77.6%) in the present study correlates with Bhattacharya et al⁸ and Kansal R et al¹⁹ (75%) where as Sinha et al²⁰ reported low prevalence of 28%.

VI. Conclusions

1. Acinetobacter infections become a common threat in Hospital Acquired Infections.
2. Despite the increasing frequency of multi resistant Acinetobacter infection, many clinicians and microbiologists still lack appreciation of the importance of these organisms in the hospitals.
3. A combined effort of surveillance and infection control protocols have to be implemented to control the increasing incidence of highly resistance Acinetobacter infections.

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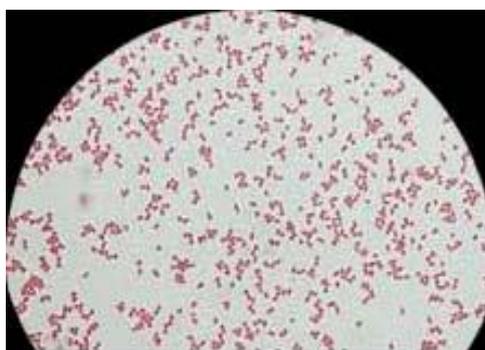


Fig 1 : Gram stain from growth



Fig 2 : Acinetobacter baumannii on MacConkey agar

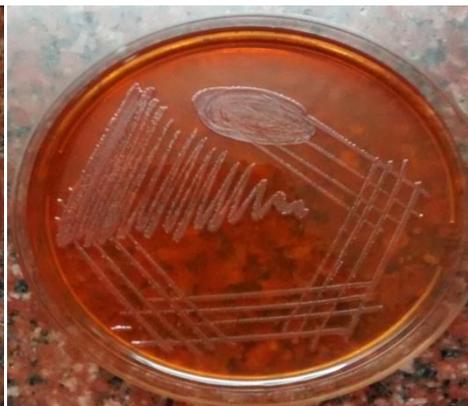


Fig 3 : Acinetobacter Iwoffii on MacConkey agar



Fig 4 : Acinetobacter hemolyticus on Blood agar

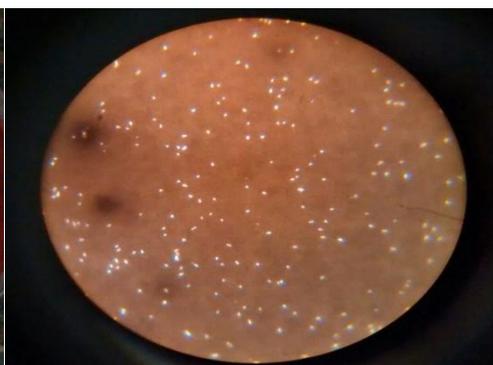


Fig 5 : Capsular staining showing Acinetobacter



Fig 6 : Utilization of 10% lactose
A. baumannii (+Ve) & A. Iwoffii(-Ve)



Fig 7: Nitrate reduction test
Escherichia Coli (+Ve) & A. baumannii (-Ve)

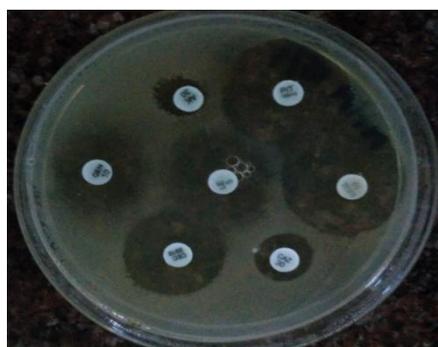


Fig 8: Antibiotic susceptibility testing