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Isolation and Diagnosis of *Acinetobacter baumannii* Isolates from Patients with Meningitis in Child Protection Hospital in Baghdad

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ABSTRACT: Environmental contamination has been estimated to account for up to 20% of all hospital-acquired illnesses. *Acinetobacter baumannii* has recently become the predominant pathogen, posing a significant risk to human health. In present study, two hundred fifteen CSF samples were collected from children between the ages of new born to 14 years who were visiting child protection hospital in Baghdad. For the period from first of Oct 2023 to the end of Jan 2024, and the samples were sent for diagnosis. Isolates were identified after culturing them on MacConkey agar, blood agar, and mannitol salt agar. A number of biochemical tests were performed to detect *Acinetobacter baumannii* bacteria. The results indicated a negative oxidase reaction, a positive catalase reaction, a negative indole reaction, a negative methyl red reaction, a negative Voges-Proskauer reaction, and a positive citrate reaction, which were performed for the samples and for final diagnosis the Vitek-2 Compact system was used. After cultivation and diagnosis, 7 isolates of *Acinetobacter baumannii* were obtained out of 40 samples that showed positive culture results with bacterial growth.

Keywords: Meningitis, *Acinetobacter baumannii*, Biochemical tests

1. INTRODUCTION

Bacteria play a significant role in the causes of HAIs, and one of the most common bacteria associated with HAIs is *Acinetobacter baumannii* (1). It is the most clinically significant and has been recognized as the causal agent of the vast majority of nosocomial infections, particularly in intensive care units (2). *A. baumannii* is responsible for a variety of illnesses, including wound infections, meningitis, bloodstream infections, urinary tract infections, pneumonia and bacteremia (3, 4). In the last few years, antimicrobial resistance among *A. baumannii* isolates has dramatically increased due to the widespread use of antibiotics. Consequently, the development of extensively drug-resistant and multi-drug-resistant bacteria (5). According to World Health Organization, *A. baumannii* is the first disease on the list of important priorities that poses the greatest harm to human health (6).

The successful development of *A. baumannii* as an endemic pathogenic organism in healthcare institutions has been attributed to its propensity to survive and spread widely in hospital environments (7). Numerous studies have reported that hospital environmental surfaces are heavily contaminated with *A. baumannii*, particularly in intensive care units (8). Many medical professionals throughout the world have identified and documented the meningitis caused by *A. baumannii*. The majority of case reports related to meningitis were linked to head trauma, external ventricular drainage (EVD), or CSF leakage (9). The pathogenicity of *A. baumannii* is due to the presence of many virulence factors such as

Lipopolysaccharides LPS, Siderophores, Capsule, Biofilm formation (10). Meningitis caused by *A. baumannii* is typically fatal and is linked to foreign material placement and neurosurgery (6). *A. baumannii* is one of the six "ESKAPE" pathogens that show virulence and multidrug resistance (11). This study aims to isolate and diagnose A. baumannii from CSF samples for patients with meningitis in child protection hospital/ medical city in Baghdad.

2. MATERIALS AND METHODS

2.1 Sample collection and Isolation

A total of 215 CSF samples were collected at the child protection hospital in Baghdad. For the period from the first of Oct 2023 to the end of Jan 2024 and for different age groups (new born to 14 years) for both genders. The samples were taken by lumbar puncture (LP) procedure and cultured on Blood agar, MacConkey agar, Mannitol salt agar and incubated at 37°C for 24-48 hours.

2.2 Diagnosis of *A. baumannii*

The growth diagnosed using biochemical tests (IMViC tests, oxidase tests and catalase tests) and Vitek-2 compact system. Samples must be transferred to the laboratory quickly and tests must be performed, because delay may produce a cell count that does not reflect the clinical situation of the patients. CSF analysis is the gold standard for diagnosis of meningitis as well as to identify its etiology (8).

2.3 Ethics approval

This study was conducted in accordance with the ethical principles set forth in the Declaration of Helsinki. Before obtaining the sample, the study was approved by the local ethics committee, according to Document No. 3/7/738 in12/6/2023).

2.4 Statistical Analysis

The Statistical Analysis System- SAS (2018) program was used to detect the effect of difference factors in study parameters. T-test was used to significant compare between means. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability). Estimate of correlation coefficient between variables in this study.

3. RESULTS AND DISCUSSION

A. baumannii is an opportunistic pathogenic organism that has emerged as one of the most capable organisms for thriving in a variety of environments, which is increasing problems in healthcare facilities (12). *Acinetobacter* meningitis usually happens after neurosurgery. Long neurosurgical procedures, CSF leaks, incision infection, foreign body implantation, external ventricle drain (EVD) placement, multiple operations, and having been on antibiotics before are all things that can make you more likely to get it (13). In this study, 215 CSF samples were collected and cultured on Blood agar, MacConkey agar, and Mannitol salt agar. The preparation of every culture medium is carried out in strict accordance with the instructions provided by the manufacturer. The sample was placed in an incubator and maintained at a temperature of 37°C for a period of 24 to 48 hours. After incubation, bacterial growth was investigated, and a diagnosis was made using the phenotypic features of the colonies—their edges, colours, sizes, shapes, and textures—all of which were closely examined to help identify them (11). And by using both biochemical tests and the Vitek-2 compact system. Seven isolates of *A. baumannii* were obtained.

A. baumannii was observed on MacConkey agar as circular colonies that were small in size, pale pink in color, and did not ferment lactose. The bacteria exhibited non-hemolytic, opaque, grey-colored colonies on Blood agar after being incubated at 37°C for 24 hours.

Figure 1: ***Acinetobacter baumannii*** **isolate appearance on MacConkey agar**

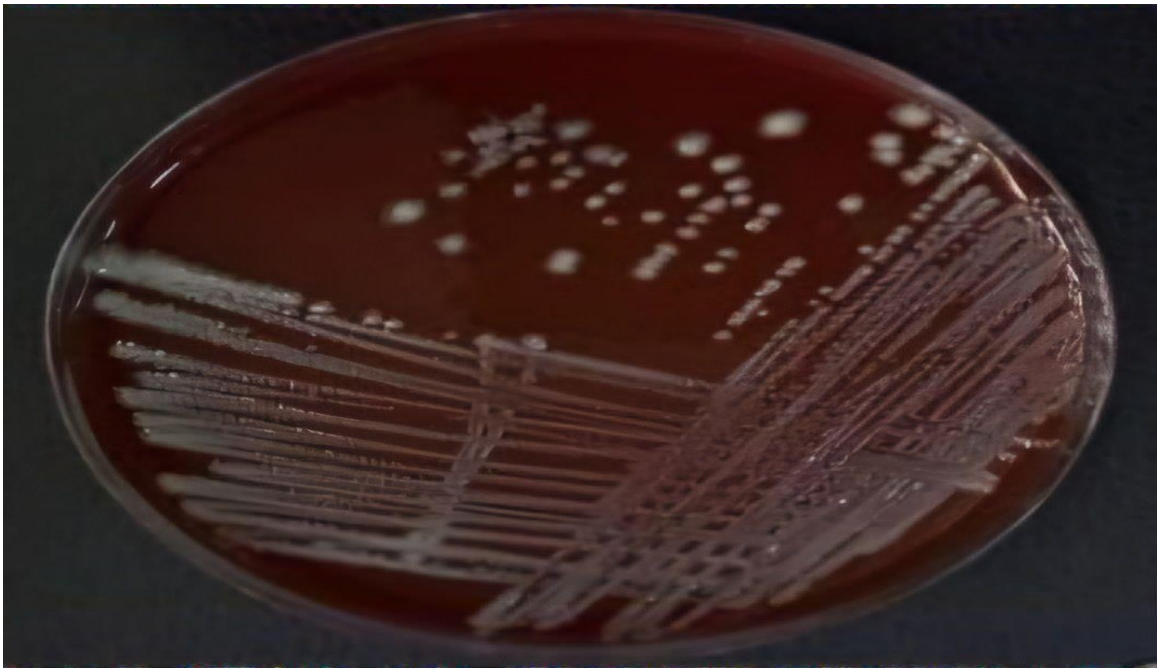

Figure 2: ***Acinetobacter baumannii*** **isolate appearance on Blood agar**

Diagnostic tests for detecting *A. baumannii* include IMViC, catalase, and oxidase assays. The results indicated a negative oxidase reaction, a positive catalase reaction, a negative indole reaction, a negative methyl red reaction, a negative Voges-Proskauer reaction, and a positive citrate reaction.

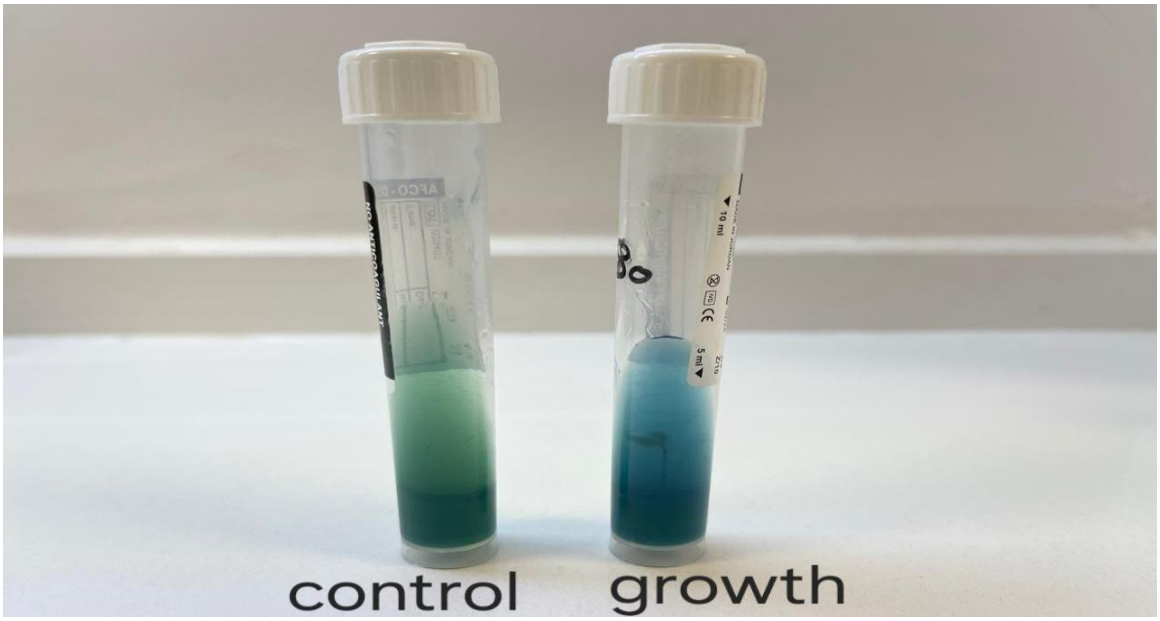

Figure 3: **Citrate utilization positive result**

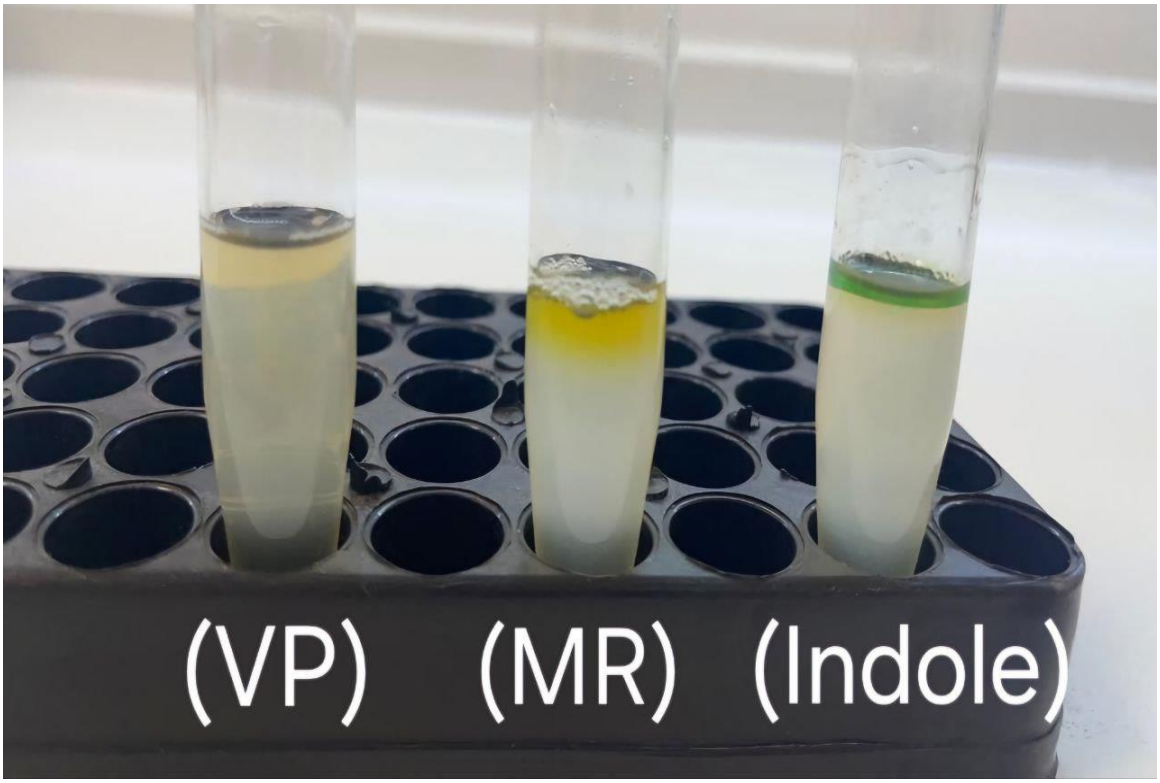

Figure 4: **Voges-Proskauer negative result, Methyl Red negative result and Indole negative result**

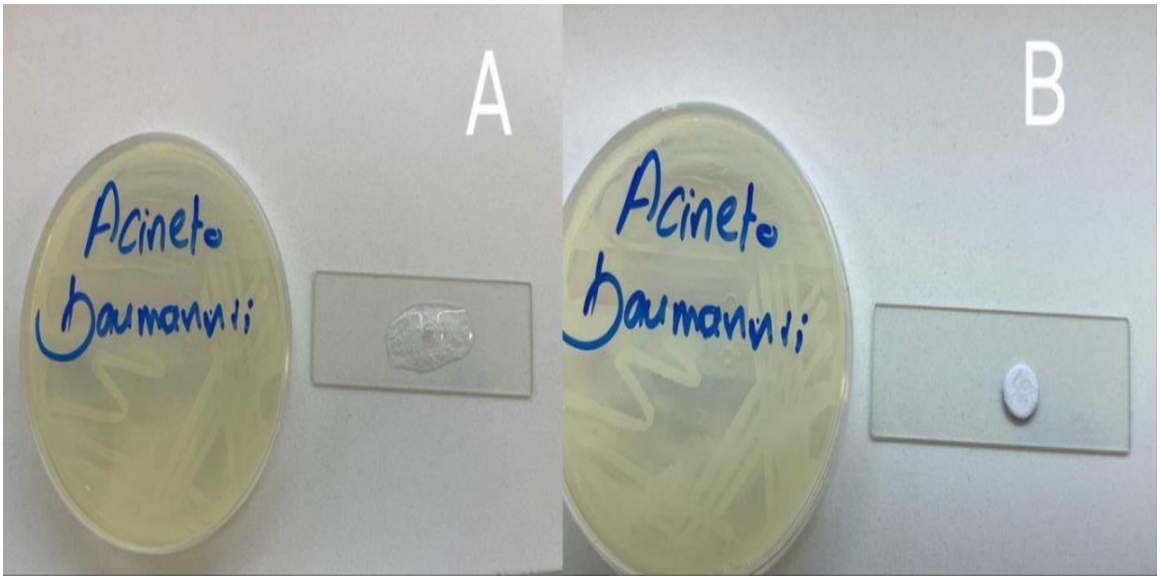

Figure 5: **Oxidase and catalase test. (A) Catalase positive result, (B) Oxidase negative result.**

The VITEK-2 system's results confirmed the diagnosis.

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Patient Name: Patient ID:
Location: Physician:
Lab ID: 13 Isolate Number: 1

Organism Quantity:
Selected Organism : Acinetobacter baumannii complex

Source: **Collected:**

Comments:	

Identification Information	**Analysis Time:**	6.03 hours	**Status:**	Final
Selected Organism	99% Probability	**Acinetobacter baumannii complex**		
	Bionumber:	0241010103500352		
ID Analysis Messages				

Biochemical Details																
2	APPA	-	3	ADO	-	4	PyrA	-	5	lARL	-	7	dCEL	+	9	BGAL	-
10	H2S	-	11	BNAG	-	12	AGLTp	+	13	dGLU	+	14	GGT	-	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	-	36	CIT	+	37	MNT	+	39	5KG	-
40	lLATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	lHISa	+	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	-	61	lMLTa	+	62	ELLM	-	64	lLATa	+			

Figure 6: **The results of vitek-2 system revealing the diagnosis of *A. baumannii***

A. baumannii has been isolated from people with secondary illnesses, including low birth weight premature infants, the elderly with long-term illnesses, cancer patients, people with tumors, people receiving chemical doses, and young people with meningitis, prolonged hospital stays, surgical patients, people with wounds, burns, or other conditions. This bacterium is more commonly caused by conditions including diabetes, overuse of medicines, particularly broad-spectrum antibiotics, and immunosuppressive therapies (14). The results of the current study are consistent with other studies conducted on spinal fluid samples from male and female children (15, 16, 17). In a study conducted on 13 males and 10 females, ranging in age from newborns to 16 years, with an average age of 57.96 ± 66.32 months, the results of the study indicated that inflammation *Acinetobacter baumannii* meningitis was associated with neurosurgery and the placement of foreign material, such as an EVD.

4. CONCLUSION

Acinetobacter baumannii meningitis is commonly linked to neurosurgery and the insertion of foreign objects, and it typically has a high fatality rate. Further research is needed to determine the efficacy of administering polymyxin directly into the spinal cord or brain ventricles for the treatment of meningitis.

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