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Unusual Culture and Microscopic Finding of *Acinetobacter* Isolate in Cerebrospinal Fluid; a Case Report

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ABSTRACT

Background: *Acinetobacter* species are the major cause of nosocomial infection especially in intensive care unit settings. This organism also gains its importance for its nature of showing resistance towards various classes of antibiotics.
Methods: Conventional biochemical test and Microscan walkway automated system was used for identification of the isolate.
Results: Here, we report an unusual finding of multidrug resistant *Acinetobacter baumanii*, isolated from cerebrospinal fluid due to the post antibiotics effect.
Conclusion: Microbiologist should be vigilant while reporting the unusual morphology of bacteria as antibiotic pressure effect may change the morphology and important findings may miss by microbiologist so antibiotic history should evaluated.

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Introduction

Members of the genus *Acinetobacter* is considered opportunistic pathogen among hospitalized patients. They are ubiquitous, free living, small, aerobic, non-motile, catalase positive and oxidase negative, Gram-negative coccobacilli with ability to colonize healthy or damaged tissues. They are responsible for various infections, virtually in every organ system (1). *Acinetobacter* is reported in about 10% of nosocomial infection among intensive care unit patients. Among the species, *Acinetobacter baumanii* shows predominance and has the ability to acquire resistance to major class of antibiotics. It readily develops resistance to antibiotics such as those of cephalosporins and fluoroquinolones (2).

Case Report

A three months old patient was admitted in neonatal intensive care unit (NICU) of a tertiary care hospital, with features suggestive of hyperkalemia and acute kidney injury. Urine and blood sample was received for aerobic bacterial culture and sensitivity testing on the same day. *Acinetobacter* spp. was isolated from both the samples. The isolate from blood sample was susceptible to cefoperazone-sulbactam (by Kirby Bauer disc diffusion method) and colistin (MIC 1µg/ml by E strip method, Himedia). The isolate from urine sample was susceptible to only colistin (MIC 1µg/ml by E strip method, Himedia laboratories, Mumbai). But, patient was taken by his parents against medical advice on the next day of hospitalization. After few days the patient was again brought back to emergency and admitted in NICU with provisional diagnosis of acute gastroenteritis with some dehydration and sepsis. Injection Meropenem and Colistin was started based on previous reports. Cerebrospinal fluid (CSF) along with blood and urine sample was received in Microbiology laboratory on the next day after hospital admission. Urine sample was reported sterile after 48 hours of aerobic incubation and blood sample was reported sterile after 5 days of aerobic incubation in automated blood culture system (BD BACTEC FX). The investigations requested for CSF was aerobic bacterial culture and fungal culture. On gross examination, CSF sample was slightly hemorrhagic. On Gram stain, Gram variable coccobacilli like structures were seen with size larger than usual, approximately 2.5µm to 3µm (Figure 1). CSF sample was inoculated on Chocolate agar, Blood agar and MacConkey's agar and incubated aerobically at 37oC for 48 hours. The aerobic bacterial culture was sterile after 48 hours of incubation. As microscopy showed Gram variable coccobacilli, so rule out any yeast it was processed for fungal culture and India ink preparation. CSF inoculated on Sabouraud's dextrose agar (SDA) with antibiotic and incubated at 25 ºC and 37 ºC. India ink was negative. There was no growth observed after one week of incubation on SDA tubes. But after 10 days creamy mucoid growth was observed on both SDA tubes (Figure 2). Gram stain was prepared from the growth and Gram negative coccobacilli were seen. The isolate was identified as *Acinetobacter* spp. on the basis of biochemical test, which was confirmed by automated identification system (Microscan walkaway plus system by Beckman Coulter) as *Acinetobacter baumanii*. Isolate was susceptible to cefoperazone-sulbactam according to Kirby Bauer disc diffusion test and for colistn, MIC was 0.5µg/ml (E strip method, Himedia laboratories, Mumbai).

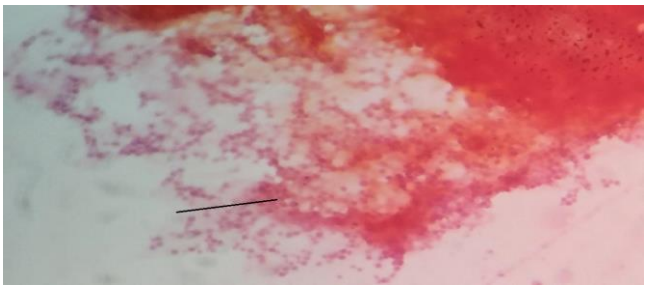

Figure 1. Gram staining showing Gram variable coccobacilli in CSF sample.

Figure 2. SDA showing creamy mucoid growth after 10 days of incubation.

Discussion

Acinetobacter spp. are important nosocomial pathogen that are often multi-drug resistant and can be associated with life-threatening infections and prolonged hospital stay. *Acinetobacter* spp. has tendency towards causing infection among patients, particularly in intensive care units. Their presence in clinical specimens should be correlated with clinical findings. *Acinetobacter* spp. are normally seen as short, plump, gram negative coccobacilli, about 1.0–1.5 µm by 1.5–2.5 µm in size during their rapid phase of growth and coccoid forms presented in pairs or long chains of variable length in their stationary phase (3). However in the present case, mostly Gram variable large size cocci were seen (Figure 1). *Acinetobacter* spp. are non-fastidious and can be easily grown on ordinary laboratory media (3). In the present case there was no growth in routine culture media after normal incubation period in which normally *Acinetobacter* spp. grows. Abnormal behavior observed in this case may be due to effect of antibiotic, as this patient received three doses of colistin before collection of specimens. In the present case *Acinetobacter* spp. was isolated previously from blood and urine samples, before observation of this unusual characteristic in CSF sample. Bacteria are strongly affected by changes in environmental conditions. Multiple species undergo morphological changes under certain conditions like low-temperature exposure, nutrient deprivation, gene expression (4). These morphological changes may be related to a transition to a metabolically inactive state or to a need to increase nutrient uptake or escape threats. Low-temperature and/or nutrient deprivation may induce a dormant viable and not-cultivable state of bacteria (4). Some Gram-negative pathogen may change its morphology from rod to coccoid forms (5). Morphological changes in some cases correlated with regulation of the expression of cell envelope or cell wall genes (6). Similar findings of altered microbiology of bacteria was also reported by Bitterman et al [2017], Rajeshwari et al [2009], Lorian et al. (1982) (7, 8, 9). Contamination of the culture media can be ruled out as internal quality control of culture media is performed after the preparation of each lot.

Conclusion

Antibiotic pressure effect may change the morphology and culture findings of bacteria, so microbiologist should be vigilant while reporting such a case as microscopy may show smear positivity but sterile culture. In such cases appropriate antibiotic history and longer incubation period of cultures can give appropriate result as microbiologist may miss such isolate when it comes sterile after 48 hours incubation period.

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Ethics approval and consent to participate

Not needed.

Conflict of interest

None declared.

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