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Isolation, Identification and β-lactamase detection of Multi-Drug Resistant *Acinetobacter* species from Patients admitted in Intensive Care Units of Hospital

Silver R. Panchal
Department of Biotechnology, Veer Narmad South Gujarat University, Surat, India

Gaurav S. Shah
Department of Biotechnology, Veer Narmad South Gujarat University, Surat, India

Niranjan P. Panchal
Government Medical College, Surat, India

ABSTRACT

Acinetobacter species are ubiquitous, free-living saprophytes, natural habitats are water and soil, and have been isolated from foods, arthropods, and the environment. *Acinetobacter* species is now a day's very important opportunistic pathogen, responsible for the fatal health care associated (Nosocomial) infection with high morbidity and mortality rates. *Acinetobacter* species infections are more frequent in critically ill patients of intensive care units (ICUs) of the hospital. Such infections are widely increasing because of its ability to develop rapid resistance towards the major groups of antibiotics. That resistance is due to some specific enzymes like extended spectrum β-lactamase (ESBL), carbapenemase and metallo β-lactamase. In our study isolation and identification of this fatal pathogen was carried out along with its β-lactamase detection. Total of 350 samples were studied out of which 99 isolates were obtained over a period of 18 months (July 2013-December 2014). Antibiotic susceptibility testing was done by Kirby-Bauer's disc diffusion method with commercially available antibiotic discs on Muller-Hinton's agar plates. In our study we found 17% of *Acinetobacter* species as multidrug resistant (MDR) strains with 57% ESBL positive.

Keywords: *Acinetobacter species, Antiboigram, ESBL, MDR strains.*

INTRODUCTION

Acinetobacter species are ubiquitous, free-living saprophytes, natural habitats are water and soil, and have been isolated from foods, arthropods, and the environment. *Acinetobacter* Infection is common in the critically ill patients and sometime it is responsible for the severity of his /her illness. Such bacterial infections are the primary concern, although some fungal infections are opportunistic in nature. It increases the ICU mortality rate, and the costs because of its multi drug resistance. Despite the efforts of many, together knowledge on microbial genetics, microbial population dynamics, and interactions between pathogen, host, environment, and clinical epidemiology, there is yet not a 'magic-bullet strategy' to control antibiotic resistance.

Acinetobacter infections have emerged as a worldwide nosocomial pathogen among hospitalized patients especially in ICUs. Although *Acinetobacter* species is regarded to have low virulence, they are now recognized as major pathogen in nosocomial infections associated with high mortality rate and long hospital stay. *Acinetobacter* spp. can survive on human skin and in dry conditions for several weeks, a characteristic that easily propagates transmission through fomite contamination in hospitals. For these reasons, *Acinetobacter* spp. are commonly found on the skin and mucous membranes of hospitalized patients, which is an important factor for the occurrence of nosocomial infections[7]. Hospital outbreaks have been described from various geographic areas, and this organism has become endemic in some of them *Acinetobacter* spp. does not have fastidious growth requirements and is able to grow at various temperatures and pH conditions.

Acinetobacter spp. are often multidrug resistant and associated with life-threatening infections especially in patients with factors that impair normal host resistance.

One of the important causes of nosocomial infections are *Acinetobacter* species[3] with *Acinetobacter baumannii* being recognized as the most common clinical isolate from nosocomial infections. *Acinetobacter baumannii* has epidemic potential and is being identified as a major cause of outbreak or sporadic cases with high mortality rates accounting for about 80% of reported infection worldwide [5].

Acinetobacter infections have been reported for almost all organ systems. It is usually an opportunistic pathogen as evidenced by the fact that 14% to 16% of infections are mixed infection [4]. *Acinetobacter* species is now recognized to be the species of great clinical importance being capable of causing life threatening infection including pneumonia, septicemia, wound sepsis, urinary tract infection, endocarditis and meningitis. It was found in the 1990 to 1992 National Nosocomial Infection Surveillance (NNIS) data that 2% of blood stream infections and 4% of nosocomial pneumonia cases were due to *Acinetobacter*. Also it is currently the most common isolate from gram negative sepsis in immunocompromised patients posing risk for high mortality. The organism prefers moist environment, therefore, its colonization among damaged tissues is common [8].

Antimicrobial resistance among *Acinetobacter* species has increased substantially in the past decade [13]. The capacity of *Acinetobacter* species for extensive antimicrobial resistance may be due in part to the organism's relatively impermeable outer membrane and its environmental exposure to a large reservoir of resistance genes [6]. Mechanisms of its resistance are impressive and rival those of other non-fermentative Gram-negative pathogens [14].

Materials and Methodology

Isolation and identification of *Acinetobacter* species was carried out at the Department of Microbiology, Surat Municipal Institute of Medical Education and Research, (SMIMER) a tertiary care public hospital in Surat. The total sample size consisted of 350 consecutive clinical specimens (blood, swab, endotracheal, pus, sputum, urine and fluid) received from different intensive care units: Neonatal ICU (NICU), Pediatric ICU (PICU), Surgical ICU (SICU), Intensive Cardiac Care Unit (ICCU) and Medical ICU (MICU) of the hospital. Samples of the patients admitted in the ICUs during July 2013 to December, 2014 were included in our study. In our study patients admitted in any of the five ICUs of the hospital during the study period, who were clinically suspected of having acquired any bacterial infection after 48 h of admission to the ICUs, were included. Patients showing clinical signs of infection on or prior to admission or transfer to the ICUs were not included [1].

Collection of samples from patients suspected for bacterial infections was done using sterile containers. Different types of samples were collected, blood samples were collected using sterile hypodermic syringe with needle and transferred to sterile blood culture bottles having Brain Heart Infusion (BHI) medium, swab samples were collected using sterile swab sticks available commercially, pus, body fluids, sputum, and urine samples were collected using sterile containers. Only bacterial infections were studied in detail. Samples were then subjected to the identification and antibiotic sensitivity with ESBL detection [2].

All the clinical samples were initially subjected to direct microscopy (e.g. gram staining, motility and wet film examination). According to the findings of the direct microscopy (wherever applicable), presumptive identification of the bacteria was done along with the selection of the culture media.

All the clinical specimens were inoculated initially on non-selective media as Nutrient agar, Blood agar, and Mac-Conkey agar. The selection of the selective media was determined by the findings of the direct microscopy. The inoculated culture plates were examined for growth after an incubation period of 24-48 h at 37°C. For the blood samples, blood cultures were done using brain heart infusion (BHI) broth as a primary culture medium. Grams staining and first blind subcultures were done by using Nutrient agar plate, Blood agar plate and Mac-Conkey's agar plate after 24 h of inoculation. In case of no growth, the blood culture bottles were further incubated for five day at 37°C after which, final subcultures were done. All the specimens were further analyzed using various biochemical tests according to the morphology observed.

The preliminary identification of *Acinetobacter* species was done by direct microscopy (e.g. gram staining, motility and wet film examination) and the oxidase test. Non-fermenting gram negative bacilli that were oxidase-negative and non-motile were identified as *Acinetobacter* species. All the isolates of *Acinetobacter* were identified up to species level by using standard biochemical test as growth and reaction on triple sugar

iron agar, urease production, citrate utilization, sugar fermentation etc. Further confirmation was done by the VITEK 2 system (bioMérieux diagnostics, USA) instrument.

Only *Acinetobacter* isolates were subjected to antibiogram study of routine antibiotics by modified Kirby Bauer Disk Diffusion Methods by commercially available discs from Hi-Media containing following antibiotics were used: Ampicillin, Cotrimoxazole, Amikacin, Gentamycin, Levofloxacin, Cefeprime, Cefoperazone, Cefuroxime, Imipenem and Piperacillin + Tazobactum.

For ESBL detection, Muller Hinton Agar plate was inoculated with 0.5 McFarland of *Acinetobacter* culture to form a lawn culture. Disc containing Ceftazidime (30µg), Ceftriaxone (30µg) available from Hi-Media were applied onto the plate according to the guidelines provided by CLSI. Plates were incubated at 37 ºC for 18 h. Results were interpreted on the basis of zone diameter above 25mm and 22mm for ceftriaxone (30µg) and Ceftazidime (30µg) respectively, this mentioned zone indicates ESBL production[11].

Results and Discussion

Acinetobacter spp. were isolated from samples collected of number of ICU patients, total 99 samples were positive for *Acinetobacter* spp. infections including blood, pus, sputum, swab, urine and fluid samples. Identification was done by using VITEK 2C system and ID-GNB card (Perfect Diagnostics, Surat, Gujarat) as well as conventional morphological and biochemical characteristics, as described in Bergey's Manual of Systematic Bacteriology.

Fig-1: Isolated *Acinetobacter* spp. on Nutrient agar, Mac Conkey's agar and Blood agar plates

Nutrient agar plate **Mac Conkey's agar** **Blood agar plate**

Table-1: Morphological characteristics of *Acinetobacter* spp.

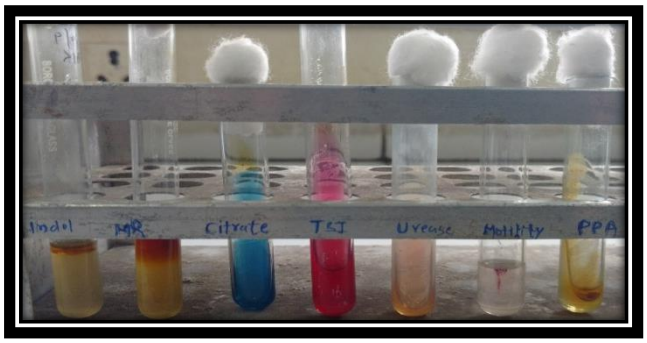
Medium	Gram negative bacilli or coccobaccilli (often diplococco-bacilli)
Nutrient Agar	Colonies were circular, convex, smooth, translucent to slightly opaque, and non- pigmented with entire margin.
MacConkey Agar	Non lactose fermenter, pale yellow colored, smooth, circular with an entire edge.
Blood Agar	Smooth, opaque, raised, white or creamy, and small, circular with an entire edge, some strains are haemolytic

Table-2 : Biochemical characteristics of *Acinetobacter* spp.

Indole	MR	VP	Citrate	TSI	Urease	Dextrose	Motility
–	–	–	+	k/k	–	+	–

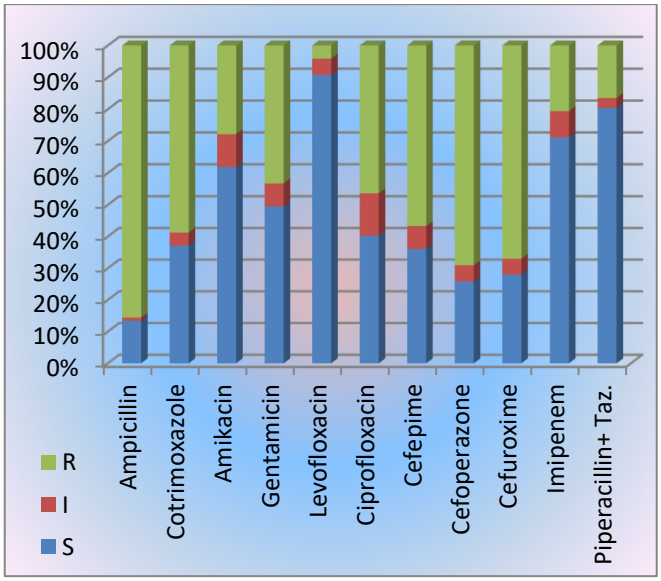
(key: — = negative, + = positive, k/k = alkaline slant/alkaline butt)

Fig-2: Biochemical characteristics of isolated *Acinetobacter* spp.



The isolates were confirmed by the above given characteristics which is shown in Table-2 and Fig-2.

Figure- 3: Antibiogram of *Acinetobacter*



Result shows that *Acinetobacter* species were more sensitive to Levofloxacin (total 88) followed by Piperacillin + Tazobactam (total 78), Imipenem (total 69) and Amikacin (total 60) while they were more resistent to Amipicillin (total 83), Cefoperazone (total 67) and Cefuroxime (total 64). In our study we find total 17 *Acinetobacter* isolates resistant to all so 17% MDR strins were isolated.

Among the *Acinetobacter* spp isolated from ICU patients, 56 isolates were ESBL producers and 43 were ESBL non producers. Antibiotic sensitivity test was performed among all these isolates against nine different antibiotics viz., Ampicillin, Cotrimoxazole, Amikacin, Gentamicin, Ciprofloxacin, Cefepime, Cefoperazone, Cefuroxime, Imipenem as well as combination of antibiotics Piperacillin+ Tazobactam and Amoxicillin + Clavulanic acid. The results were interesting which indicated the occurrence of antibiotic resistance was higher among all the isolates which were ESBL producers as compared to those which were Non-ESBL producers.

The therapeutic options for the infections which are caused by β- Lactamase producing organisms have also become increasingly limited. In our study, we determined the enzymatic profile of the *Acinetobacter* spp. isolated from the clinical cases of nosomocomial infections to understand the cause of developing resistance against the antibiotics. All the strains selected were shown to exhibit varied degree of resistance. From previous studies from India, we found that prevalence of the ESBL producers to be 6.6% to 64%.In our study we found 57% of ESBL producer among isolates.

Conclusion

Nosocomial infections and antimicrobial resistance in the ICUs is a major deterrent to patient's outcome, increasing duration of patient stay as well as expense. Reduction of the same is both challenge and goal of all intensive care units around world.

From the above study it can be concluded that *Acinetobacter* species have prime role in nosocomial infections especially in ICUs of the hospital. *Acinetobacter* species is most frequent nosocomial pathogen especially in ICUs of the tertiary care hospital. The number of *Acinetobacter* species are isolated from blood samples is highest than other i.e. pus, urine, body fluids and swabs. According to resistance pattern of the *Acinetobacter* species isolated Ampicillin resistant species are most frequent. This information will be helpful in designing of a new drug to overcome predominance of *Acinetobacter* species as

nosocomial pathogen especially in ICUs of the tertiary care hospital. Antibiotic resistance and ESBL production was consistent not just in *Acinetobacter* spp. Further study of these isolates can widen the insight to understand the mechanism of developing antibiotic resistance among the nosocomial pathogens.

According to these data combinations of two or more antibiotics can also prove effective remedy to overcome *Acinetobacter* species nosocomial infections. Patients who were in Incubation Period of nosocomial infections on discharge, who manifests it after discharge, were not covered in our study. Contribution of their load to our study prevalence is unknown. To overcome those limiting factors further research is advocated.

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