

Original Research Article

Acinetobacter Species an Emerging Nosocomial Pathogen: It's Isolation Pattern, Biofilm Formation and Antibiotic Susceptibility Profile

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ABSTRACT

Introduction: Bacteria of genus *Acinetobacter* are aerobic, nonfermentative, Gram negative bacilli that cause wide variety of illnesses. *Acinetobacter* species isolates are frequently resistant to multiple antibiotic classes through an array of resistant mechanism. Biofilm production by bacteria is associated with chronic nosocomial infection.

Aim and objective: To study the isolation pattern, biofilm formation and antibiotic susceptibility profile of *Acinetobacter* species isolated from various clinical specimens.

Materials and methods: The study included 166 *Acinetobacter* species isolates isolated from various clinical specimens. All the isolates were subjected to antibiotic susceptibility using modified Kirby- Bauer disc diffusion method. Detection of biofilm was done by tube adherence method and Congo red agar method.

Result: Out of 166 *Acinetobacter* species isolates, 70 isolates (42.1%) showed biofilm formation by either method. Biofilm producer *Acinetobacter* isolates showed significantly higher resistance than non-biofilm producer.

Conclusion: In the present study *Acinetobacter* species isolates producing biofilm showed high degree of resistance to most of the drugs including carbapenem. Detection of biofilm producer strains can help in appropriate selection of antibiotic. The *Acinetobacter* species needs to be considered as an essential pathogen and steps must be taken to control such infections.

Key words: *Acinetobacter*, Biofilm, Congo red method, Tube adherence method, Multi-drug resistance

INTRODUCTION

Acinetobacter species has emerged as an important cause of health care associated infections. It can be recovered from variety of sources like soil, water, food

products, arthropods and medical environment. Most alarming is the organism's ability to accumulate diverse mechanisms of resistance, the emergence of strains that are resistant to all commercially

available antibiotics. [1] Over the past two decades, *Acinetobacter* infections have become an increasingly common nosocomial problem in temperate zone. The emergence and spread of multidrug resistant *A. baumannii* and its genetic potential to carry and transfer diverse antibiotic resistant determinants pose a major threat in hospitals. [2]

Health care associated infection is an ever rising threat which needs to be expeditiously managed. Majority of the all the cases of health care associated infection are device related. Device related infections may not only require removal of device but at most of the time may be potentially fatal.

Biofilms are produced when microorganism adhere to a surface and produce extra cellular polymers. Microbial biofilms have been associated with a variety of persistent infections which respond poorly to conventional therapy. Antibiotic resistance spread in biofilm producing bacteria is owed to its ability to increase mutation rate by exchanging genes responsible for drug resistance and also to an elevated expression of efflux pump. [3] Despite the rising clinical importance of *Acinetobacter* infection compared to other nosocomial pathogens, this organism has been widely overlooked.

The present study was designed at rural tertiary care hospital of western Maharashtra with an aim to determine antibiotic susceptibility profile and biofilm forming ability of *Acinetobacter* species isolated from various clinical specimens.

MATERIALS AND METHODS

The present study was cross-sectional study conducted in Department of Microbiology, Rural Medical College and hospital, Pravara Institute of Medical Sciences, Loni.

During the period 166 *Acinetobacter* species isolated from various clinical samples

included in the study. Identification of isolates was done as per standard bacteriological protocol. The antibiogram of the isolates was determined by the Kirby Bauer disk diffusion method on Muller-Hinton agar.

The isolates were tested for sensitivity against ampicillin (10µg), gentamicin (10µg), amikacin (30µg) Co-trimoxazole (1.25/23.75µg), chloramphenicol(30 µg), norfloxacin (10µg), nitrofurantoin (300µg), ciprofloxacin (5µg), cefazoline (30µg), cefuroxime (30µg) cefotaxime (30µg), cefepime(30 µg), imipenem (10µg). The *Acinetobacter* strains resistant to imipenem were tested against colistin (10 µg) and tigecycline (15 µg). [4-7]

The biofilm forming ability of the isolates was studied by tube adherence method and Congo red agar method. The efficiency of these two methods for determination of biofilm formation evaluated.

Tube adherence (TM) method given by Christensen's et al was followed. Suspension of isolate was incubated in the glass tube containing Brain Heart Infusion Broth (broth) aerobically at the temperature of 35°C for a period of two days. Then the supernatant discarded and the glass tube was stained by 0.1% safranin solution for 7 min. The glass tube then washed with distilled water three times and dried. A positive result is defined as the presence of a layer of stained material adhered to the inner wall of the tubes. The exclusive observation of a stained ring at the liquid-air interface was considered negative. [8]

Congo red agar (CRA) method given by Freeman et al was followed. Suspension of *Acinetobacter* strain was inoculated onto a specially prepared solid medium - brain heart infusion broth (BHI) supplemented with 5% sucrose and Congo red. The medium was composed of BHI (37 gms/L), sucrose (50 gms/L), agar no.1 (10 gms/L)

and Congo red stain (0.8 gms/L). Congo red was prepared separately as concentrated aqueous solution and autoclaved at 121°C for 15 minutes. This ready Congo red solution added in the media when the agar had cooled to 55°C. Plates were inoculated and incubated aerobically for 24 to 48 hours at 37°C. [9]

A positive result was indicated by black colonies with a dry crystalline consistency. The experiment was performed in triplicate. The biofilm producer *S. epidermidis* ATCC 35984 (positive control) and a known non-biofilm producer was used as negative control.

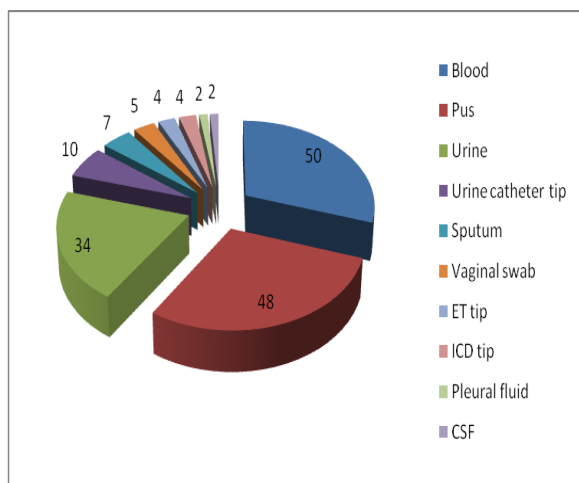


Figure 1 Clinical specimen wise distribution of Acinetobacter spp.
* ICD tip- Intercostal drainage tip **ET tip- Endotracheal tube tip.

RESULTS

Out of a total of 166 isolates of Acinetobacter species, maximum isolates were from blood, followed by pus and urine. (Figure- 1) Biofilm production was observed in 70 Acinetobacter isolates by either method, while 96 isolates were negative for the same (Figure 2). All the Acinetobacter strains were showing resistance to most of the commonly used antibiotics including third and fourth generation cephalosporins. The carbapenem resistance was seen in 40 (24%) isolates under study. Overall percentage of resistance observed among all the isolates including biofilm producer and biofilm non producer is shown in Table 1.

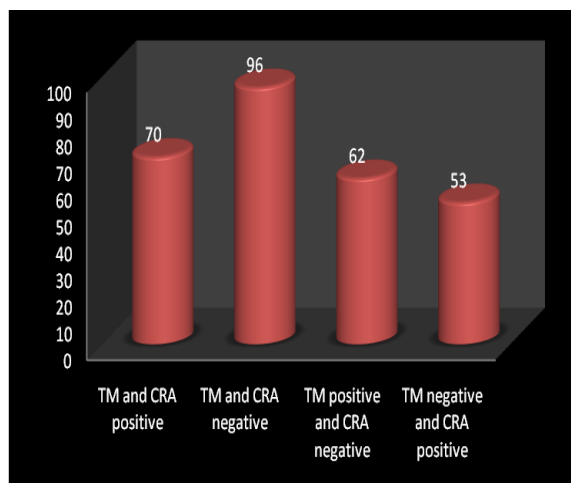


Figure 2: Results of screening of Acinetobacter isolates for biofilm production.

Table 1: Antibiotic resistance profile of biofilm and non-biofilm producer Acinetobacter isolates

Antibiotic	Biofilm producer n=70 Resistance (%)	Biofilm nonproducer n=96 Resistance (%)	Resistance of all isolates N=166 Resistance (%)
Ampicillin	70 (100)	96(100)	166(100)
Gentamicin	64 (91.4)	58 (60.4)	122(73.4)
Amikacin	61 (87.14)	52 (54.15)	113(68.0)
Norfloxacin	58 (82.8)	68(70.8)	126(75.9)
Nitrofurantoin	40(57.1)	43(44.7)	83(50)
Ciprofloxacin	57(81.4)	48(50)	105(63.2)
Co-trimoxazole	60(85.7)	77(80.2)	137(82.5)
Chloramphenicol	52(74.2)	57(59.3)	109(65.6)
Cefazolin	70(100)	96(100)	166(100)
Cefuroxime	70(100)	96(100)	166(100)
Cefotaxime	70(100)	96(100)	166(100)
Cefepime	70(100)	96(100)	166(100)
Imipenem	30 (42.8)	10(10.4)	40(24.0)

DISCUSSION

Acinetobacter species are among the most common causes of device related nosocomial infection that results when the organism is able to resist physical and chemical disinfection. In the present study maximum Acinetobacter species were isolated from blood cultures and different indwelling medical devices, which indicate the possibility of nosocomial infections.

In the present study, 70 (42.1%) of total Acinetobacter isolates showed biofilm production by either Congo red agar method or tube adherence method. The study by Nahar et al reported 87.5% and 55% biofilm production among ICU and non-ICU Acinetobacter isolates respectively. [10] The study by Dheepa et al reported 60% *A. baumannii* strains as biofilm producer while Rao et al reported as 62%. [11,12]

In the present study TM method detected more number of biofilm producer than CRA method. The superiority of TM method for biofilm detection over CRA method is supported by many authors. [13-15]

Acinetobacter isolates have propensity to readily develop resistance to second, third and newer generation of antibiotics. Currently MDR term used to denote resistance to three or more classes of drugs that would otherwise serve as treatments for Acinetobacter infection (quinolones, cephalosporin, and carbapenem). Panresistance has been used to describe strains of Acinetobacter spp. that are resistant to all standard antimicrobial agents tested (except colistin). [16]

In the present study imipenem resistance was seen as 24% which is comparable to study conducted in Pune and Korea. [17,18] The study by Rao et al reported 100% carbapenem resistance, [12] while study by Vijaya et al in U.S.A. reported it as 79.51% in Acinetobacter species under study. [19]

In the present study all carbapenem (imipenem) resistant Acinetobacter isolates were sensitive (100%) to colistin and tigecycline drugs, in contrast the study by Taneja et al reported 16% resistance in carbapenem resistant strains. [20]

All the Acinetobacter isolates showed resistance to all four generations of cephalosporin which is alarming to clinicians and microbiologists. Biofilm producer Acinetobacter isolates showed higher resistance to fluoroquinolones (ciprofloxacin, norfloxacin) aminoglycosides (gentamicin, amikacin) and carbapenem (imipenem) drugs than non biofilm producer.

Patwardhan et al studied antibiotic sensitivity of *A. baumannii* cured isolates and confirmed plasmid borne nature of antibiotic resistance marker. [21] Transfer of antibiotic resistant plasmids from Acinetobacter to other nosocomial pathogen can generate complications in the treatment of the patient.

Smith et al studied microbial synergy and observed that the Acinetobacter infection with yeast cells may increase the resistance of bacteria to toxic effects of salt and can also increase pathogenicity. [22]

The present study underscores emergence of multi drug resistance in the biofilm producing isolates of Acinetobacter spp., which represent a severe threat in the treatment of hospitalized patients.

The colistin and tigecycline drugs showed promising results in the study. The combination therapy with drugs having synergistic activity in vitro may be useful in treating MDR Acinetobacter infection. This will enhance clinical efficacy and prevention of emergence and spread of resistant strains. [2]

CONCLUSION

The present study showed correlation between biofilm production and multidrug

resistance, where strains producing biofilm were multidrug resistant phenotypes than non biofilm producer. The high rate of in vitro antibiotic resistance of Acinetobacter strains indicates importance of judicious antibiotic usage and strict appliance of hospital infection control practices. Antibiotic resistance pattern shown by Acinetobacter isolates limits the treatment alternatives to colistin and tigecycline which are kind of reserved drugs. The study of Acinetobacter in respect to virulence factors and antibiotic susceptibility profile is very essential in prevention and control of nosocomial infections.

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