Multi-drug resistant *Acinetobacter* species from various clinical samples in a tertiary care hospital from South India

Vijayan Sivaranjani, Sivaraman Umadevi, Sreenivasan Srirangaraj, Arunava Kali, Seetha KS

Department of Microbiology, Mahatma Gandhi Medical College & Research Institute, Pondicherry

RESEARCH

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Corresponding Author:
Arunava Kali
Dept. of Microbiology, Mahatma Gandhi Medical College and Research Institute, Pondicherry, India.
Email: ak.arunava@gmail.com

Abstract

Background

Acinetobacter species are gram-negative coccobacilli belonging to the group of Non-Fermenting Gram-Negative Bacilli, which are ubiquitous in nature. They cause outbreaks in intensive care units and healthcare settings, and are becoming increasingly drug resistant.

Aims

To determine the prevalence of multi-drug resistant *Acinetobacter* species from various clinical samples.

Method

Clinical samples were processed as per standard microbiological techniques. Antibiotic susceptibility testing was carried out on all the *Acinetobacter* isolates by Kirby-Bauer disc diffusion method as per CLSI guidelines.

Results

A total of 122 *Acinetobacter* spp. were isolated. 110 (90.16 per cent) were from inpatients, and 12 (9.83 per cent) were from outpatients. Out of 122 isolates, 44 (36.06 per cent) were from the ICU. The majority of the isolates, 47 (38.52 per cent), were from pus samples followed by 25 (20.49 per cent) from endotracheal tube aspirate. Out of 122 isolates, 87 (71.31 per cent) were multi-drug resistant of which 15 (12.29 per cent) were resistant to all drugs tested.

Conclusion

Acinetobacter infections associated with multi-drug resistant and pan-resistant strains have emerged as important nosocomial pathogens in our setting.

Key Words

Acinetobacter; multi-drug resistance; clinical samples

What this study adds:

1. *Acinetobacter* species are emerging nosocomial pathogens, especially in critical care units.
2. We found that a significant proportion of *Acinetobacter* infections in our hospital were multi-drug resistant and nosocomial in origin.
3. Antibiotic susceptibility testing is critical in the treatment of infections caused by *Acinetobacter,* particularly in those with inadequate response to antibiotic therapy.

Background

Acinetobacter is an important emerging nosocomial pathogen.[1] The organism is ubiquitous in nature; its ability to survive in varying temperatures, pH conditions, and on dry, moist surfaces helps in the transmission and propagation of this organism in the hospital setting.[2,3] In addition, intrinsic resistance and multi-drug resistance (MDR) pose a global medical challenge.[4] Carbapenems which were once the mainstay of therapy, are no longer effective in controlling the infections caused by this organism.[5]

Infections caused by this organism include ventilator-associated pneumonia, bacteraemia, surgical site infections, meningitis, urinary tract infections with the most common risk factor being long hospital stays.[6]

Method

A prospective study was conducted from January to December 2012. In our laboratory, we processed 7182 clinical samples from patients from various sources like pus (n=1870), blood (n=1131), urine (n=3286), sputum (n=622) and endotracheal aspirates (n=273). A total of 122 *Acinetobacter* isolates were isolated and identified by the

following method.

The samples were sub-cultured onto blood agar, Mac Conkey's agar, and incubated at 37^{O}C. After 24 hours, Gram staining was done from the colonies, which showed presence of gram-negative cocco-bacilli by microscopy.

Further identification was done using bio-chemical tests as per standard operating procedures.[7] After identification, antimicrobial susceptibility testing was done by the Kirby-Bauer disk diffusion method to determine the drug resistance, as per CLSI guidelines.[8]

The isolates were tested against ampicillin, amoxicillin-clavulunic acid, ceftazidime, ciprofloxacin, amikacin, co-trimoxazole, piperacillin-tazobactam, imipenem, colistin, and polymyxin B. Isolates showing resistance to at least three categories of drugs i.e. penicillins and cephalosporins, fluroquinolones, and aminoglycosides, were considered multi-drug resistant.[1,3,9] Extensive drug resistant (XDR) *Acinetobacter* were isolates displaying resistance to carbapenems in addition to resistance to penicillins and cephalosporins, fluroquinolones, and aminoglycosides.[3] Pan-resistant *Acinetobacter spp*. was defined as *Acinetobacter* isolate that is resistant to the whole panel of antibiotics tested.[3]

Results

Out of 122 isolates, 110 isolates were from inpatients (90.16 per cent) and 12 were from outpatients (9.83 per cent). We found, 50.80 per cent (n=62) isolates were from females, and 49.18 per cent (n=60) were from males. The mean age of the study population was 39.91±23.22 years. In male and female patients it was 45.98±22.39 and 34.03±22.66 years respectively. The proportion of isolates was more in the age group between 20–40 years (Table 1).

Table 1: Age-wise distribution of *Acinetobacter* isolates

Age groups	Number of patients (%)
< 5 years	15 (12.29%)
5—20 years	6 (4.91%)
20—40 years	42 (34.42%)
40—60 years	32 (26.22%)
> 60 years	27 (22.13%)

A total of 122 *Acinetobacter* isolates were analysed, out of which 87 (71.31 per cent) were multi-drug resistant. Of these MDR isolates, 15 (17.24 per cent) were pan-resistant. *Acinetobacter spp.* were isolated from different wards in our hospital. Most of the MDR isolates, 68.9 per cent (n=60), were from the intensive care units (ICU) and general surgery (Table 2).

Table 2: Distribution of *Acinetobacter* isolates in hospital wards.

Ward	Number of non-MDR isolates (n=35)	Number of MDR isolates (n=87)
ICU	7	37
General Surgery	7	23
Obstetrics and Gynaecology	7	15
Orthopaedics	1	4
General Medicine	6	1
Paediatrics	1	0
Urology	0	1
Out patients	6	6

Acinetobacter spp. were isolated from various clinical samples like pus, endotracheal (ET) aspirate, urine, blood, sputum, and other body fluids. Pus samples showed the greatest isolation rate of 38.52 per cent, followed by endotracheal aspirate at 20.49 per cent. Sources of isolation of *Acinetobacter* spp. from various clinical samples are shown in Table 3.

Table 3: Sample-wise distribution of *Acinetobacter* isolates

Samples	Number of non-MDR isolates (n=35)	Number of MDR isolates (n=87)
Pus	10	37
ET aspirate	3	22
Urine	12	12
Sputum	5	6
Blood	1	6
Others	4	4

Antibiotic susceptibility testing was carried out by the Kirby-Bauer disc diffusion method. More than 90 per cent of isolates displayed resistance to ampicillin, amoxicillin-clavulunic acid, ceftazidime, and amikacin (Table 4). Resistance to gentamicin, co-trimoxazole and ciprofloxacin were also common. Least resistance was seen to piperacillin-tazobactam and imipenem.

Table 4: Comparison of antibiotic resistance pattern of MDR and non-MDR *Acinetobacter* isolates

Drug	Non-MDR (n=35)	MDR (n=87)
Ampicillin	19	87
Amoxycillin – clabulanic acid	11	87
Ceftazidime	6	85
Amikacin	1	62
Gentamicin	1	73
Co trimoxazole	3	70
Ciprofloxacin	7	78
Piperacillin - tazobactam	2	46
Imipenem	1	31

Discussion

Acinetobacter has emerged as an important nosocomial pathogen, especially in the ICU set-up.[6] In our study prevalence was more among the inpatients (90.16 per cent), which clearly reflects the nosocomial origin of this pathogen. Similar prevalence was observed in other studies.[1,3] We found no gender difference in *Acinetobacter* infections.

Among 122 isolates, 87 (71.31 per cent) isolates displayed resistance to three or more categories of antibiotics; 15 (17.24 per cent) of MDR isolates were resistant to all antibiotics tested (pan-resistant). Increased isolation of this organism was seen in ICU (42 per cent). This finding is comparable to other studies.[1,3,10,11]

Abbo et al, stated that isolation was more from respiratory tract, which was 32 per cent, followed by wound (19.5 per cent), urine (9 per cent), and blood was (16 per cent).[1] In our study increased isolation was from pus samples (38.52 per cent) followed by endotracheal aspirates, which was 20.49 per cent, and urine 19.67 per cent as shown in Table 3. Percentage of isolation from blood was only 5.73 per cent, which was contrary to the findings in a study done by Mastofi et al, which showed high isolation rates from blood.[10]

Acinetobacter is resistant to many antibiotics with more isolations from areas under increased antibiotic pressure such as ICUs. This has decreased the therapeutic options available to treat them.[3,6,12] Our isolates showed high resistance to ampicillin (86.8 per cent), amoxicillin–clavulunic acid (80.3 per cent), ceftazidime (74.5 per cent), amikacin (51.6 per cent%), gentamicin (60.6 per cent), co-trimoxazole (59.8 per cent), and ciprofloxacin (69.6 per cent). Similar findings were reported in a study done in Tehran from three different hospitals.[10]

In the present study, the least resistance was shown to piperacillin-tazobactam and imipenem, 39.3 per cent and 26.2 per cent, respectively. Another study reports a resistance percentage of 73.3 per cent to imipenem, increased resistance to piperacillin-tazobactam, and high resistance to third-generation cephalosporins.[13]

The above findings clearly show the emerging resistance to co-trimoxazole and ciprofloxacin followed by imipenem and piperacillin-tazobactam, which remain the main stay of treatment for these infections. This is comparable to another study done by Valentia et al.[6] Hence, stringent infection control measures and judicious use of antibiotics are essential for treatment and prevention of *Acinetobacter* infections.

Emerging resistance to antibiotics could not be ascertained by determining the minimum inhibitory concentration (MIC) for the drugs tested. Risk factor assessment for the MDR *Acinetobacter* could not be evaluated. Only 12 isolates could be tested for susceptibility to Colistin and were found to be sensitive. This was due to non-availability of the disc for testing. These were the major limitations of this study.

Conclusion

Multi-drug resistant *Acinetobacter* has emerged as an important nosocomial pathogen, especially in critical care units. A significant proportion of the isolates were multi-drug resistant. Antibiotic susceptibility testing is critical in the treatment of infections caused by *Acinetobacter,* particularly in those with inadequate response to antibiotic therapy.

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CONFLICTS OF INTEREST

The authors declare that they have no competing interests.