Prevalence & Antimicrobial Profile of Acinetobacter Spp. Isolated from Tertiary Care Hospital

Rupinderjit Kaur[1], Shailpreet Kaur[2], Loveena Oberoi[3], Kanwardeep Singh[4], Neelu Nagpal[5], Manpreet Kaur[6]

ABSTRACT

Introduction: *Acinetobacter* is an opportunistic pathogen and can cause a wide range of healthcare associated infections like ventilator associated pneumonia, meningitis, and bacteremia, urinary tract infections, peritonitis, etc. *Acinetobacter* infections are often extremely difficult to treat because of their widespread resistance to the major groups of antibiotics and are difficult to treat pathogens and can lead to treatment failure.

Material and Methods: Our study was conducted to determine the prevalence and antimicrobial profile of Acinetobacter species from various clinical samples. The isolates were identified by standard protocols and further tested for antimicrobial resistance by Kirby-Bauer disk diffusion method as per CLSI guidelines.

Results: From 142 *Acinetobacter* isolates, majority were from urine (26.1%), vaginal swabs (23.2%), pus (18.3%), blood (17.6%) followed by other samples. The present study, *Acinetobacter Spp.* showed high level of resistance to, Cephalosporins(ceftadizime 88.6%, ceftriaxone 88.1%, cefpodoxime 83.3%) Flouroquinolones (81.8% Ciprofloxacin & 66.7% Levofloxacin). Among Aminoglycosides, Gentamicin showed lesser resistance (38.2%) than Amikacin (47.6%). Amongst combination drugs more resistance was seen to Piperacillin + Tazobactum (65.7%) than Ampicillin + Sulbactum (55.6%). However, among carbapenems, meropenem (55.6%) showed more resistance as most appropriate solution. This hospital-based epidemiological data will help to implement better infection control strategies and improve the knowledge of resistance pattern in our region compared to imipenem(49.4%). Out of 142 isolates, 73.24% were MDR & 8.5 % of the *Acinetobacter* species(more prevalent in *Acinetobacter baumanii*) isolates were pan drug resistant. All the isolates were found to be sensitive to colistin.

Conclusion: Multi drug resistant isolates are increasing day by day, due to indiscriminate use of these antibiotics in healthcare settings. Reducing and restricting the use of antimicrobials to only those situations where they are warranted, at proper dose and for the proper duration is the most appropriate solution.

Keywords: *Acinetobacter* species, nosocomial infection, antimicrobial resistance

INTRODUCTION

Acinetobacter species is, gram negative, strictly aerobic, non-fastidious, non-fermenting coccobacilli which is mostly responsible for hospital acquired infections including nosocomial pneumonia, meningitis, endocarditis, skin and soft tissue infections, urinary tract infection, conjunctivitis, burn wound infection and bacteremia. It is responsible for wide spectrumof infections due to its high capacity to colonize the human body and the environmental reservoirs.[1] According to most recent scientific literature, it's the second most common non-fermenting gram negative pathogen isolated from clinical samples after *Pseudomonas aeruginosa* and is listed by the American Society of Infectious Diseases (IDSA) as one of the six most hazardous microorganism.[1] It's most important representative is *Acinetobacter baumannii* and other species such as *Acinetobacter lwoffii, Acinetobacter haemolyticus and Acinetobacter johnsonii* are rarely isolated from patients. *Acinetobacter* species are opportunistic pathogens predominantly found in immunocompromised patients.[2] The increased risk of infection is associated with the severity of patient's illness, length of exposure to invasive devices and procedures, increased risk of patient contact with health care personnel and length of stay in ICU.[3] In addition to infection among hospitalised patients, community acquired *Acinetobacter* infection is increasingly reported. Depending on the geographical localization and the patient's socio-economic status, the prevalence of *Acinetobacter* infection is variable. *Acinetobacter* species are resistant to many antibiotics because of the low permeability of its outer cell membrane & constitutive expression of certain efflux pumps, and it can accumulate components of resistance mechanisms encoded on plasmids, integrons, and transposons in hospital settings associated with high antibiotic consumption.[4] Acinetobacter has the ability to survive for a long period of time on hospital equipments, therefor making multidrug-resistant *Acinetobacter* infection, a great problem in hospital settings.[5] The trend in increasing antimicrobial resistance(AMR) limits the choice of effective antimicrobial agents. Thus, continuous surveillance of AMR of *Acinetobacer* is very important for the selection and implementation of proper empirical therapy for seriously ill

[1]Senior Resident, Department of Microbiology, [2]Associate Professor, Department of Microbiology, [3]Professor & HOD, Department of Microbiology, [4]Professor, Department of Microbiology, [5]Assistant Professor, Department of Microbiology, [6]Junior Resident, Department of Microbiology, Department of Microbiology, GMC, Amritsar, India

Corresponding author: Dr.Shailpreet Kaur, Department of Microbiology, GMC, Amritsar, India

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patients.

The aim of this study was to determine the prevalence & antibiotic resistance pattern of *Acinetobacter* species isolated from various samples.

MATERIAL AND METHODS

The retrospective study was carried out in the Microbiology department of a Tertiary Care Hospital (Government Medical College, Amritsar) from January 2019 to December 2019. A total of 20,138 clinical samples were received, processed and the *Acinetobacter* species were identified by characteristic colony appearance, non lactose fermenting, gram negative coccobacilli, non motile, oxidase negative, catalase positive and alkaline reaction on Triple Sugar Iron Agar test using standard microbiological methods.[6,7] The isolates were subjected to antibiotic susceptibility testing by standard Kirby Bauer Disc Diffusion methods[8] and their Susceptibility patterns were determined following panel of antimicrobial agents as recommended by CLSI(Clinical laboratory of standard institute).[9]

RESULTS

A Total of 142(0.71%) *Acinetobacter* Spp. was isolated from 20,138 sampls received in the microbiology laboratory. From 142 *Acinetobacter* isolates, majority were from Urine(26.1%), Vaginal Swabs(23.2%), Pus(18.3%), Blood(17.6%) followed by other samples(Figure 1).

Among the *Acinetobacter* isolates (142), majority

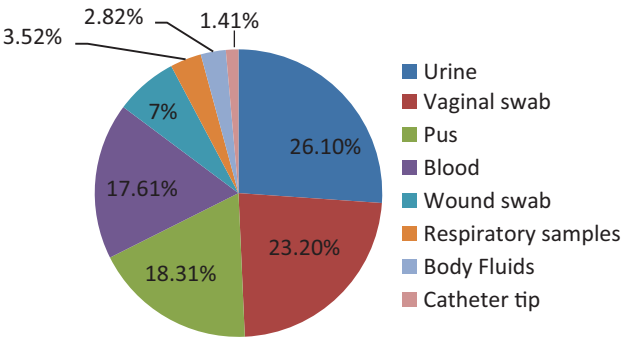

Figure-1: Acinetobacter species isolated from various samples

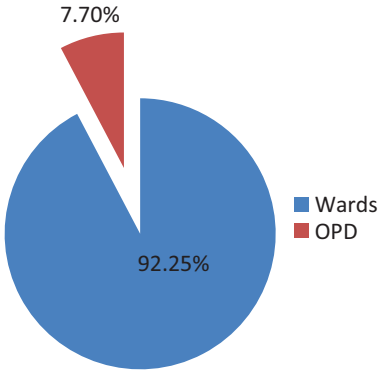

Figure-2: Distribution of Acinetobacter spp. isolated from Wards & OPD

S. No.	Department	Indoor cases (n=131)	Outdoor cases (n=11)
1.	Obstetrics & Gynaecology	61(46.6%)	07(63.6%)
2.	Paediatrics	29(22.1%)	00(0%)
3.	Orthopaedics	17(12.9%)	00(0%)
4.	Intensive care unit	15(11.45%)	00(0%)
5.	Medicine	06(4.5%)	01(9.09%)
6.	Surgery	01(0.76%)	00(0%)
7.	Tb & chest	01(0.76%)	01(9.09%)
8.	ART	01(.76%)	00(0%)
9.	Skin & VD	00(0%)	01(9.09%)
10.	Radiotherapy	00(0%)	01(9.09%)

Table-1: Department wise distribution of Acinetobacter species isolates (n=142)

S.No	Antibiotics	Resistant isolates (percentage)	Intermediate isolates (percentage)	Sensitive isolates (percentage)
1	Ceftazidime (30µg)	88.6%	2.8%	8.6%
2	Ceftriaxone (30µg)	88.1%	2.4%	9.5%
3	Cefpodoxime (10µg)	83.3%	0%	16.7%
4	Amikacin (30µg)	47.6%	3.5%	44.0%
5	Gentamicin (10µg)	38.2%	3.6%	58.2%
6	Ciprofloxacin (5µg)	81.8%	1.85%	16.4%
7.	Levofloxacin(5µg)	66.7%	0%	33.3%
12	Meropenem (10µg)	55.6%	0%	44.4%
13	Imipenem (10µg)	49.4%	3.8%	46.8%
14	Piperacillin+Tazobactum (100/10µg)	65.7%	1.4%	32.8%
15	Ampicillin+Sulbactum (10/10µg)	55.6%	11.1%	33.3%
16	Polymixin B	0%	0%	100%

Table-2: Sensitivity pattern of Acinetobacter species to different Antimicrobial drugs

131(92.25%) were detected from patients admitted in different wards of the hospital (Figure 2) giving a probability of increased hospital acquired infections. Gender ratio was 2.1:1(Female:Male) thus, a slight female preponderance was observed in our study. *Acinetobacter spp.* reported in our study, 91.6% were *Acinetobacter Baumanii &* 5.6% *Acinetobacter lwoffi,* 2.8%*Acinetobacter hemolyticus.*

In our study out of 131(92.25%) indoor cases, maximum number were reported were from department of Obstetrics & Gynaecology 46.6%(29), Paediatrics 22.1%(29) & Orthopaedics 12.9%(17). Fifteen (11.45%) cases were reported from intensive care unit (Table 1).

In the present study, *Acinetobacter Spp.* showed high level of resistance to, Cephalosporins(ceftadizime 88.6%, ceftriaxone 88.1%,cefpodoxime 83.3%) Flouroquinolones(81.8% Ciprofloxacin & 66.7% Levofloxacin) (Table 2). Among Aminoglycosides, Gentamicin showed lesser resistance (38.2%) than Amikacin (47.6%). Amongst combination drugs more resistance was seen to Piperacillin + Tazobactum (65.7%) than Ampicillin + Sulbactum (55.6%). However, among carbapenems, meropenem(55.6%) showed more resistance as compared to imipenem(49.4%). Almost all the isolates showed in vitro resistance to one or more of the antibiotics mentioned earlier. Multidrug resistant (MDR) *Acinetobacter* spp. are defined as those isolates resistant to more than three classes of antibiotics, An isolate was classified as pan resistant when it was resistant to all of the commonly used antibiotics.[10] Out of 142 isolates, 73.24% were MDR & 8.5% of the *Acinetobacter* species(more prevalent in *Acinetobacter baumanii*)isolates were pan drug resistant. All isolates of *Acinetobacter spp.* were sensitive to Polymyxin B.

DISCUSSION

In the recent years, *Acinetobacter* species infection has become a critical challenge to healthcare systems, contributing to increased morbidity and mortality . *Acinetobacter* species especially *A. baumannii* are causing more hospital-acquired infections due to increased use of invasive procedures, overuse of broad spectrum antimicrobials and prolonged duration of stay in the hospital and development of resistance against antimicrobials poses major problem in the treatment of *Acinetobacter* infections. Although they are considered as pathogen of mild virulence, they can rapidly acquire resistance.[11] This organism has been reported as the most frequent cause of respiratory tract infections, with strains being isolated from 3 to 5% of patients with nosocomial pneumonia.[12] *Acinetobacter* normal inhabitant of soil and water and has also been isolated from foods and animals. In humans, *Acinetobacter* can colonize skin, wounds, respiratory and gastrointestinal tracts.[13] It is a pathogen of tropical and humid environment, but some species can survive environmental dessication for weeks, a characteristic that promotes transmission through fomite contamination in hospitals.[14]

In our study, total of 142(0.71%) *Acinetobacter* spp. was isolated from 20138 samples. Approximately similar prevalence of 2.9% *Acinetobacter* species were reported in a study by Saha S et al in Manipur. Prevalence of 3% and 3.36% of total organisms isolated was reported by Dash et al in Odisha and Gupta et al in Pune.[15,16] Higher prevalence rates of 14% and 9.6% was reported by Mostofi et al, in Tehran, Iran and Joshi et al in Pune.[17,18] In the present study maximum isolates were isolated from wards 119(83.8%) This is probably related to increasingly invasive diagnostic procedures used, greater quantity of broad spectrum antimicrobials used and prolonged duration of stay in hospital as reported by various authors.[15,19] In the present study *A.baumannii* (91.6%) was found to be the frequent cause of infections. Like our study, W. Nageeb et al., also proved that *A.baumannii* was the only *Acinetobacter spp.* encountered in clinical specimens and this supported the finding that infections by other *Acinetobacter spp.* are infrequent.[20] There are some other studies which also found that among different *Acinetobacter spp, A.baumannii* was the most prevalent in clinical specimens and the most often responsible for nosocomial infections.[21,22,23]

Hospital environment is heavily contaminated with these organisms. The carriage rate is much higher among hospital staff than community. Unhygienic practices in hospital (contaminated hands of staff) and warm hospital environment (summers) promote colonization. *Acinetobacter* persist on inanimate surfaces for prolonged periods of time ranging from 3 days to 5 months and can be detected on various equipment including bedrails, curtains, ventilation equipments(e.g. AMBU bags, ventilator filter).[24] Patients with underlying diseases or immunosuppression are predisposed to invasion and pathogenesis. Virulence factors attributed to pathogenesis are Outer membrane protein (OmpA), lipopolysaccharide, lipases ability to form biofilm and siderophores.[25] Out of 142 isolates of *Acinetobacter* species,92.25% of Acinetobacter isolates were obtained from patients admitted to various wards, whereas only 7.70%% were obtained from OPD cases and most of the cases were reported from department of Obstetrics & Gynaecology 46.6%(29), Paediatrics 22.1%(29), Orthopaedics 12.9%(17) & intensive care unit 11.4%(15).

In our study out of 142 acinetobacter isolates, majority were from urine(26.1%),Vaginal swabs(23.2%), pus(18.3%) & blood(17.6%).The higher isolation rates 26.1% of *Acinetobacter* spp from the Urine samples are not in agreement with the results reported in other studies where higher isolation rates were most often from respiratory samples.[19] However, a study by Lone et al. in Srinagar, India reported that majority (39.6%) of the *Acinetobacter* isolates from urine, followed by pus and wound exudates (29.5%).[26] Hospital outbreaks caused by problematic microorganisms, like multidrug-resistant *Acinetobacter baumannii*, resulting in increased morbidity and mortality, especially in intensive care units, surgical wards in a big hospital complexes, have been reported worldwide.[26] Also there are many reports, showing that persistent hospital environmental contamination with *A. baumannii* strains may play an important role in nosocomial dissemination of these

organisms.[27,28] Management of *Acinetobacter* infections is huge challenge because of the broad array of antimicrobial resistance. The resistance patterns of *Acinetobacter* isolates towards various antimicrobial agents were determined by disc diffusion method as recommended by CLSI guidelines.

In the present study, *Acinetobacter* species were found to be resistant to most commonly used antibiotics. *Acinetobacter* isolates were extremely resistant to Ceftazidime (88.6%) and ceftraixone (88.1%) which correlates with the studies by Saha S et al.[2] Resistance to levofloxacin is found less in comparison to other fluoroquiniolones in our study and similar finding was also found by Bhattacharya et al in their study.[29] In our study resistance towards Meropenem & Imipenem was recorded to be 55.6% and 49.4% respectively which were comparatively higher than in a studies by Dash et al that reported resistance towards Meropenem (22%) and Imipenem (19%) and Saha S et al which showed resistance towards Imipenem and Meropenem to be 25.3% and 29.7% respectively.[2,15] No resistance was seen in Colistin in our study which is similar to the study published by Dash et al and Shareek et al, whereas isolates were sensitive to colistin.[15,30]

In our study, out of 142 *Acinetobacter* species isolates 73.24% were found to be Multi drug resistant(MDR). In a study conducted by Saha S et al, 62.1% isolates were multidrug resistant (MDR).[2] The other studies conducted by Dash et al, in Odisha and Rekha et al in Kolar, Karnataka reported MDR isolates to be 55% and 74% respectively[15,19] Bhattacharya et al, Gupta et al, and Mostofi et al, reported MDR isolates to be 29%; 40% and 54% respectively.[16,17,30]

In a review comparing hospitals of 10 Asian countries, 1.2-87% of all *Acinetobacter* isolates from patients with Hospital Acquired Pneumonia (HAP) were MDR, with MDR strains most prevalent in India and Thailand.[31] In a study from Pune, about 48% to 68.6% *A.baumanni* isolates were MDR.[18] *Acinetobacter* appears to have a propensity to develop antibiotic resistance extremely rapidly, perhaps as a consequence of its long term evolutionary exposure to antibiotic producing organisms in soil environment. Notably, our findings show that Gentamicin and Amikacin is effective against *Acinetobacter spp.* showing 38.2% & 47.6% and resistance respectively which can be a cost effective therapeutic option against *Acinetobacter* isolates, especially in this part of India. The other studies conducted by Dash et al in Odisha and Mostofi et al in Tehran reported MDR isolates to be 54.7% and 54% respectively.[15,17] One report from U.S.A has quoted imipenem resistance in *Acinetobacter baumanni* in the order of 23.1%.[15,17] Taneja et al. in Chandigarh, India studied 224 *A.baumanii* isolates, out of which 22.3% isolates were resistant to carbapenems.[33] Our results show that about 23.6% were resistant to imipenem which is similar to reports from U.S.A and India. We found that imipenem and piperacillin/tazobactum were most potent antibiotics against this pathogen. Differences observed between the studies could be due to the methods and the resistance patterns that are influenced by the environmental factors and the antimicrobial patterns used.Colistin retains activity against *Acinetobacter spp*. in the face of broad-spectrum antimicrobial resistance and have become the last resort of treatment. A delay in the administration of colistin, has the potential to increase the risk of mortality. However, *A.baumannii* can develop resistance to colistin, and thus extreme vigilance is required to diagnose the development of resistance during treatment. Unfortunately, resistance to colistin has emerged with its increasing use, and the recent observation of heteroresistance to colistin among clinical strains of MDR *A.baumannii* is also a significant cause of concern.

Pan drug resistance typically is the result of the convergence of multiple resistance mechanisms.[34] Pan drug resistant *A.baumannii* isolates, i.e isolates resistant to all antimicrobial agents in-vitro, have been reported from Asia and the Middle-East. In our study 8.5% isolates were pan drug resistant. Colistin retains activity against PDR *Acinetobacter spp*. but side effects like nephrotoxicity and ototoxicity limits its use.[35] Our study did not find any *Acinetobacter* isolate resistant to colistin, which may be due to its selective use only in case of carbapenem- resistant gram-negative bacteria. Colistin activity can be enhanced when combined with some other modes of action such as carbapenems, rifampicin and ceftazidme. Combination therapy of colistin and meropenem has synergistic effect/additive effect. Colistin acts on outer membrane of cell wall and creates pores allowing the other drugs to enter into the bacterial cell. Meropenem has bactericidal activity and binds to PBP of cell wall and inhibits cell wall synthesis.[34]

Susceptibilities of *Acinetobacter* against antimicrobials are considerably different among countries, centers and even among different wards of the same hospital. Therefore, such types of local surveillance studies are around important in deciding the most adequate therapy for *Acinetobacter* infections.[2]

The primary goals for the control of multidrug resistant *Acinetobacter* infection are recognizing its presence in a hospital at an early stage and controlling its spread. Hospital acquired infections are best controlled by following appropriate sterile techniques and infection control guidelines and by implementing effective protocols for the sterilization and decontamination of medical supplies. Infection control measures such as improved hand hygiene are essential to prevent nosocomial infections due to *Acinetobacter*.

CONCLUSION

Acinetobacter is an important nosocomial pathogen causing significant morbidity and mortality. MDR isolates are increasing day by day, probably due to indiscriminate use of these antibiotics in healthcare settings. Reducing and restricting the use of antimicrobials to only those situations where they are warranted, at proper dose and for the proper duration is the most appropriate solution. Susceptibility testing should be done to help select the best antimicrobial drugs for therapy. A combination of a review of handwashing practice, education about spread of bacteria via hands and contaminated environment, and the revision of infection

control procedures would help in the control of this organism in hospitals.

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