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Original Research Article

Prevalence and Antibiogram of *Acinetobacter* spp. Isolated from Various Clinical Samples in a Tertiary Care Hospital, Bathinda

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ABSTRACT

Introduction: *Acinetobacter*, once considered as an opportunistic pathogen has recently emerged as an important nosocomial pathogen. An increase in antibiotic resistance among isolates of the organism during recent years has made these infections difficult to treat.
Material & methods: The study was conducted to determine prevalence and antibiotic susceptibility pattern of *Acinetobacter*spp isolated from various clinical samples collected from patients admitted in Intensive care unit and various wards of the hospital over a period of one year (July 2015 to June 2015).
Results: Out of 950 clinical samples, 460 (48.4%) yielded significant growth and out of these positive cultures; 47 (10.2%) *Acinetobacterspp* were isolated. Majority of isolates (68.1%) were obtained from Intensive Care Unit. Maximum sensitivity of *Acinetobacter*spp was seen towards polymyxin B (97.8%), colistin (95.8%) followed by imipenem (57.4%) and meropenem (44.6%). Thirty six (76.6%) isolates were found to be multidrug resistant.
Conclusion: To avoid resistance, antibiotics should be used judiciously and empirical therapy should be determined for each hospital according to the resistance rates of the hospital.

Key words: *Acinetobacter*spp, antimicrobial resistance, Intensive care unit.

INTRODUCTION

Members of genus *Acinetobacter* are ubiquitous, free-living and saprophytic bacilli that can be obtained easily from soil, water, food and sewage. *Acinetobacter* has undergone significant taxonomic modification over last 30 yrs. 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. [1] *Acinetobacter*, once considered as opportunistic pathogen has recently emerged as an important nosocomial pathogen world over, mostly involving patients with impaired host defense. [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] Human infections caused by *Acinetobacter*spp include pneumonia, which is most often related to endotracheal tubes or tracheostomies, endocarditis, iatrogenic meningitis, skin and wound infections, peritonitis in patients receiving peritoneal

dialysis, UTI and bacteremia. [1] In addition to infection among hospitalised patients, community acquired Acinetobacter infection is increasingly reported. [4] An increase in antibiotic resistance among isolates of the organism during recent years has made these infections difficult to treat. [5] Resistance mechanisms that are expressed frequently in nosocomial strains of *Acinetobacter* include: β-lactamases which are either chromosomally encoded or borne on plasmids or transposons, alteration in cell wall channels (porins) and efflux pumps. [2] Because of frequent resistance to amino glycosides, fluoroquinolones, ureidopenicillins and third generation cephalosporins, carbapenems have been the drug of choice for treating *Acinetobacter* infections. [4-6] However, there has been alarming increase in reports of carbapenem resistant *Acinetobacter*spp. Over the last decade. [6] carbapenem resistance in *Acinetobacter* is attributed to various causes such as reduced expression of outer membrane proteins (29kDa, 33-36kDa) and carbapenamases β-lactamases. *A .baumannii* is known to produce following metallo β-lactamases (MBL's)-IMP-1, 2, 4, 5 and VIM-1 and 2. [6]

The most active agents in vitro against multidrug resistant *A. baumannii* are polymyxins-Polymyxin B and Polymyxin E (colistin) and tigecycline. [7] Clinicians abandoned polymyxins in 1960's and 1970's due nephrotoxicity and neurotoxicity, The emergence of multidrug resistant(MDR) gram negative bacilli has brought polymyxins back into use in lower doses and different drug formulations. [2] There is a significant difference in behaviour and spread of MDR *Acinetobacter*spp recovered from various geographical locations. [8] Since several factors cause resistance in *Acinetobacter*spp, treatment of infections caused by this organism should be based upon susceptibility tests. Therefore, having information regarding prevalence and pattern of bacterial resistance to various antibiotics is important. [9,10] Thus the objective of the study was to determine antibiotic sensitivity pattern of *Acinetobacter*spp isolated from various clinical samples collected from patients admitted in ICU and various wards of hospital of Adesh Institute of Medical Sciences and Research (AIMSR), Bathinda. The study was conducted after due approval was obtained from Institutional Ethical Committee and Research Degree Committee.

MATERIALS AND METHODS

The present study was conducted in Department of Microbiology, Adesh Institute of Medical Sciences and Research, Bathinda and included *Acinetobacter*spp isolated from various clinical samples over a period of one year. (July 2014 to June 2015) A total of 950 clinical samples such as pus, urine, blood, tracheostomy and endotracheal tube tips, tracheal aspirate CSF and other body fluids were collected from patients admitted in ICU and different wards of hospital. The samples were inoculated on Blood Agar and MacConkey Agar plates. All isolates obtained were further processed and identified by routine microbiological and biochemical tests. Genus *Acinetobacter* was identified by Gram staining as Gram negative coccobacilli, colony morphology, non-motile, oxidase negative, catalase positive, TSI reaction K/K and citrate utilisation test positive. Speciation of Acinetobacter (*A. baumannii and A. lwoffii*) was done on the basis of glucose oxidation(OF test) and growth at 37 C and 44 C. [11,12] Antibiotic susceptibility testing was performed by standard Kirby Bauer disc diffusion method [13] for following antimicrobial agents-ceftazidime (30µg), cefepime (30 µg)), piperacillin-tazobactam (100µg)/10 µg)), Ampicillin-sulbactam (10 µg)/10 µg)), Imipenem (10 µg)), Meropenem (10 µg)),Gentamicin (10 µg)), Amikacin (30 µg)), Cotrimoxazole (25 µg)), Ciprofloxacin (5 µg)), Norfloxacin (30 µg)) (for urinary isolates), Polymyxin B (300 units) and Colistin (10 µg). The zones of inhibition were measured and interpreted

according to CLSI guidelines. [14] All dehydrated media and antibiotic discs were procured from Hi Media Labs, Mumbai, India.

RESULTS

Out of total 460 culture positive samples, 47 (10.2%) infections were due to *Acinetobacter*. [Table I] There was higher incidence of *Acinetobacter* infection in males (56%) than females (44%). *Acinetobacter*spp was more common in patients with age group of 21-40yrs (28; 59.4%). The mean age group of patients infected with *Acinetobacter*spp was 38 yrs.

Total samples processed	950
Total culture positive samples	460(48.4%)
Gram negative bacteria isolated out of culture positive samples	315(68.4%)
Total Non-fermenting Gram negative bacilli (NFGNB) isolated among all Gram negative bacteria	151(47.9%)
Acinetobacterspp isolated among NFGNB	47(31.1%)
Prevalence of Acinetobacterspp among total culture positive samples	10.2%

*Acinetobacter*spp were predominantly isolated from respiratory samples (24; 51%) followed by pus (13; 27.6%); urine (6; 12.7%); Intercostal drain tube (2; 4.2%) blood (1; 2.1%) and CSF (1; 2.1%) [Table II]

Table II: *Acinetobacter*spp isolated from various samples (N=47)

Type of sample	No. of isolates n (%)
Tracheostomy tube tips	13(27.6%)
Endotracheal tube tips6	(12.7%)
Tracheal aspirate	4(8.5%)
Endotracheal secretion	1(2.1%)
Pus	13(7.6%)
Urine	6(12.7%)
Intercostal drain tube tips	2(4.2%)
Blood	1(2.1%)
CSF	1(2.1%)

A. baumannii (39; 87.2%) was the predominant species followed by *A. lwoffii* (6; 12.8%) [Table III]

Table III: *Acinetobacter*spp isolated (N=47)

Name of species	No. of isolates	Percentage
Acinetobacter baumannii	41	87.2%
Acinetobacter lwoffii	06	12.8%

Maximum *Acinetobacter*spp were isolated from ICU (32; 68.1%) followed by surgery ward (8; 17%), medicine ward (2; 4.25%), gynaecology ward (2; 4.25%), orthopaedic ward (3; 6.4%). Maximum resistance was recorded to ceftazidime (97.8%) and maximum sensitivity of *Acinetobacter*spp was seen towards polymyxin B (97.8%) and colistin (95.8%) followed by imipenem (57.4%) and meropenem (44.6%). Norfloxacin was tested only in urinary isolates and 66.6% isolates were resistant to this antibiotic. [Table IV]

Out of 47 isolates, 36 (76.6%) were MDR (Isolates resistant to at least one agent in three or more antimicrobial categories penicillins, cephalosporins, amino glycosides, fluoroquinolones and carbapenems) [15,16] *Acinetobacter baumannii* was found to be more resistant than *Acinetobacter lwoffii*, therefore maximum resistance was observed in ICU isolates in comparison to wards where *A. baumannii* was more prevalent.[Table V]

Table IV: Antibiotic sensitivity and resistance pattern of *Acinetobacter*spp to various antibiotics

Antibiotic tested	No. of sensitive isolates (%Sensitivity)	No. of resistant isolates (%Resistance)
Ceftazidime	01(2.2%)	46(97.8%)
Cefepime	02(4.2%)	45(95.8%)
Ampicillin-sulbactam	08(17.1%)	39(82.9%)
Imipenem	27(57.4%)	20(42.6%)
Meropenem	21(44.6%)	26(55.4%)
Piperacillin-Tazobactam	07(14.8%)	40(85.2%)
Cotrimoxazole	03(6.4%)	44(93.6%)
Ciprofloxacin (in 41 isolates)	03(7.3%)	38(92.6%)
Norfloxacin(in 6 urinary isolates)	02(33.3%)	04(66.6%)
Gentamicin	08(17.1%)	39(82.9%)
Amikacin	06(12.7%)	41(87.3%)
Polymyxin B	46(97.8%)	01(2.2%)
Colistin	45(95.8%)	02(4.2%)

Table V: Comparison between antibiotic resistances of *Acinetobacter*spp isolated from ICU and wards

Name of antibiotic	Total no. of resistant isolates	ICU (N=32) n(%)	Wards (N=15) n (%)
Ceftazidime	46	32(100%)	14(93.3%)
Cefepime	45	32(100%)	13(86.6%)
Ampicillin-sulbactam	39	28(87.5%)	11(34.3%)
Imipenem	20	16(50%)	4(25%)
Meropenem	26	19(59.3%)	7(46.6%)
Piperacillin-Tazobactam	40	28(70%)	12(80%)
Cotrimoxazole	44	31(96.8%)	13(86.6%)
Ciprofloxacin (in 41 isolates)	38	30(78.9%)	8(53.3%)
Norfloxacin (in 6 urinary isolates)	04	03(75%)	01(25%)
Gentamicin	39	29(90.6%)	10(66.6%)
Amikacin	41	30(93.7%)	11(73.3%)
Polymyxin B	01	01(3.1%)	0(0%)
Colistin	02	02(6.2%)	0(0%)

DISCUSSION

In our study, from 460 bacterial isolates, 47 (10.2%) *Acinetobacter*spp were obtained. Similar prevalence of 14% and 9.6% was reported by Mostofi *et al.* in Tehran, Iran and Joshi *et al.* in Pune. [17,18] Lesser prevalence rates of 3.36% and 3% of total organisms isolated was reported by Guptaet *al*. in Pune and Dash *et al* in Odisha. [19,4] Our prevalence of *Acinetobacter*spp from total Gram negative bacteria is 15% which is slightly higher than Jaggi *et al.* (9.4%) [20] In the present study, 32 (68%) isolates were from critical care setting and source was most often respiratory samples which is similar to studies by Rekha *et al.* (70%) and Nahar *et al.* (68%) [5,21] This is probably related to increasingly invasive diagnostic procedures used ,greater quantity of broad spectrum antimicrobials used and prolonged duration of stay in hospital [4,5] 32% isolates were obtained from various wards; predominantly surgery ward. This is comparable to studies by Jaggi *et al.* and Rekha *et al.* [Table VI]

Table VI: *Acinetobacter*spp isolated from ICU and IPD compared with other studies

Department	Jaggi et al	Rekha et al	Present study
ICU	76.8%	76.5%	68.1%
IPD	23.2%	23.5%	31.9%

According to literature, amongst *Acinetobacter*spp, commonest species isolated in human clinical specimens *is A. baumannii.* [1] We also observed that 87.2%isolates were *A. baumannii* whereas remaining 12.8% isolates were A. lwoffii. This is also in concordance with study done by Parandekrar PK and Peerapur BV, Bijapur, Karnataka, who have reported 86.3% isolates as *A. baumannii* and 13.7% isolates as *A. lwoffii* [22]

In the present study, *Acinetobacter*spp were found to be resistant to most commonly used antibiotics. *Acinetobacter* isolates were extremely resistant to ceftazidime (97.8%); cefepime (95.8%) and cotrimoxazole (93.6%). Higher level of resistance was also recorded for Ampicillin-sulbactam (82.9%) Piperacillin - tazobactam (85.2%); amikacin and ciprofloxacin (92.6%). This correlates with the studies by JaggiN *et al.* [20] and Kalidas *et al.* [23] Resistance towards imipenem and meropenem was recorded to be 42.6% and 55.4% respectively. A study by Dash *et al.* [4] also reported more resistance towards meropenem (22%) as compared to imipenem (19%). Sinha Mand Srinivasa H reported carbapenem resistance in *Acinetobacter* isolates as 50% [6] Lower resistance (2.2%-4.2%) was seen in Polymyxin B and Colistin in our study. Various authors have reported resistance rates of *Acinetobacter* towards colistin between 1.8% to 2.0%. [4] Taneja *et al.* [24] Nahar *et al.* [21] and Kalidas *et al.* [23] recorded 3.5%; 10.5% and 5% resistance of *Acinetobacter* towards colistin respectively. In a study published by Dash *et al* .and Shareek *et al.* all isolates were sensitive to colistin. [4,25] Comparison of percentage resistance of *Acinetobacter*spp isolated in present study and other studies in India by Jaggi *et al*. in Gurgaon [20] and Kalidas *et al.* [23] in Eastern India is shown in Table VII

Table VII: Comparison of resistance of *Acinetobacter*spp with other studies in India

Antibiotic	Present study	Jaggi et al Gurgaon	Kalidas et al Eastern India
Ceftazidime	97.8%	92.1%	73.8%
Cefepime	95.8%	90.3%	Not tested
Imipenem	42.6%	89.6%	13.7%
Meropenem	55.4%	89.6%	Not tested
Piperacillin+ Tazobactam	85.2%	89.7%	62.3%
Cotrimoxazole	93.6%	Not tested	93.5%
Ciprofloxacin	92.6%	91.6%	80.4%
Gentamicin	82.9%	85.8%	75.5%
Amikacin	87.3%	90.3%	37.8%
Polymyxin B	2.2%	2.0%	Not tested
Colistin	4.2%	2.0%	5%

Out of total isolates 36 (76.6%) were multidrug resistant (MDR) in our study. 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. [4,5] Bhattacharya *et al.;* [26] Gupta *et al.* [19] and Mostofi *et al.* [17] reported MDR isolates to be 29%; 40% and 54% respectively.

In this study, 95% isolates obtained from intubated patients were MDR. Emine *et a*l from Turkey reported that all isolates obtained from intubated patients were MDR. [27]

Acinetobacter appears to have a propensity to develop antibiotic resistance extremely rapidly, perhaps as a consequence of it's long term evolutionary exposure to antibiotic producing organisms in soil environment. The emergence of antibiotic resistant strains in ICU is because of higher of use of antimicrobial agents per patient and per surface area. [19] 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. [21]

CONCLUSION

Acinetobacter is nowadays common threat in hospital acquired infections especially in critically ill patients admitted to ICU. It is a great challenge for the physicians to treat MDR *Acinetobacter*spp. *Acinetobacter*spp in our study were found to be resistant to most commonly used antibiotics. Emergence of carbapenem resistance is worrisome. Lower resistance was only in Polymyxin B and colistin. However, colistin resistant *Acinetobacter*spp are emerging slowly. Rational use of antibiotics is necessary to prevent microbial resistance catastrophe. Resistant antibiotics after sensitivity report should be discontinued and in place sensitive drug should be chosen. Though the organism has developed multidrug resistance, it has largely remained susceptible to disinfectants and antiseptics. Thus, the prevention involves aseptic care of vascular catheters and endotracheal tubes, proper disinfection of surfaces with which the patient comes in contact and through hand hygiene of health care workers. To avoid resistance, antibiotics should be used judiciously and empirical therapy should be determined for each hospital according to the resistance rates of the hospital.

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