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Original Research Article

A study of Acinetobacter infections in a tertiary care hospital in Northeast India

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ABSTRACT

Background: *Acinetobacter* is an important opportunistic pathogen and is a common cause of hospital acquired infections. *Acinetobacter* infections are often extremely difficult to treat because of their widespread resistance to the major groups of antibiotics. The study was conducted to determine prevalence and antibiotic susceptibility pattern of *Acinetobacter* species isolated from various clinical samples.
Methods: Clinical specimens over a period of 2yrs from May 2015 to April 2017 were collected from the patients attending the hospital. *Acinetobacter species* isolates were identified, and antibiotic susceptibility test was done following standard operative procedures.
Results: From 9979 clinical specimens, 3715 were positive for significant bacterial growth of which 111 (2.9%) were culture positive for *Acinetobacter spp*. Among 111 isolates 109 (98.2%) isolates were *Acinetobacter baumanni* and 2 (1.8%) were *Acinetobacter lwoffii*. Maximum isolates were isolated from urine samples 36 (32.4%) and majority of the isolates were from wards (56.7%) giving a probability of increased hospital acquired infections. Maximum resistance was shown by cefipime (80.1%). Imipenem and Meropenem shows resistance of 25.3% and 29.7% respectively. ICU isolates showed extensive resistance in comparison to wards and OPD.
Conclusions: Increasing trend of resistance pattern to a large range of antibiotics is a matter of concern. 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. Infection with MDR *Acinetobacter species* is independently associated with high mortality, emphasizing the need for aggressive infection control strategies.

Keywords: Antibiotic susceptibility, Empirical therapy, Hospital acquired infections, Infection control strategies

INTRODUCTION

Acinetobacter spp. are Gram negative, strictly aerobic, non-fastidious, non-fermenting encapsulated coccobacilli causing mostly hospital acquired infections. According to most recent scientific literature, *Acinetobacter spp.* are the second most common non-fermenting gram negative pathogen isolated from clinical samples after *Pseudomonas aeruginosa*.[1] *Acinetobacter* has undergone significant taxonomic modification over the 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.[2] *Acinetobacter species* are opportunistic pathogens predominantly found in immunocompromised patients. They are widespread in nature, and regarded as commensal microbes of human skin and respiratory tract, however, they may cause serious infections, such as endocarditis, urinary tract infections, pneumonia, wound infections, meningitis, and septicemia, especially in individuals with impaired host defenses.[3] 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.[4] In addition to infection among hospitalised patients, community acquired Acinetobacter infection is increasingly reported.[5] An increase in antibiotic resistance among isolates of the organism during recent years has made these infections difficult to treat.[6] *Acinetobacter* species are becoming increasingly resistant to nearly all routinely prescribed antimicrobial agents, including aminoglycosides, fluoroquinolones, and broad-spectrum β-lactams. The majority of strains are resistant to cephalosporin class of antimicrobials, whereas the resistance to carbapenems is increasingly reported.[7] The objective of the study is to determine the prevalence of the Acinetobacter infections and antibiotic sensitivity pattern of *Acinetobacter* species isolated from various clinical samples collected from patients attending Regional Institute of Medical Sciences Hospital.

METHODS

This study was conducted in the Department of Microbiology, Regional Institute of Medical Sciences, Imphal, Manipur, and included *Acinetobacter species* isolated from various clinical samples over a period of two year (October 2014 to September 2016). A total of 9979 clinical samples such as pus, urine, blood, catheter tips, tracheal aspirate, sputum and other body fluids were collected from patients attending OPD, admitted in ICU and different wards of hospital. The samples received in the laboratory were inoculated on 5% Sheep Blood Agar and Mac Conkey agar and incubated overnight aerobically at both 37◦C. All isolates obtained were further processed and identified by routine microbiological and biochemical tests.

In case of urine samples, the isolates were subjected to biochemical tests only if the colony count was significant (>10^5 CFU/ml). Genus *Acinetobacter* was identified by characteristic colonies (Non Lactose-fermenting, glistening, small mucoid colonies), Gram staining pattern as Gram negative coccobacilli, Motility as non-motile, and standard biochemical reactions (Catalase, oxidase, oxidation-fermentation test, indole production, citrate utilization, urease activity, reaction in triple sugar iron medium), speciation of Acinetobacter (*A. baumannii and A. lwoffii*) was done on the basis of glucose oxidation (of test) and citrate utilization test.[8] After identification by phenotypic methods, antibiotic susceptibility was performed for each isolate by the Kirby-Bauer disc diffusion method on Mueller-Hinton agar using 0.5 MacFarland turbidity standard and comparing zone sizes with control strain *Pseudomonas aeruginosa* ATCC 27853.[9] The antimicrobial agents used were-ceftazidime (30µg), cefepime (30µg)), ceftazidime and clavulinic acid, piperacillin-tazobactam (100µg)/10µg)), imipenem (10µg)), meropenem (10µg), gentamicin (10µg), amikacin (30µg), cotrimoxazole (25µg), ciprofloxacin (5µg)), levofloxacin, norfloxacin (30µg) and nitrofurantoin (300) (for urinary isolates), and colistin (10µg). Antibiotic susceptibility results were interpreted by measuring the zone diameters produced and correlating them with the CLSI standards.[10] ESBL production was tested using the double-disc approximation method using ceftazidime and ceftazidime-clavulinic acid discs.[11] All the analysis was performed using simple percentage method.

RESULTS

During the study period, out of 9979 specimens received from hospital 3715 were positive for bacterial growth of which 111 (2.9%) were culture positive for *Acinetobacter spp.* Among all the 111 isolates, 109 (98.2%) are *Acinetobacter baumanni* and 2 (1.8%) are Acinetobacter lwoffii. Table 1 shows the distribution of the isolates in various clinical samples. Maximum isolates were isolated from urine samples 36 (32.4%).

Table 1: Isolation from different samples.

Clinical samples	No. of isolates (%)
Pus	15(13.5%)
Urine	36(32.4%)
Swab	12(10.8%)
Sputum	24(21.6%)
Blood	7(6.3%)
Tracheal aspirate	12(10.8%)
Catheter tube tips	3(2.7%)
Drain	2(1.8%)

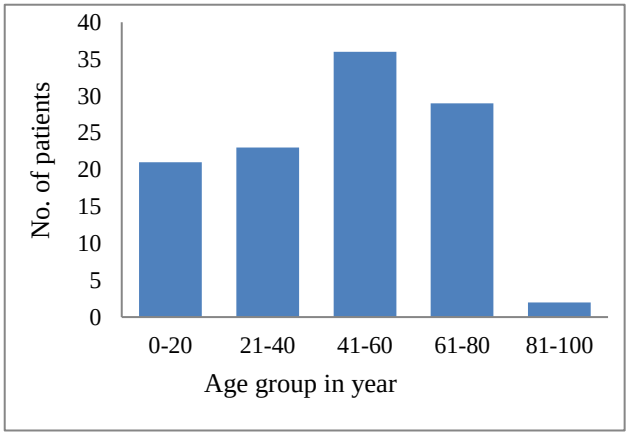

Figure 1: Age-wise distribution of *Acinetobacter* isolates.

Figure 1 shows that *Acinetobacter* infection mainly occurred in population aging between 41-60 yrs. Gender ratio was 1.2:1 (Female:Male) thus, a slight female preponderance was observed in our study.

Highest isolates were isolated from wards 63 (56.7%) followed by OPD and ICU as shown in (Figure 2). giving a probability of increased hospital acquired infections.

Sensitivity pattern of *Acinetobacter species* to different antimicrobials showed higher resistance to cefipime 80.1% and ceftadizime 74.8% and lower resistance was observed to drugs gentamicin 20.7%. Resistance pattern for other drugs was co-trimaxazole-55%, piperacillin/tazobactum 50.5%, ceftazidime and clavulinic acid 61.2%, amikacin 24.3%, ciprofloxacin 45.9%, levofloxacin 44.1%, imipenem 25.3%, meropenem 29.7%, nitrofurantoin 55.5%, norfloxacin 58.3% as shown in (Table 2). None of the isolates was resistant to colistin. Almost all the isolates showed in-vitro resistance to one or more of the antibiotics mentioned earlier.

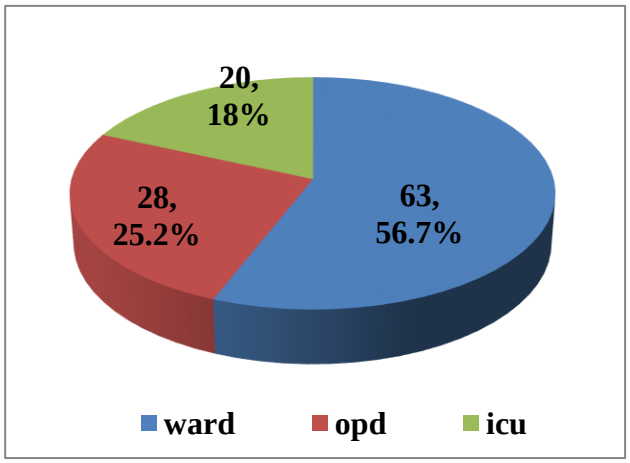

Figure 2: Distribution of isolates from wards, ICU, OPD.

Table 2: Antibiotic resistance pattern of the *Acinetobacter species* isolates.

Antibiotics	Resistant isolates (%Resistance)	Sensitive isolates (%Sensitivity)
Imipenem	28(25.3%)	83(74.7%)
Meropenem	33(29.7%)	78(70.3%)
Piperacillin/tazobactum	56(50.5%)	55(49.5%)
Ceftazidime and clavulinic acid	68(61.2%)	43(38.8%)
Ceftadizime	83(74.8%)	28(25.2%)
Cefipime	89(80.1%)	22(19.9%)
Co-trimaxazole	61(55%)	50(45%)
Gentamicin	23(20.7%)	88(79.3%)
Amikacin	27(24.3%)	84(75.7%)
Ciprofloxacin	51(45.9%)	60(54.1%)
Levofloxacin	49(44.1%)	62(55.9%)
Norfloxacin ((n=36)	21(58.3%)	15(41.7%)
Nitrofurantoin (n=36)	20(55.5%)	16(44.5%)
Colistin	0(0%)	111(100%)

Out of 111 isolates, 69 (62.1%) were MDR (Isolates resistant to resistance to at least one agent in 3 or >3 different classes of antibiotics).[12,13] Maximum resistance was observed in *Acinetobacter baumannii* was found to be more resistant than *Acinetobacter lwoffii*, therefore maximum resistance was observed in ICU isolates followed by Ward and OPD isolates where *A. baumannii* was more prevalent (Table 3).

Table 3: Comparison between antibiotic resistances of *Acinetobacter species* isolated from ICU, Wards and OPD.

Name of antibiotic	Total no. of resistant isolates	ICU (n=20) n (%)	Wards (n=63) n (%)	OPD (n=28) n (%)
Imipenem	28	10(50%)	18(28.6%)	0(0%)
Meropenem	33	11(55%)	17(30%)	5(17.8%)
Piperacillin/tazobactum	56	17(85%)	35((55.5%)	4(14.3%)
Ceftazidime and clavulinic acid	68	16(80%)	47(74.6%)	5(17.8%)
Ceftadizime	83	20(100%)	52(82.5%)	11(39.3%)
Cefipime	89	20(100%)	54(85.7%)	15(53.6%)
Co-trimaxazole	61	18(90%)	37(58.7%)	6(21.4%)
Gentamicin	23	10(50%)	13(20.6%)	0(0%)
Amikacin	27	12(60%)	15(23.8%)	0(0%)
Ciprofloxacin	51	18(90%)	29(46%)	4(14.3)
Levofloxacin	49	15(75%)	28(44.4%)	6(21.4%)
Norfloxacin (n=36)	21	2(100%) n=2	12(63.2%) n=19	7(46.7%) n=15
Nitrofurantoin (n=36)	20	2(100%) n=2	10(52.6%) n=19	8(53.3%) n=15
Colistin	0	0(0%)	0(0%)	0(0%)

DISCUSSION

In our study, from 3715 bacterial isolates, 111 (2.9%) *Acinetobacter* species were obtained. Similar prevalence of 3% and 3.36% of total organisms isolated was reported by Dash et al in Odisha and Gupta et al in Pune.[5,14] Higher prevalence rates of 14% and 9.6% was reported by Mostofi et al, in Tehran, Iran and Joshi et al in Pune.[15,16] In the present study maximum isolates were isolated from wards 63 (56.7%). This is probably related

to increasingly invasive diagnostic procedures used, greater quantity of broad spectrum antimicrobials used and prolonged duration of stay in hospital.[5,6]

The higher isolation rates 36 (32.4%) of *Acinetobacter spp* from the Urine samples are not in agreement with the results reported previously in other studies where higher isolation rates were most often from respiratory samples.[6] According to literature, amongst *Acinetobacter* spp, commonest species isolated in human clinical specimens *is A. baumannii.*[2] We also observed that 109 (98.2%) isolates were *A. baumannii* whereas remaining 2 (1.8%) isolates were A. lwoffii.

In the present study, *Acinetobacter* species were found to be resistant to most commonly used antibiotics. *Acinetobacter* isolates were extremely resistant to Cefepime (80.1%) and ceftazidime (74.8%) which correlates with the studies by Guckan R et al.[17] Resistance to levfloxacin is found less in comparison to other fluoroquiniolones in our study and similar finding was also found by Bhattacharya et al in their study.[18] Resistance towards imipenem and Meropenem was recorded to be 25.3% and 29.7% respectively. A study by Dash et al also reported more resistance towards Meropenem (22%) as compared to imipenem (19%).[5] 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.[5,19]

Out of total isolates 69 (62.1%) 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.[4,6] Bhattacharya et al, Gupta et al, and Mostofi et al, reported MDR isolates to be 29%; 40% and 54% respectively.[18,11,14] Maximum resistance in our study was observed in ICU isolates in comparison to wards and opd. In ICUs most, sensitive drug was colistin (100%) followed by imipenem (50%) and gentamicin (50%). *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. The emergence of antibiotic resistant strains in ICU is because of higher of use of antimicrobial agents per patient and per surface area.[14]

Notably, our findings show that Gentamicin and Amikacin is effective against Acinetobacter spp. showing 20.7% and 24.3% resistance respectively which can be a cost effective therapeutic option against Acinetobacter isolates, especially in this part of India. 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.[20]

CONCLUSION

Acinetobacter is nowadays a common threat in hospital acquired infections especially in critically ill patients admitted to ICU. *Acinetobacter* species in our study were found to be resistant to most commonly used antibiotics. It is a great challenge for the physicians to treat MDR *Acinetobacter spp.* which is independently associated with high mortality, emphasizing the need for aggressive infection control strategies. Emergence of carbapenem resistance is worrisome. Only drug which is sensitive is colistin. 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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