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Original Research Article

Prevalence of *Acinetobacter Species* in Various Clinical Samples and its Antibiotic Sensitivity Pattern in Tertiary Care Hospital, Vadodara

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ABSTRACT

Background: *Acinetobacter species* are Gram negative, non-fermentative bacteria commonly present in soil and water as free living saprophytes. They are isolated as commensals from skin and throat. *Acinetobacter* has emerged as an important nosocomial pathogen involved in outbreaks of hospital infections in hospitalized patients like septicaemia, pneumonia, wound sepsis, endocarditis, meningitis and urinary tract infections. Moreover, most of those outbreaks were caused by multi-drug resistant (MDR) strains of this organisms.

Material and Methods: Various samples like blood, urine, swab, cerebro-spinal fluid (CSF), pleural fluid, body fluid, pus, catheter tip were taken from clinically suspected cases for culture and antimicrobial sensitivity testing. A total of 164 *Acinetobacter species.* were isolated from these samples, which were included in this study.

Results: Out of 6555 culture positive isolates, 164 (2.50%) were *Acinetobacter species.* Out of total 164 isolates, 77% (126 isolates) were *Acinetobacter baumannii* and 23% (38 isolates) were *Acinetobacter lwoffii*. The rate of isolation of *Acinetobacter* was more in males (58%) & in infants (25%). Highest number of *Acinetobacter species* were isolated from the blood (30%) & from the Extramural-NICU (24%). *Acinetobacter* isolates from various samples other than urine samples show highest sensitivity to colistin (100%) & for urine samples, sensitivity to cotrimoxazole, cefotaxime, cefuroxime, levofloxacin and doxycycline was 25%. The prevalence of *Acinetobacter spp* in the present study is 2.50%.

Conclusions: To prevent the spread of the resistant bacteria, it is critically important to have strict antibiotic policies while surveillance programmes for multidrug resistant organisms and infection control procedures need to be implemented.

Keywords: *Acinetobacter Species,* Multidrug Resistant.

INTRODUCTION

Acinetobacter species are Gram negative non-fermentative bacteria commonly present in soil and water as free living saprophytes. They are isolated as commensals from skin and throat.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] Their taxonomy has undergone several modifications, and it is only recently that their harmful role has come to light. A significant nosocomial pathogen that has been linked to hospital infection outbreaks is *Acinctobacter*. Moreover, most of those outbreaks were caused by multi-drug resistant (MDR) strains of this organism.

Acinetobacter has the capacity to endure over a long time period of time on hospital equipments, therefore making multidrug-resistant *Acinetobacter* infection, a great problem in hospital settings.[2]

The commonplace organism has been found in hospital environments, on staff members, or on colonized or infected patients (Hand carriage).[3] *Acinetobacter species* are resistant to many antibiotics because of low permeability of it's outer cell membrane and constitutive expression of certain efflux pump. It can accumulate components of resistance mechanism encoded on plasmid, integrons & transposons in hospital setting associated with high antibiotic consumption.[4]

Because of these organisms' unclear taxonomic position, many physicians and microbiologists continue to underestimate the importance of multi-drug resistant *Acinetobacter* infections, despite their growing significance and frequency. Very few studies on *Acinetobacter species* have been published in India; nevertheless, given their growing significance in nosocomial infections, further research is necessary in this region of the world.[5] Studies regarding *Acinetobacter* pathogen role in various countries have illustrated that the most frequent are urinary and tracheo-bronchial. *A. baumannii* is the second most commonly isolated non-fermenting germ, after Pseudomonas *spp.*[6,7,8]

The treatment of infections caused by *Acinetobacter species*. can be difficult because it has intrinsic resistance to certain antibiotics and can acquire resistance to many others. During the last decade, nosocomial infections caused by multi-drug resistant A.*baumannii* have been reported. For the multi-resistant strains of *Acinetobacter species.* Imipenem and Meropenem are considered the most effective antimicrobial agents. Carbapenems are better against *Acinetobacter* than most other antibiotics, but carbapenemases have begun to emerge in the genus.[6,9,10] Many studies have demonstrated that microbes are vulnerable to sulbactam. The drugs with sulbactam are indicated in the therapy of severe infections produced by *Acinctobacter species.*[11]

In the present study, an attempt is made to know the prevalence of *Acinetobacter species.* in various samples and also to determine their antimicrobial susceptibility.

MATERIAL AND METHODS

This descriptive-cross sectional study was conducted over a period of 10 months from December-2021 to September-2022 in the Department of Microbiology in Medical College Baroda & SSG Hospital, Vadodara, Gujarat, India. The study population comprised of all clinically suspected indoor and outdoor patients. Various clinical samples like blood, pus and wound swabs, urine , body fluids, sputum, endotracheal tube and secretions were collected from the indoor and outdoor patients under aseptic condition in sterile containers and sent to the Microbiology laboratory for culture and sensitivity testing along with the requisition forms filled with relevant clinical details of patients.19,069 samples were received and analyzed at the Medical College Baroda Diagnostic Laboratory, Department of Microbiology, during this time.

A total of 164 *Acinetobacter species* were isolated from these samples, which were included in this study. Every one of these samples was handled in accordance with standard clinical laboratory practices on respective agar plates and incubated aerobically at 37°C in incubator and inoculated blood agar and chocolate agar plates were incubated in candle jar for overnight (18-24hours). Blood specimens incubated overnight at 37°C for 18-24 hours after the early subculture was put up in incubator were sub cultured on respective agar plates. If early subculture showed no growth, then all blood samples were further incubated in incubator at 37°C for 3 consecutive subculture. Blood specimens which received in automated blood culture bottle (BACTEC) is placed into the system location. Any blood culture bottle with red alert/flag in the instrument within 24-28 hours after insertion is consider Positive and manual subculture was done on respective agar plates and incubated. Any blood culture bottle with Green alert/ No flag within 5 days of insertion in instrument is consider negative for bacterial growth and reported Negative. On day 02, all inoculated plates were assessed for the colony morphology of the culture isolates under investigation, specifically *Acinetobacter spp*. The isolates that were non-lactose fermenting and exhibited an alkaline change (K/K) reaction in triple sugar iron agar media were tentatively classified as non-fermenting Gram-negative bacilli (NFGNB), which were subsequently identified using established protocols and biochemical tests, like gram staining, motility, indole test, citrate test, TSI test, MR-VP test, arginine decarboxylase test, nitrate reduction test, cytochrome oxidase test, oxidative fermentative test. [1,12]

Antimicrobial susceptibility of all the isolates was performed by the disc-diffusion (Modified-Kirby Bauer disc diffusion method) according to CLSIs guidelines (CLSI M100)[14]. The following antibiotics were tested by disc diffusion method, gentamicin (10 µg), levofloxacin (5 µg), meropen (10µg), cefepime (30µg), ceftriaxone (30µg), cotrimoxazole (1.25/23.75µg), piperacillin+tazobactum (100/10µg), doxycycline (30 µg). Colistin MIC was detected by using E strip test. For reference following quality control strains were used ATCC *Acinetobacter baumannii* 19606 and ATCC *Acinetobacter lwoffii* 15309.[13,14]

The diameter of the inhibitory zone of growth was measured using millimeter scale or calipers. The zone size was compared with CLSI guideline and interpreted as per Clinical laboratory standard institute (CLSI) guidelines .[13]

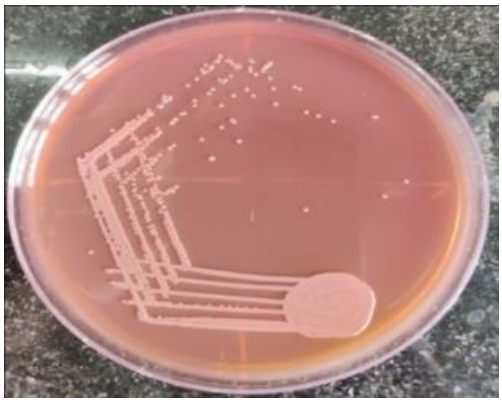

Figure-1: *Acinetobacter species* colony on Macconkey agar

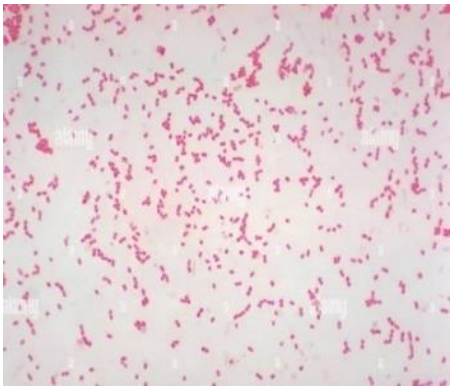

Figure-2: Gram stain of *Acinetobacter species.*

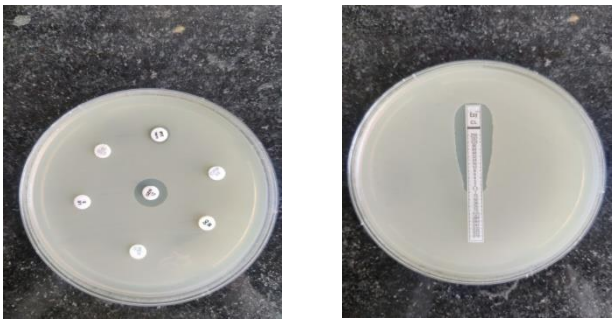

Figure-3: Antibiotic sensitivity test of *Acinetobacter species*

Table-1: Zone diameter interpretive standards for *Acinetobacter species*[14]

Antimicrobial agent	Disc contain	Zone interpretation		
		Sensitive	**Intermediate**	**Resistant**
Gentamicin	10 µg	≥15	13-14	≤12
Levofloxacin	5 µg	≥17	14-16	≤13
Meropenem	10 µg	≥18	15-17	≤14
Cefepime	30 µg	≥18	15-17	≤14
Ceftriaxone	30 µg	≥21	14-20	≤13
Cotrimoxazole	1.25/23.75 µg	≥16	11-15	≤10
Piperacillin+ Tazobactum	100/10 µg	≥21	18-20	≤17
Doxycycline	30 µg	≥13	10-12	≤9
Colistin MIC	0.016-256 µg/ml		≤2	≥4

RESULTS

In the current investigation the total 19069 clinical specimens were processed in the Microbiology laboratory of Medical College Baroda & S.S.G Hospital, Vadodara during the study period of December 2021 to September 2022. Out of which, 6555 culture positive isolates were obtained. Out of 6555 culture positive isolates, 164 were *Acinetobacter species*. Thus prevalence of *Acinetobacter species* in the present study is 2.50%.

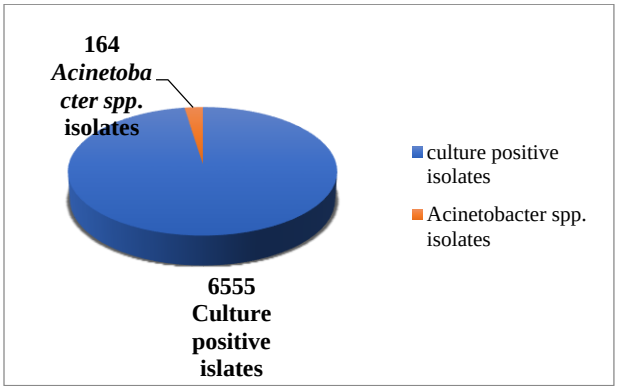

Chart-1: Number of *Acinetobacter species*. Isolates among culture positive isolates

Table-2: *Acinetobacter baumannii* and *Acinetobacter lwoffii* Isolation

Species	Total number of isolates	Percentage (n=164)
Acinetobacter baumannii	126	77%
Acinetobacter lwoffii	38	23%

Out of total 164 isolates, 77% (126 isolates) were *Acinetobacter baumannii* and 23% (38 isolates) were *Acinetobacter lwoffii*.

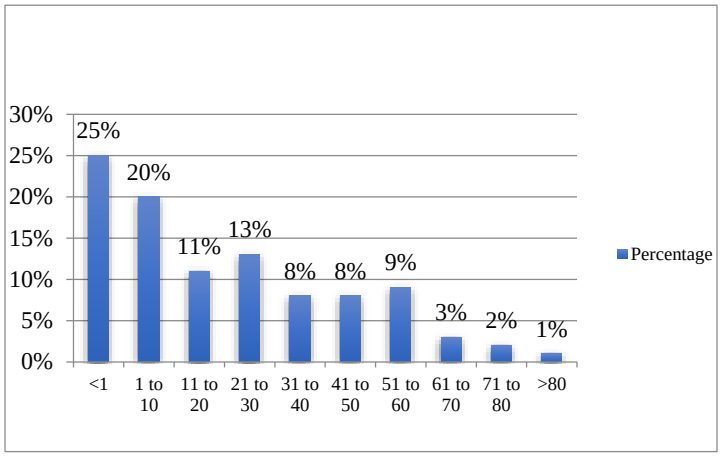

Chart-2: Distribution of *Acinetobacter species* within different age groups

In present study, majority of the *Acinetobacter species* were isolated from infants (25%) followed by age group of 1-10 years (20%), 21-30 years (13%), 11-20 years (11%) followed by other age groups.

Table-3: Patient distribution according to gender with *Acinetobacter* isolates

Gender	Total number of isolates	Percentage (n=164)
Male	95	58%
Female	69	42%

The rate of isolation of *Acinetobacter spp* was more in male (58%) than female (42%).

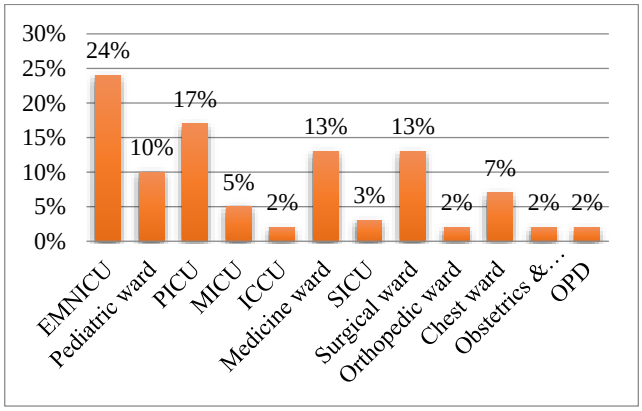

Chart-3: Ward wise distribution of *Acinetobacter species* isolates

Maximum species of *Acinetobacter* identified from the Extramural-NICU (24%) followed by pediatric ICU (17%), medicine & surgical wards (13%), pediatric ward (10%), TB ward (7%), MICU (5%), SICU (3%) followed by others. In present study maximum number of *Acinetobacter species* isolated from Intensive Care Units (51%).

The majority of *Acinetobacter species that were identified for this study came from* the blood (30%) followed by CSF & pus (18%), sputum (11%) followed by other specimens.

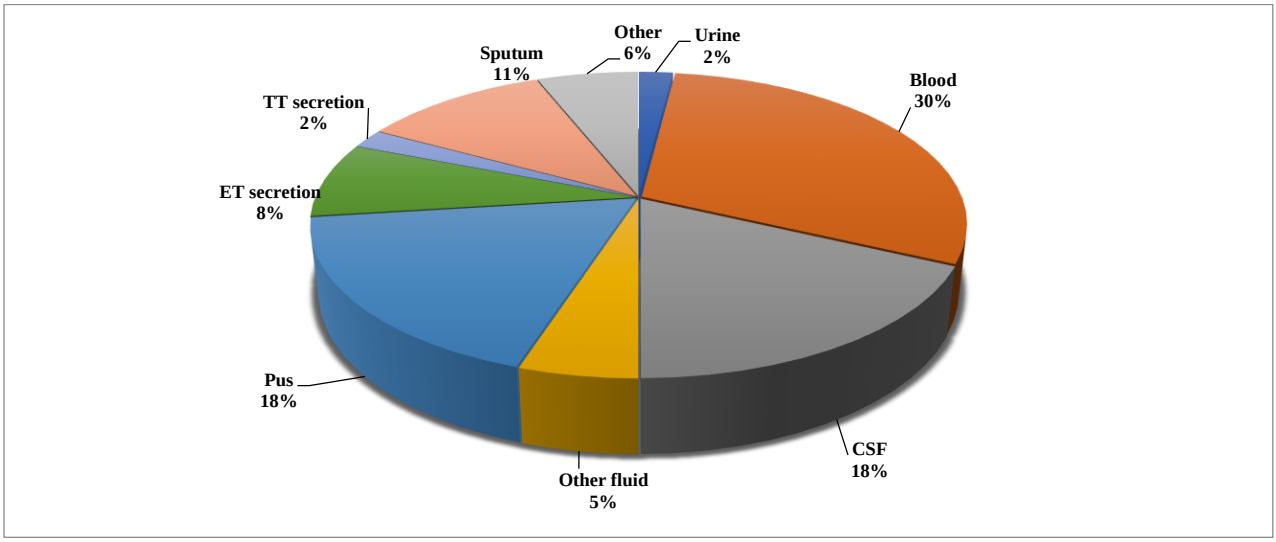

Chart-4: Distribution of *Acinetobacter species* among various types of specimens

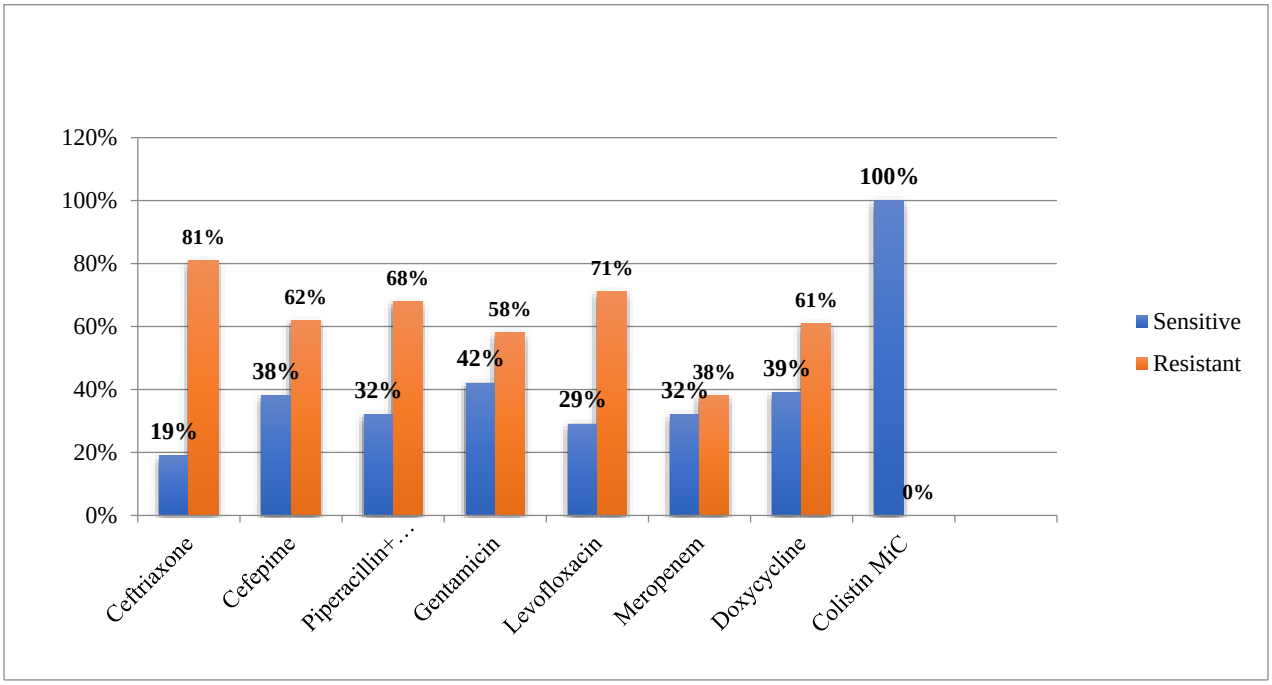

Chart-5: Pattern of susceptibility for *Acinetobacter species* isolates to different antibiotics

In present study, *Acinetobacter* isolates from various samples other than Urine samples show highest sensitivity to Colistin MIC (100%) followed by gentamicin (42%), doxycycline (39%), cefepime (38%) ,meropenem & iperacillin + tazobactum (32%), levofloxacin (29%) & ceftriaxone (19%).

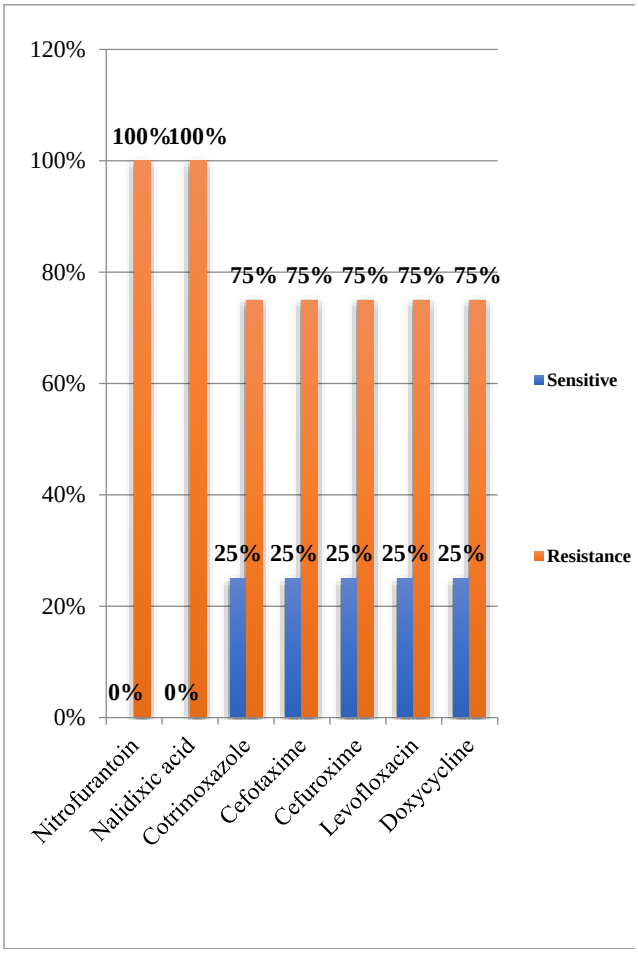

Chart-6: Pattern of susceptibility for *Acinetobacter species* isolates to different antibiotics

In present study, *Acinetobacter* isolates from urine samples show sensitivity to co-trimoxazole, cefotaxime, cefuroxime, levofloxacin and doxycycline (25%) & 100% resistances to nitrofurantoin and nalidixic acid.

DISCUSSION

During routine clinical microbiology work in most laboratories, non-fermentative Gram negative bacilli other than *Pseudomonas aeruginosa* are not taken seriously as a pathogen They are written off as pollutants and not sought out for identification.[15] We started our investigation since we frequently came across NFGNB isolates in a variety of clinical samples based on conventional criteria, *Acinetobacter spp*. were identified from these isolates.[12] Out of the total 164 cases, 77% of isolates were of *Acinetobacter baumannii* and only 23% isolates were *Acinetobacter lwoffii* which was in agreement with the findings of the studies by Saha S *et al* [16], Gupta N *et al*[17],Kamble R *et al* [18],Shridhar S *et al*[19] & Suryawanshi *et al*[20] which *shows Acinetobacter baumannii* was the isolate with the greatest number.

In this study, majority of the *Acinetobacter species* were discovered in infants (25%) followed by age group of 1-10 years (20%) followed by age group of 21-30 years (13%) which was differ from Kamble R *et al*[18] & Saha S *et al*[16], Lone R *et al*[21] & Tadvi J *et al*[22]. Different *Acinetobacter* species were discovered in 0-10 years (29%) & 41-60 years (33%) & >60 years (38.4%),1-10 years (33.69%) respectively.

In present study, the rate of isolation of *Acinetobacter* was more in males (58%) than female (42%). In Kamble R *et al*[18] & Pandya N *et al*[25] similar result found that isolation rate was higher in male (63% & 69% respectively). In Kaur R *et al*[23] study & Saha S *et al*[16] & Rebic V *et al*[24] isolation rate was higher in female which was 67% & 53% & 50% respectively. The majority of *Acinetobacter species* that were identified for this study came from the blood (30%) which was similar to the Kamble R *et al*[18] & McCraken M *et al*[26] study. In Kaur R *et al* [23] study & Saha S *et al*[16]. The greatest amount of *Acinetobacter species* were found in isolation from the urine 26% and 32% respectively while in Mindolli PB *et al* [27], *Acinetobacter species* with the greatest number of isolations came from the pus samples. In Dent LL *et al* [28] study several *Acinetobacter species* were found to be isolated from the sputum (31%). The present study shows that the strains were sensitive to gentamicin (42%) which was lower than Saha S *et al*[16](79%) & Kaur R *et al*[23](52%).
Sensitivity to levofloxacin in present study was 29% which was lower than Saha S *et al*[16](56%) & Kaur R *et al*[23](33%).

Sensitivity pattern for meropenam was 68% in present study which was higher than study done by Kamble R *et al*[18] (45%) and Kaur R *et al*[23] (44%), lower than Saha S *et al*[16] (70%). In present study sensitivity of piperacillin-tazobactam was 32 % which was lower than study done by Kamble R *et al* [18] (54%), Saha S *et al*[16] (49%) & Kaur R *et al*[23] (34%). In present study *Acinetobacter spp* 100% sensitive to colistin which was similar to

other three study Kamble R *et al*[18], Saha S *et al*[16] & Kaur R *et al.*[23] Colistin/ polymyxin B & tigecyclin need to use cautiously to prevent resistance development against them as they are reserved antibiotic.

The major limitations of this study are:

1) Ideal method for colistin MIC is broth micro-dilution which was not carried out in this study.
2) Molecular techniques were not performed to differentiate the various species of *Acinetobacter*.

CONCLUSIONS

Every day, the number of isolates resistant to multiple drugs rises, due to indiscriminate use of these antibiotics in healthcare settings. The best course of action is to minimize and limit the use of antimicrobials to only those circumstances in which they are justified, at the right dose, and for the right amount of time. Traditional typing methods like phenotyping and antibiogram typing have an advantage over genotyping as they are readily available in all clinical microbiology laboratories. Simple identification schemes and antimicrobial susceptibility testing provide a cost effective approach for typing *Acinetobacter spp*. Although above systems have certain limitations when compared to molecular methodologies, the distinction between resistant and susceptible *Acinetobacters* at least, is useful for effective clinical management the illness brought on by this group of organisms.

The genus *Acinetobacter* is becoming increasingly important as a human pathogen due to its high potential for developing antibiotic resistance, which gives it a significant selective advantage in environments where antibiotics are widely and heavily used, particularly in relation to hospital environments and nosocomial infections. This is impressively demonstrated by the overall infections caused by *Acinetobacter spp*. To prevent the spread of the resistant bacteria, it is critically important to have strict antibiotic policies while surveillance programmes for multidrug resistant organisms and infection control procedures need to be implemented.

In the meantime, it is desirable that the pattern of antibiotic susceptibility of bacterial pathogens like *Acinetobacter spp*. in specialist clinical units should be regularly observed and the findings promptly communicated to clinicians in order to reduce resistance. The solution can be planned by continuous efforts of microbiologist, clinician, pharmacist and community to promote greater understanding of this problem. Frequent hand washing to prevent spread of organism should be encouraged. Better surgical and medical care should be provided to patients during hospital stay.

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