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Incidence of multi drug resistant *Acinetobacter* species in intensive care units at a tertiary care hospital

Dr. Udayasri B, Dr. Swati Prakasham

Department of Laboratory Medicine, Yashodha Hospitals, Somajiguda, Hyderabad, Telangana, India

Abstract
Acinetobacter species are nonfermenting Gram negative coccobacilli once considered to be opportunistic pathogens has recently emerged as an important nosocomial pathogens. The purpose of this study is to know the incidence of Acinetobacter species and their antibiotic resistance patterns from various clinical samples collected from patients admitted in Intensive care units at Yashodha hospitals, somajiguda. Over a period of 6 months from June 2016 to November 2016. A total number of 1036 samples were collected from various ICUs. The samples included were endotracheal secretions, blood, sputum, urine and pus. The samples were inoculated on Blood agar, Mac conkey agar and incubated at 37 ºC over a period of 24 hours. Identification of Acinetobacter species were made on basis of phenotypic criteria recommended by Gerner-smidt. (Gram stain, colony morphology, oxidase, catalase and urease production, citrate utilization, gelatin hydrolysis, glucose and lactose fermentation and growth at 42 ºC etc. Antimicrobial susceptibility testing was performed on Muller Hinton agar by disc diffusion method according to CLSI guidelines. A total number of 36 Acinetobacter species were isolated from endotracheal secretions 28(82.5%), followed by urine 2(5.8%), blood2 (5.8%), pus 2 (5.8%), bronchoalveolar lavage 2(5.8%). All isolates were resistant to ceftazidime. High level of resistance was also recorded for cefepime (95%), ciprofloxacin (94%), levofloxacin (94.4%). Resistance towards piperacillin tazobactam was (91%), doripenem was (91%), meropenem was (91%). Minimum resistance towards cotrimoxazole (77.7%), Cefoperazone sulbactam (83.3%), Imipenem (88%). All isolates were sensitive to colistin. Acinetobacter species are emerging as predominant healthcare associated multidrug resistant pathogen in ICUs. Good hospital infection control practices will help to reduce the spread of these organisms.

Keywords: Acinetobacter, multidrug resistant, Intensive care units

1. Introduction
Acinetobacter species are non – fermenting, Gram negative coccobacilli once considered to be an opportunistic pathogen has recently emerged as an important nosocomial pathogen [1]. There are many species in this genus but only three species Acinetobacter baumanii, Acinetobacter calcoaceticus and Acinetobacter lwoffii are associated with clinical diseases [2]. All the three are included under the term Acinetobacter calcoaceticus- Acinetobacter baumanii complex and usually reported as Acinetobacter species [2]. The organism is ubiquitous in nature and often isolated from clinical specimen. Acinetobacter once considered as an opportunistic pathogen of low virulence is being frequently reported from nosocomial infections mostly involving criticall ill patients in intensive care units [3]. Increased risk of Acinetobacter infections in critically ill patients may be due to prolonged exposure to invasive procedure, increased contact with healthcare personnel, mechanically ventilated patients and prolonged ICU stay [1].

The Acinetobacter isolates from these patients showing increased resistance to various groups of antibiotic. The resistance mechanism of Acinetobacter are multiple. They include production of beta lactamases alteration in cell wall channels and efflux pumps by which it becomes resistant to beta lactam antibiotic [3].

Acinetobacter species are showing resistance to aminoglycosides and quinolones due to production of aminoglycoside modifying enzymes and mutation in gene coding for gyr A and par C genes respectively. There fore the management of infections caused by these multidrug resistant Acinetobacter species became a challenge [4]. The success of treatment depends upon the choice of appropriate antimicrobial agent, which inturn is based on prior knowledge of susceptibility pattern of the agent.

The purpose of the present study is aimed to examine the antimicrobial sensitivity patterns of Acinetobacter isolates obtained from various clinical samples collected from patients admitted in intensive care units.

2. Materials and methods
The present study was conducted over a period of 6 months from June 2016 to November 2016at Yashodha hospitals, somajiguda, Hyderabad. A total number of 1036 samples were collected from patients admitted to intensive care units having clinically suspected cases of pneumonia, urinary tract infections, septicemia, skin and soft tissue infections and other infective conditions. All the age groups and either sex were included in the present study. Patients attending out patient departments and wards or rooms were excluded from the study. The samples included were endotracheal secretions, blood, urine, sputum, body fluids, Broncho alveolar lavage and pus. All samples were collected under aseptic conditions as per standard protocols. The samples were inoculated on blood agar and Mac Conkey agar plates and incubated at 37 ^{0}C for 24 to 48 hours. The plates were observed for growth and the suspected colonies of Acinetobacter were identified by standard microbiological techniques. Presumptive identification was done by Purplish hue colonies on Mac Conkey agar, Gram negative coccobacilli in pairs on staining which are Non motile and Oxidase negative. Identification was done by alkaline / alkaline reaction in TSI, Citrate Positive, Oxidative growth in OF test, Growth at 42 °C [5, 6].

All the Acinetobacter isolates were subjected to antimicrobial susceptibility testing by using Kirby- bauer disc diffusion testing as per Clinical Laboratory Standards Institute (CLSI) guidelines. Antimicrobial used were Ceftazidime (30ug), Cefipime (30ug), Gentamicin (10ug), ciprofloxacin (5ug), Levofloxacin (5ug), piperacillin tazobactum (100ug), Cefperazone sulbactum (100ug), dorepenem (10ug), Meropenem (10ug), Imipenem (10ug), cotrimaoxazole (25ug), Colistin (10ug). The zone diameters were mesured and results were interpreted according to Clinical Laboratory Standards Institute (CLSI) guidelines 2016 [7].

3. Results

A total number 1036 samples were collected from various ICUs. In sample distribution the maximum samples obtained were urine (n=387) comprising up to 37%, followed by blood (n=223) comprising up to 22%, followed by ET secretions (n=193) comprising up to 16%. The remaining are swabs 9% (n=96), sterile body fluids6% (n=66), sputum 3.4% (n=36), Catheter Tips 1.73% (18) and Broncho alveolar lavage1.64% (17) (Ref table No1). Out of 1036 samples Acinetobacter was isolated from 36 samples making up to 3.5% of isolation rate. Out of these 36 samples 28 were isolated from ET secretions, 2 from Broncho alveolar lavage, 2 from pus, 2 from urine and 2 from blood (Ref table No 2). Maximum number of Acinetobacter species were isolated from respiratory samples including ET secretions and Broncho alveolar lavage making up to 87.5%.

All these Acinetobacter isolates were resistant to ceftazidime. High level resistance was observed against cefipime and quinolones (94.4%). Resistance towards Piperacillin/ tazobactum was 91%, dorepenem was 91%, Meropenem was 91%. Relatively less resistance was observed towards cotrimoxazole 77.7%, imepenem 88% and cefperazone /sulbactum 83.3%. All the Acinetobacter isolates in present study are sensitive to Colistin (100%). (Refer figure No: 1)

4. Discussion

In the present study 3.5% of Acinetobacter isolates were obtained from different ICU samples and it is low compared to other stydies like Dr Amardeep *et al*, Satnam sing *et al*, in 2014 8.2% [3], Patwardhan *et al* and Dhakephalkar PK *et al*, in 2008 13.2% [8].

In the present study Acinetobacter species were predominantly isolated from respiratory sample comprising up to 27% which is being correlated with Villers D *et al*, Espase E *et al*, in 1998 24.8% [9]. Isolation rate is low Amardeep *et al*, Satnam sing *et al*, in 2014 and Jaggi N *et al*, Sissoda P *et al* and Sharma L *et al*, in 2012 where it is 68% and 60% [10]. The high prevalence in respiratory secretions may be attributed to high risk factors like prolonged intubation and hospitalization, head injury patients and other underlying conditions like CVA and COPD.

In the present study a very low isolation rate of Acinetobacter species was reported from urine samples comprising 0.5%, which is correlating with other studies like Jaggi N *et al*, Sissoda P *et al* and Sharma L *et al*, in 2012 where it is 1% and Amardeep *et al*, Satnam sing *et al*, in 2014 where it is 2%. This shows Acinetobacter is having relatively low prevalence in causation of Urinary tract infections.

In the present study 100% isolates are resistant to ceftazidime which is correlating with Amardeep *et al*, Satnam sing *et al*, in 2014. High level resistance is observed against ciprofloxacin (94.4%), Levofloxacin (94.4%) which is correlating with Rahbar *et al* were Quinolone resistance was (90.4%) [11]. High level resistance was also observed against imipenem (88.8%) and Gentamycin (91.6) which is correlating with Nahar *et al* [12]. Most of the isolates in present study were showing a relatively better sensitivity towards Cefperazone + Sulbactum (20%) and Cotrimoxazole (23%) which is correlating with Amardeep *et al*, Satnam sing *et al*, in 2014 i.e 25%. All the isolates (100%) in the present study are sensitive to Colistin which is correlating with Amardeep *et al*, Satnam sing *et al*, in 2014.

5. Tables and figures

Table 1: Distribution of samples

Sample	Number	Percentage
Sputum	36	3.47%
Urine	387	37.35%
BAL	17	1.64%
Swabs	96	9.26%
ET secretions	193	18.62%
Fluids	66	6.37%
Catheter Tips	18	1.73%
Blood	223	21.50%
Total	1036	100.00%

Table 2: Distribution of Acinetobacter isolates

Sample	Number	Percentage
Sputum	0	0.00%
Urine	2	5.80%
BAL	2	5.80%
Swabs	2	5.80%
ET secretions	28	82.50%
Fluids	0	0.00%
Catheter Tips	0	0.00%
Blood	2	5.80%
Total	36	100%

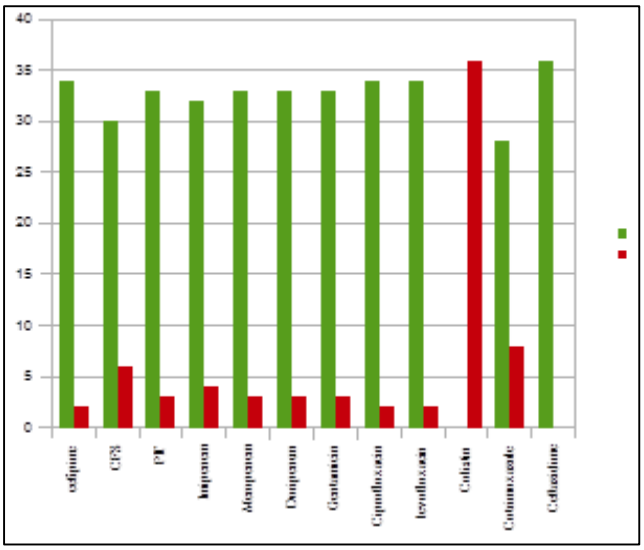

Fig 1: Antibiotic suseptibility pattern of isoletes

6. Conclusion

In conclusion most of the isolates of acinetobacter species in present study from ICUs are often from respiratory samples might be due to various underlying factors which have already been mentioned, as well as the microorgnism can survive in

dry conditions and persist for prolonged period on the surfaces making them as source of infection. Repeated handling by healthcare personnel for suction and other things could be the cause of increased prevalence.

High proportion of antibiotic use in ICU might explain the multi drug resistance observed in theses isolates and, further more carbapenem resistance can be transferred horizontally, so major mode of transmission from one patient to other is through contaminated hands of healthcare providers. This high lights the need for implementation of vigorous infection control program by through cleaning and disinfection and by improving the hand hygiene compliance among healthcare providers. Last but not the least rational use of antibiotics is necessary to prevent microbial resistance.

The data provided by the present study will help our clinician to prescribe empiric antimicrobial therapy. The information on incidence of Acinetobacter species and their resistance pattern will provide base line data, which can guide us in improving hospital infections control measures. Any increase in the number of Acinetobacter species from ICU settings in near future above this base line will give an early alarm either pertaining to out break or break in hospital infection control practices, which can be rectified immediately.

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