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A Cross Sectional Study Characerization and Antimicrobial Susceptibility Pattern of Clinical Isolates of Acinetobacter at a Tertiary Health Care Teaching Hospital, Bhavnagar, Gujarat

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Abstract

Introduction: *Acinetobacter is considered an important nosocomial pathogen because of its intrinsic as well as acquired antibiotic resistance. Hospital acquired infection is most common. The genus Acinetobacter consists of Gram negative, facultative aerobic organisms that are ovoid in shape and may appear as gram negative cocco-bacilli. This study conducted in a tertiary care centre from August 2014 to March 2016.*

Material and Method: *Sample like blood pus and other body fluid are collected from 72 patients into wide mouth open sterile container. Samples processed by microscopy by wet mount and gram stain. Samples cultured on Mac Conkey and Blood Agar media by standard loop method for semi-quantitative culture and incubated aerobically for 24 hrs at 37°C.Antibiotic sensitivity testing by Kirby Bauer method according to CLSI guidelines.*

Result: *All 72 cases observed more commonly in patients with hospital admitted patients. Bacteria isolated included A.baumanii with a prevalence of 88.88%, followed by A. Lwoffii spp 11.11% were also isolated.*

Conclusion: *Risk factor associated with nosocomial infections are associated with duration of hospital stay, more common among patients with catheterization and Mechanical ventilation, Surgical site infection subjects. Further, long term studies investigating the occurrence of complications in patients with XDR and PDR studies for studying the efficacy of antimicrobial therapy in preventing further complications in patients with Hospital acquired infections.*

Keyword: *Acinetobacter Baumanii, Acinetobacter Lwoffii, CLSI- Clinical laboratory standard institute. XDR – Extended Drug Resistance. PDR- Pan Drug resistance.*

INTRODUCTION

In 1911 Beijerinck, a dutch microbiologist working in delft, isolated and described the organism which is now recognized as Acinetobacter (2008)[4]. Brison and prevot proposed the generic designation, Acinetobacter in 1954. In 1971, the subcommittee on Taxonomy of moraxella & accompany bacteria suggested that the genus Acinetobacter shall include only oxidase negative bacteria, non motile, non fermenting, gram negative cocco bacilli.

DNA homology has led to recognition of at least 25 genomo species by various workers[108]. To date only 10 are named by Dijkshoorn L, Nemec A., Seifert H(2007)[29] .

Genomospecies 1: A.calcoaceticus
Genomospecies 2: A.Baumannii
Genomospecies 4: A.Hemolyticus
Genomospecies 5: A.Junii
Genomospecies 7: A.Jonsanii
Genomospecies 8: A.Lwoffii
Genomospecies 12: A.Radioresistens
A.Ursingii, A. Schindleri, A.Venctianus are the others.
The other genomospecies are unnamed.

Acinetobacter species are saprophytic and ubiquitous and can be found in natural environment like soil, water, food and commonly in hospital environment. Acinetobacer is consider as a part of commensal flora of man in groin, axilla and digit spaces. In this parts flora is residing as opportunistic pathogens. Cutaneous colonization can be seen in approximately 25% OF Popultion. 7 % of adults and children shows transient pharyngeal colonization. It is difficult to differentiate between colonization and infection with this organism and hence attribute the exact morbidity and mortality associated with infections due to this organism.

Acinetobacter species are increasingly being recognized as a major pathogen causing nosocomial infections, including Ventilator associated pneumonia, particularly in patients admitted to intensive care units. Carbapenems are often used as last source due to multidrug resistant gram negative bacilli.

Indian study shows that major organism casuing hospital acquired infections are pseudomonas spp, acinetobacter spp, candida spp, Escherichia coli and klebsiella are most common in tertiary care hospital. Among this bugs acinetobacter is a growing problem and widely spread among worldwide.

Carbapenem resistant organism have the ability to rapidly disseminate within institute and may lead to poor patient outcome when infections occurs. Therefore early detection and identification of these multidrug resistant acinetobacter species is of great clinical importance. Monitoring of bacterial infection and drug sensitivity pattern of the organism is important for empirical therapy. Therefore , the purpose of this study was to examine antibiotic sensitivity pattern of Acinetobacter isolates from Blood , pus, sputum, various body fluids and catheters of patients admitted in tertiary care hospital Bhavnagar over an period of August 2014 to March 2016 has been taken in to consideration.

Most reports in india do not report the species involved in human infections and address the infections at only genus level. Speciation of isolates is important in the epidemiology of acinetobacter infections. In view of the increasing challenges posed by this organism in health care institutes, this present study was undertaken to isolate and speciate acinetobacter from clinical samples and to determine their antibiogram.

AIMS AND OBJECTIVES

1. To study HAI and VAP cases and correlation in patients admitted in tertiary care hospital, bhavnagar.
2. To study HAI in control healthy group.
3. To find out various species of Acinetobacter prevalent in & around Bhavnagar city.
4. To find out the antibiotic sensitivity pattern among those isolates.
5. To find out age & sex preference of Acinetobacter infections.
6. To find out the correlation between the duration of stay in hospital and the Acinetobacter infections.

REVIEW OF LITRACTURE

1. Current taxonomy

The genus *Acinetobacter* is characterized by a long history of taxonomic changes. These organisms have been moved from the family Neisseriaceae to the family Moraxellaceae [33]. There are at least 25 different *Acinetobacter* strains which fulfill the criteria to be considered distinct species. These have been identified by DNA-DNA hybridization studies[33]. These studies have also been used to delineate 15 genomic species (gen. sp.) which do not yet have valid names[29]. These genomic species are

commonly labeled by the initials of their authors e.g. Tjernberg and Ursing (TU) or Bouvet and Jeanjean[29].

Acinetobacters are Gram negative coccobacilli, that are strictly aerobic and non-motile (occasionally showing twitching motility)[20]. The organisms exist as bacilli during rapid growth and coccobacilli in the stationary phase and have a tendency of retaining crystal violet, thus may be incorrectly identified as Gram-positive cocci.

It is difficult to differentiate *Acinetobacter* isolates according to their phenotypic characteristics[33,83]. This has led to the use of the term *A. calcoaceticus – A. baumannii* complex[33]. The complex includes genomic species 1 (*A. calcoaceticus*), 2 (*A. baumannii*), gen. sp. 3, and 13TU, which show an extremely close relationship[20]. *A. baumannii* seems to be the species of greatest clinical importance. Repeated isolation of other species from the *A. calcoaceticus – A. baumannii* complex might be significant, especially if clinical symptoms are also present[20/83]. *A. calcoaceticus* is an environmental species that has been recovered from soil and water but has not been implicated in serious clinical disease[83].

2. Species of clinical importance

Acinetobacter spp. may form part of the human skin flora. Not all species of the genus *Acinetobacter* have their natural habitat in the environment. The skin carriage rate of all *Acinetobacter* species can be as high as 75% among hospitalized patients, and up to 25% among healthy individuals[92]. *A. baumannii* and gen. sp. 13TU, on the other hand, were found only rarely on human skin in the study by Seifert et al., which looked at the distribution of *Acinetobacter* spp. on human skin of 40 cardiology patients and 40 healthy controls[92].

Figure 1 below shows the delineation of *Acinetobacter* genomic species.

TABLE 1. Delineation of *Acinetobacter* genomic species

Species	Genomic species[a]	Type or reference strain	Reference(s)
A. baumannii	2	ATCC 19606^T	51, 542
A. baylyi		DSM 14961^T	72
A. bouvetii		DSM 14964^T	72
A. calcoaceticus	1	ATCC 23055^T	51, 542
A. gerneri		DSM 14967^T	72
A. grimontii		DSM 14968^T	72
A. haemolyticus	4	ATCC 17906^T	51, 542
A. johnsonii	7	ATCC 17909^T	51, 542
A. junii	5	ATCC 17908^T	51, 542
A. lwoffii	8/9	ACTC 15309^T ATCC 9957	51, 542
A. parvus		NIPH384^T	393
A. radioresistens	12	IAM 13186^T	51, 401, 542
A. schindleri		NIPH1034^T	392
A. tandoii		DSM 14970^T	72
A. tjernbergiae		DSM 14971^T	72
A. towneri		DSM 14962^T	72
A. ursingii		NIPH137^T	392
"*A. venetianus*"[b]		ATCC 31012	573
	3	ATCC 19004	51, 542
	6	ATCC 17979	51, 542
	10	ATCC 17924	51, 542
	11	ATCC 11171	51, 542
	13TU	ATCC 17903	542
	13BJ, 14TU	ATCC 17905	53, 542
	14BJ	CCUG 14816	53
	15BJ	SEIP 23.78	53
	15TU	M 151a	542
	16	ATCC 17988	53
	17	SEIP Ac87.314	53
	Between 1 and 3	10095	190
	Close to 13TU	10090	190

[a] Unless indicated otherwise, genomic species delineation is according to Bouvet and Grimont (51) and Bouvet and Jeanjean (53). BJ, Bouvet and Jeanjean; TU, Tjernberg and Ursing.
[b] "*A. venetianus*" is found in marine water but does not yet have formal species status.

Figure 1: Delineation of *Acinetobacter* genomic species.
Reproduced from: (Peleg A.Y., 2008)[83]

A. baumannii is the main genomic species associated with nosocomial outbreaks[20] Many reports of infection due to *A. baumannii* however do not include the necessary tests for specific identification to species level, but give a presumptive identification[20]. There is a need for further investigations to define the clinical significance of Acinetobacter species other than *A. baumannii*, because these isolates are often considered as contaminants derived from the environment[20]. However, genomic species 3 and 13TU have been implicated in nosocomial infections and *A. johnsonnii* has been reported to cause catheter related bacteremia. The main sites of infections due to *A. baumannii* are the lower respiratory tract and the urinary tract[20].

3. Epidemiology[2]

Acinetobacter may form part of the skin flora, mostly in moist regions such as the axillae, groin

and toe webs. They have also been isolated from the oral cavity and respiratory tract of healthy adults. The carriage rate in non-hospitalized patients, apart from on the skin, is generally low[20]. Debilitated hospitalized patients have a high rate of colonization, especially during nosocomial Acinetobacter outbreaks. The predominant site of colonization in these patients is the skin, but respiratory tract or digestive systems may also be colonized. The differences between carriage rates between outpatients and hospitalized patients suggest that infecting or colonizing organisms in hospital patients may be acquired from cross-transmission or from hospital environmental sources and is usually not derived from endogenous patient sources[20]. Colonization or infection with multidrug – resistant Acinetobacter is associated with the following risk factors: prolonged hospital stay, admission to an intensive care unit (ICU), mechanical ventilation, and exposure to broad spectrum antibiotics, recent surgery, invasive procedures, and severity of the underlying disease[66].

To investigate the environmental habitat of Acinetobacter, the distribution and frequency of *Acinetobacter* species in a variety of purchased and harvested fresh fruit and vegetables have been studied. Acinetobacter was isolated in 17% (30 of 177) samples of the produce. *A. baumannii* complex formed 56% of all isolates from cucumbers, peppers, mushrooms, lettuces, potatoes, corns, cauliflowers, radishes, mushrooms, melons, cabbages, apples, and beans. According to this study hospital food could be a natural habitat and a source for *A. baumannii* acquisition and subsequent colonization of the digestive tract of hospitalizedpatients[21]

A. baumannii has also been isolated from wounds of injured American and British soldiers from Afghanistan and Iraq. These strains were multidrug-resistant and mostly were part of polymicrobial infections[78]. The sources for these infections were unknown, but it was suggested that prolonged environmental contamination of military field hospitals played a role as *Acinetobacter* species can survive in both moist and dry environments [38]. Interestingly, in a study done in France, *A. baumannii* strains were isolated from body lice of homeless people. The researchers demonstrated that body lice were vectors of A. *baumannii*. This indicated that *A. baumannii* was epidemic in human body lice. *A. baumannii* association with body lice is likely due to undiagnosed transient *A. baumannii* bacteremia in people infested with body lice[57].

A review article by R B Patvardhan[105] provided examples of locations in the hospital environment where Acinetobacter has been isolated. Common sources for this organism included ventilator tubing, suction catheters, humidifiers, containers of distilled water, urine collection jugs, intravenous nutrition, multi-dose vials of medication, potable water, moist bedding articles, and inadequately sterilized reusable arterial transducers[105] .In an outbreak of Acinetobacter infection in burns patients, wet mattresses served as environmental reservoirs of Acinetobacter[89]. Contaminated bedding materials may play an important role in the nosocomial spread of these organisms[20]. Medical equipment can get contaminated through contact with both the patients and staff during handling. Therefore hospital staff may be responsible for contaminating equipment if they do not adhere to infection control measures. In respiratory ICUs, respiratory equipment can be a source of persistent outbreaks due to inadequate decontamination after use[20].

Acinetobacter has an ability to persist in the hospital environment, thus are able to cause extended outbreaks [20]. In one outbreak, the presence of airborne *Acinetobacter* species was demonstrated by settle plates[16]. The source of these organisms was probably the skin of infected or colonized patients, and/or contaminated fomites, e.g. bed linen and curtains. Airborne Acinetobacter produces extensive environmental contamination, and was found to persist in the environment for up to 13 days after patient discharge[16]. Thus, there is a possible interchange between patients, hospital

staff and inanimate items, allowing the survival of nosocomially important pathogens [36].

Acinetobacter differ from other gram negative bacteria in that they spread easily in the environment surrounding infected or colonized patients. In an in vitro study it was shown that the ability of *A. baumannii* strains to survive under dry conditions varied greatly[103]. This ability correlated well with the source of the strain. Those strains which were isolated from dry sources tended to survive longer than the ones derived from wet sources [103]

4. Pathogenesis of *Acinetobacter* infections

Acinetobacters cause opportunistic infections because of limited number of virulence factors and are thus considered as low grade pathogens [20]. Recently, there have been a number of case-reports of fulminating community – acquired pneumonia which indicated that these organisms may sometimes be highly pathogenic and cause invasive disease[47]. There are certain characteristics of this organism that can enhance its virulence. These include the presence of a capsular polysaccharide which makes the organism to be hydrophilic, the ability to adhere to human epithelial cells in the presence of fimbriae and/or capsular polysaccharides, the production of lipases which can damage tissue lipids and the presence of cell wall lipopolysaccharide and lipid A which are potentially toxic[20]. The lipopolysaccharide causes resistance to complement in human serum and acts synergistically with capsular exopolysaccharide[47]. Little else is known about Acinetobacter's lipopolysaccharide endotoxigenic potential in humans. The capsule is a major virulence factor and is presumed to protect bacteria from host defenses[47]. Quorum – sensing as a widespread regulatory mechanism in gram negative bacteria has been found in clinical isolates of Acinetobacter. It might be a central mechanism for auto-induction of multiple virulence factors in Acinetobacter [47].

Mixed infections involving Acinetobacter and other bacteria are more virulent than infections with *Acinetobacter* species alone[20]. *Acinetobacter* species have the ability to obtain the necessary iron for growth in the human body. This is also an important virulence determinant[20]

5. Clinical manifestations of *Acinetobacter baumannii* infections

a. Nosocomial bloodstream infections

The major and frequent manifestation of infection caused by *A. baumannii* is bacteremia, followed by respiratory tract and surgical wound infections. During a nationwide, concurrent surveillance study done in the USA (1995 – 2002), to examine trends in the epidemiology and microbiology of nosocomial bloodstream infections, *A. baumannii* was the 10th most common etiologic agent. This organism was responsible for 1.3% of all monobacterial nosocomial bloodstream infections (0.6 bloodstream infection per 10 000 admissions).The mean interval between admission and infection was 26 days for *Acinetobacter* species and most of theinfections were in patients admitted in intensive care unit. *A. baumannii* bloodstream infection had a crude mortality rate of 34% - 43.4% in ICU, and 16.3% in the general wards. *Pseudomonas aeruginosa* (crude mortality - 38.7%) and Candida species (crude mortality - 39.2%) were the only organisms with crude mortality rates above *A. baumannii* (crude mortality 34-43.4%) in ICU patients.

b. Nosocomial pneumonia

There is a persistent seasonal variation in the rate of Acinetobacter infection. This variation tends to increase in late summer for all major infection sites[68]. Presently the most important role of Acinetobacter is as a cause of nosocomial pneumonia, mostly following the use of mechanical ventilation in ICU patients[47]. The role played by *Acinetobacter* species in ventilator associated pneumonia (VAP) appears to be increasing [20]. An increase from 0.64% to 6.4% in the incidence of nosocomial pneumonia due to Acinetobacter between 1976–1990 has been reported in a surveillance program of the Nosocomial Infections Surveillance (NNIS)

System in the USA which involved adult and pediatric patients[68].

Today, there are many major advances in the management of ventilated patients and there is routine use of effective procedures to disinfect respiratory equipment. These have not affected the increased incidence of VAP due to Acinetobacter [20]. Although it is often very difficult to distinguish upper respiratory tract colonization from true pneumonia, ventilator-associated pneumonia due to *A. baumannii* does occur[80]. The acquisition of *A. baumannii* infection in the ICU is associated with a high APACHE II score, cardiovascular failure, respiratory failure, previous infection, previous antibiotic therapy, use of mechanical ventilation and the presence of a central venous or urinary catheter[62].

The prognosis associated with nosocomial pneumonia is considerably worse than that due to other Gram- negative or Gram-positive bacteria, except for *Pseudomonas aeruginosa*[20]. Acinetobacter nosocomial pneumonia is a severe disease in ventilated patients. It is not easy to ascertain whether such critically ill patients would have survived if nosocomial pneumonia had not occurred[20]. Fagon et al. looked at the extent to which nosocomial pneumonia increased mortality and hospital stay in ventilated patients by performing a matched retrospective cohort study in a Paris hospital[31]. The authors diagnosed pneumonia by use of quantitative culture of samples from protected specimen brush and observation of intracellular organisms from bronchoalveolar lavage. They were able to match cases and controls for confounders like severity of underlying illness, age and reason for ventilation. VAP caused by *Pseudomonas* or *Acinetobacter* species was associated with considerable mortality in excess of that due to the underlying disease alone [31]. The mortality attributed to Acinetobacter or Pseudomonas infection exceeded 40%, with a relative risk of death of 2.5. There was also a significantly prolonged hospital stay in the ICU by more than 10 days in patients diagnosed with pneumonia[3]

c. Traumatic battlefield and other wound infections

Injured and ischemic tissue in trauma patients facilitates colonization with *A. baumannii*[75]. *A. baumannii* has been isolated from wounds of war casualties from Iraq and Afghanistan. In one study *A. calcoaceticus-baumannii* complex formed 32.5% of initial wound cultures. It did not appear to directly contribute to any substantial morbidity (viz. persistent nonunion or amputation), thus signifying that this organism is of low pathogenicity in wound infections[46]. Gunshot wounds and external fixation tend to be associated with increased risk of Acinetobacter infection[82].

Most Acinetobacter infections in war casualties are caused by highly antibiotic-resistant strains. These infections occur in critically ill patients with severe traumatic injuries. These organisms are acquired through nosocomial transmission in field hospitals [87]. Murray et al.[71] found that *Acinetobacter* species were not isolated from wounds immediately after or soon after injury from casualties who were treated at a US Military field hospital in Iraq. In a study by Petersen et al[82], which looked at trauma related infections in Iraqi war casualties, Acinetobacter followed by Pseudomonas species, and *Escherichia coli* were the most common wound isolates. Environmental contamination and transmission of organisms within healthcare facilities seem to play a significant role in acquiring Acinetobacter wound infection[87].

The circumstances of combat are extremely challenging[106]. Infection control measures such as cohorting, isolation and even proper hand washing techniques are very difficult, especially in mass casualty situations. This leads to ongoing colonization, and at the end, to wound infection[106]. Complicated soft tissue and bone infection may follow. An increase in the rate of osteomyelitis caused by Acinetobacter was described in soldiers stationed in southwest India[106]. Traumatic wounds related to natural disasters may also involve Acinetobacter. *A. baumannii* has been isolated from traumatic

wounds sustained during an earthquake in Marmara, northwest of Turkey[75].

d. Urinary Tract Infections (UTI)

In many cases *A. baumannii* isolated from respiratory secretions and urinary tract specimens collected from hospitalized patients signify colonization rather than infection[33]. Most infections due to this organism are from organ systems with a high fluid content, e.g. respiratory tract, peritoneal fluid and the urinary tract. These infections are associated with indwelling devices[33]. *A. baumannii* is not usually implicated in uncomplicated UTI in healthy outpatients[80]. In a study looking at trends in Gram-negative pathogen distribution in ICUs (1986 – 2003)[35], *Acinetobacter* isolates comprised 1.6% of pathogens associated with UTIs in the ICU. Investigators in Spanish hospitals looked at 206 patients colonized or infected with *A. baumannii*. UTIs constituted 23%, second only to respiratory tract infections (39%)[85]. *A. baumannii* UTIs tend to show seasonal variability[33]. The reason for the seasonal variability is unknown, but was observed also in a study done by McDonald[68].

e. Meningitis

There is a steady increase in cases of nosocomial, post-neurological surgery *A. baumannii* meningitis [80,52]. Community-acquired meningitis due to *A. baumannii* on the other hand is very rare[52]. Patients with post-neurological surgery central nervous system (CNS) infection tend to be young, acquire the infection in hospital, commonly have no severe underlying diseases, and have a slow clinical course[63]. Mortality due to Acinetobacter meningitis has been cited to be 23%[90]. In this case series, patients were predominantly adult males and the most significant risk factor was the presence of a continuous connection between the ventricles and the external environment. The median time to develop Acinetobacter meningitis following a neurosurgical procedure was 12 days (range 1-40 days)[90]. These types of infections can be prevented by maintaining a closed drainage system together with timely removal of the ventricular catheters. Furthermore, the selective pressure of the antibiotics used in the neurosurgical ICU favors the growth of multi-drug resistant Acinetobacter.

Pseudomeningitis may occur, where the CSF culture is positive for Acinetobacter in the absence of clinical and laboratory features of meningitis [52]. Contamination of the CSF may occur during specimen collection as this organism is increasingly prevalent in the hospital environment and may colonize the skin. The specimen may also become contaminated due to contaminated specimen tubes and in the laboratory, contaminated pipettes and media. In a study by Chen[23], lumbar puncture derived *Acinetobacter* isolates were more clinically insignificant than those obtained from previously placed ventricular drains. Differentiating between clinically significant and insignificant isolates enables clinicians to avoid unnecessary antibiotic treatments and helps with timely and accurate treatment of infected patients[23]. Most significant cases are associated with neurosurgical procedures [23,52]. With regard to clinical signs and symptoms in Acinetobacter meningitis, fever was the most common presentation in a study by Siegman-Igra[90]. Neck stiffness and other symptoms suggestive of meningitis were frequently absent. In the study by Chen *et al*[23], the absence of fever, meningeal signs and seizures correlated with the isolation of insignificant CSF Acinetobacter isolates. Most cases of Acinetobacter meningitis (20 – 50%) were found to be polymicrobial[90,23]

f. Other clinical presentations

There are a limited number of case reports of Acinetobacter endocarditis in the literature. The precise species identification remains an issue in these case reports[81]. Cases reported in the literature involved both native and prosthetic valves [95,74,39]. Risk factors for gram negative infective endocarditis are diabetes mellitus type I, endoscopy of the gastrointestinal or genital tract,

patients with congenital heart diseases, dental surgery, and patients with right-sided endocarditis[56]. Any breach of the integument can lead to Acinetobacter seeding of a heart valve [39]. A maculopapular rash involving the palms and soles has been reported in cases of Acinetobacter endocarditis. Splenomegaly seems to be a common, but not a dominant feature of Acinetobacter endocarditis [39]. The prognosis of Acinetobacter prosthetic valve endocarditis (PVE) has been more favorable than PVE due to other pathogens. This might be due to the low virulence of *Acinetobacter* species[74].

Acinetobacter can cause ulcerative keratitis and corneal ulcers. These infections may be related to the use of contact lenses or follow eye surgery [51/28]. There is an association between high levels of contamination of contact lenses with Acinetobacter and occurrence of adverse responses[28]. Acinetobacter are not regarded as normal flora, but there is a small proportion of the general population that carries low numbers of this organism on their skin. Acinetobacter causing eye infections may have been transferred to the eye by the hands or from the hands to the contact lens[28].

There was a single case report about a Shiga toxin-producing *A. haemolyticus* strain from Uruguay [41]. This involved a 3-month old baby who presented with bloody diarrhea of 12 hours' duration without pyrexia or other previous illnesses. Fecal samples were inoculated onto MacConkey sorbitol plates. All sorbitol negative colonies were recovered after 48h of incubation. These were then analyzed by PCR to detect the presence of shiga-toxin 1/ shiga-toxin 2(Stx 1/Stx 2) – encoding organisms. The presence of *stx2* –related sequence was then confirmed by PCR. A specially designed PCR suggested that the Shiga toxin genes of *A. haemolyticus* were carried in an infective bacteriophage. The usual enteropathogenic pathogens were not detected from the patient's stool samples[41].

6. Laboratory identification.

Acinetobacters are non-fastidious organisms that grow well on common laboratory media [20]. Clinical isolates, mostly *A. baumannii*, gen spp. 3, and 13TU, grow at 35 - 37 ˚C or higher, whilst some other genomic species grow only at lower temperatures. Most *Acinetobacter* strains can grow in a simple mineral medium containing a single carbon and energy source[20]. Some *Acinetobacter* species outside the *A. Calcoaceticus- A. baumannii* complex may not grow on McConkey agar. Some species may show hemolysis on sheep blood agar (e.g. *A. haemolyticus*). Members of the *A. calcoaceticus – A. baumannii* complex are never hemolytic on sheep blood agar. However, there is no single metabolic test which enables unambiguous identification of *Acinetobacter* species from other similar bacteria[83].

7. Antimicrobial Susceptibility Testing[14]

Antibiotic susceptibility testing of the isolates are performed by Kirby-Bauer disc diffusion method. The antibiotic concentration of disc used and zone size interpretation is in accordance with Clinical Laboratory Standards Institute (CLSI).These drugs are Ampicillin-Sulbactum, ceftriaxone-Sulbactum, tobramycin, ceftazidime ,ciprofloxacin, pipracillin-tazobactum, ofloxacin, gentamycin and amikacin. Commercially available six mm disks are used. All antibiotics were used for sensitivity testing only for gram negative bacteria as per Clinical Laboratory Standards Institute (CLSI) guidelines.

IDENTIFICATION BY AUTOMATED SYSTEMS

Precise species identification of Acinetobacter is not necessary in the routine clinical laboratory. The term *A. baumannii* group is sufficient for laboratory diagnosis. Exact strain identification may be required for epidemiologic purposes to identify strain relatedness. Various methods are available for molecular typing of strains for epidemiological purposes. There are also molecular methods which have been validated for

the identification of Acinetobacter. Examples of these molecular methods are: amplified 16S rRNA gene restriction analysis, high resolution fingerprint analysis by amplified fragment length polymorphism, ribotyping, tRNA spacer fingerprinting, restriction analysis of the 16S – 23S rRNA intergenic spacer region and sequencing of the *rpoB* gene. All the above mentioned methods are too labor intensive to be used routinely in the clinical microbiology laboratory[83].

Manual and semi-automated commercial identification systems are currently being used for species identification in the clinical microbiology laboratory. Examples are the API 20NE, Vitek 2, Phoenix, and Microscan Walk Away systems. The problem with these systems is their limited database content and the fact that they use identification substrates which have not been tailored specifically for Acinetobacter identification[83]. All the currently available commercial methods cannot differentiate clinically relevant members of the *A. calcoaceticus – A. baumannii* complex. *A. baumannii*, Acinetobacter genomic species 3, and Acinetobacter genomic species 13TU are uniformly identified as *A. baumannii*. It is thus advisable to use the term *A. baumannii* group instead of *A. calcoaceticus – A. baumannii* complex when referring to these species. The distinction between *A. baumannii* group and *Acinetobacter* spp. outside the *A. baumannii* group has important infection control implications. Acinetobacter spp. outside the *A. baumannii* group rarely causes nosocomial outbreaks and therefore do not necessitate infection control measures[83].

DNA – DNA relatedness is used to classify *Acinetobacter* isolates into genomic species. The different DNA hybridization methods which have been employed are the nitrocellulose filter method, the S1 endonuclease methods, the hydroxyapatite method and a quantitative bacterial dot filter method. The latter method is the simplest, with the others being more labor intensive and not suitable for routine microbiological use[20]. The DNA – DNA hybridization method is the gold-standard among the few validated methods for identification of *Acinetobacter* species[83].

METHODOLOGY

Study area and period

A study conducted, in a tertiary care hospital attached to a teaching Institution, to study the occurrence of Acinetobacter infection among patients admitted and treated at tertiary health care institute , Bhavnagar.

The study was conducted from August 2014 to March 2016 in Sir Takhtsinhji General Hospital, Bhavnagar, Gujarat.

Source population

Patient was randomly selected child, adult to old patients (0-85 years) diagnosed with Acinetobacter infection and presenting to inpatient department of tertiary health care Institute were included in the present study.

Sample size

This study comprised of 72 patients diagnosed with acinetobacter infections.

Inclusion criteria for Acinetobacter cases:-

- Patients coming to inpatient department of Sir T Hospital, Bhavnagar and resides in health care for more than 48 hours.

Exclusion criteria for Acinetobacter Cases:-

One or more combination of the following

- Asymtomatic patients
- Outdoor patients department.
- Stool samples
- Patients who had already taken antibiotic before sample taken

Selection of type cases/subjects:

A detailed history was taken with emphasis on age, sex, occupation and duration of symptoms, socio economic status, personal hygiene were recorded. Latest reports all cases were collected from the patient. The samples were collected at inpatient department of medicine at Sir T Hospital, Bhavnagar, after explaining in detail about clean catch sample to the patient.

Specimen collection

Patient was asked to collect sample in a wide mouth sterile container. In case of Blood in Hartley's Broth and for Pus in sterile swab stick and all other samples were collected in sterile wide mouth container.

Transporting the Specimen:

The sample was transported to microbiology laboratory with in 1 hrs so that overgrowths of bacteria do not occur.

Direct smear Examination[11]

Smear was prepared from sample, stained by gram stain technique and examined under oil immersion lens for presence of bacteria and pus cells.

Culture[10]:

Within 2 hrs of Sample collection, culture was done by strict aseptic universal precautions on Mac Conkey and blood agar media using standard measured wire loop technique. Plates were incubated for 24 hrs under aerobic condition. After 24 hrs of incubation all samples showing colony appearance of 4 same type was considered to be significant for processing. Those colonies developed on Mac Conkey media was further preceded by gram stain, motility check by wet mount preparation and biochemical reaction and then antibiotic sensitivity test on Muller Hinton agar by Kirby-Bauer's disc diffusion method following Clinical Laboratory Standards Institute (CLSI) guidelines. Results were not considered for more than three clinical isolates obtained on isolation and the sample was considered to be contaminated[14].

Identification of organism:

Colony appears as smooth, small, dome shaped, pale with pinkish tinge on Mac Conkey media and Creamy white, smooth on Blood agar[5], they were preceded for gram stain. By help of gram stain colonies were divided into two groups and further tested for biochemical reaction as per standard guideline for identification[5].

Figure: Mac Conkey agar Shows smooth colorless colony with dome shaped appearance Gram Stain Preparation [11]

In Gram stain Acinetobacter appear as Gram negative cocco-bacilli

Biochemical Reactions[12]:

As Acinetobacter is Gram negateve cocco-bacilli, were further tested for oxidase test, indole production, methyl red test, citrate utilization, urease test, Triple sugar iron test and OF (glucose) test for identification[12]

Biochemical reaction of Acinetobacter species

Test	A.Baumanii	A.lwoffii
TSI	K/K	K/K
Citrate test	+Ve	-Ve
Urease	Variable	-Ve
Indole	-Ve	-Ve
MR	-Ve	-Ve
Glucose	+Ve	-Ve
Galactose	+Ve	-Ve
Rhamnose	+Ve	-Ve
Lactose	+Ve	-Ve
Maltose	Variable	-Ve
OF Test	Oxidative & Non-fermentative	Non-oxidative & Fermentative

All Media were used in this study are Readymade Dehydrated base from Hi Media, Laboratories Ltd; India, was prepared as per manufacturer's instructions.

Antimicrobial Susceptibility Testing[14]

Antibiotic susceptibility testing of the isolates was performed by Kirby-Bauer disc diffusion method. The antibiotic concentration of disc used and zone size interpretation was in accordance with Clinical Laboratory Standards Institute (CLSI). Drugs used and their concentration are Ampicillin-Sulbactum (20:15mcg), ceftriaxone-Sulbactum(30:15mcg), tobramycin (20mcg), ceftazidime (30mcg), ciprofloxacin (5mcg), pipracillin-tazobactum (100:10mcg), ofloxacin (5mcg), gentamycin (10mcg) and amikacin (30mcg). Commercially available six mm disks (Himedia Laboratories, Mumbai) were used. All antibiotics were used for sensitivity testing only for gram negative bacteremia as per Clinical Laboratory Standards Institute (CLSI) guidelines.

Quality controls[11] :

Standard operational procedures were followed with sterile precaution during processing of each

sample. All the instruments used for sample processing were checked every day for proper functioning before processing. *Pseudomonas Aeruginosa* ATCC 27853 was used as a reference strain.

Observation and Results

Figure 1: Age distribution of Acinetobacter cases

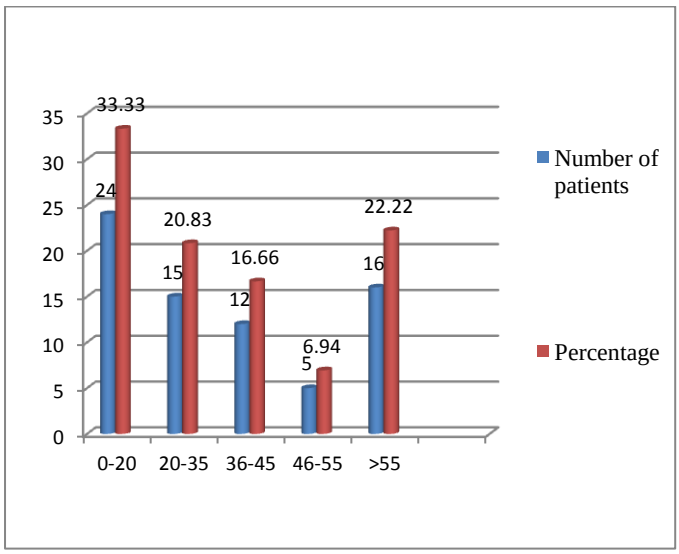


Figure – 2: Sex distribution in Acinetobacter cases

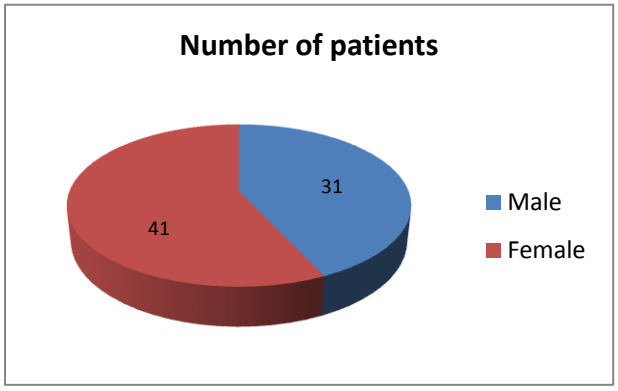
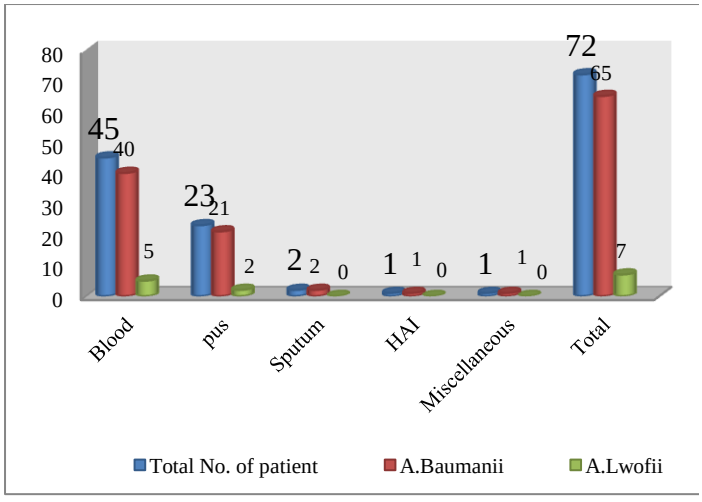


Figure – 3 sample wise distribution of Acinetobacter species



Figure–4: Species of Acinetobacter found in male and female.

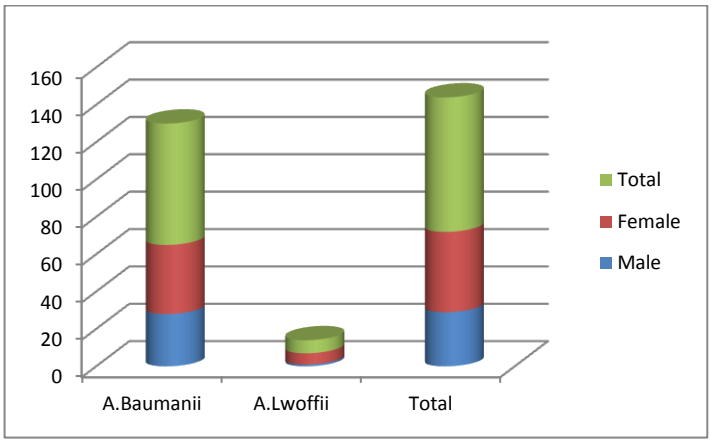


Figure 5: Result according to Hospital Stay group:-

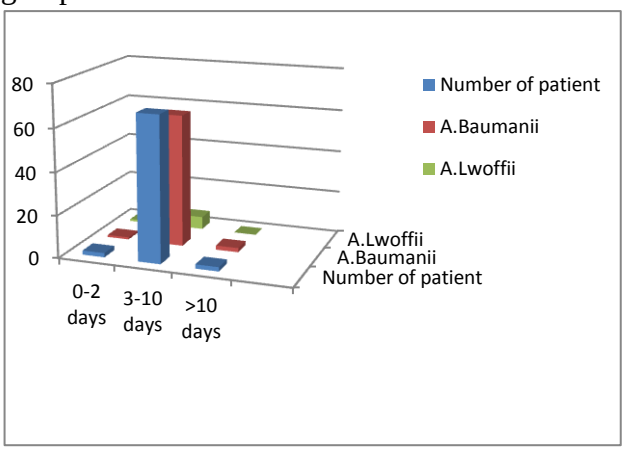


Figure – 6: Distribution according to Hospital Area

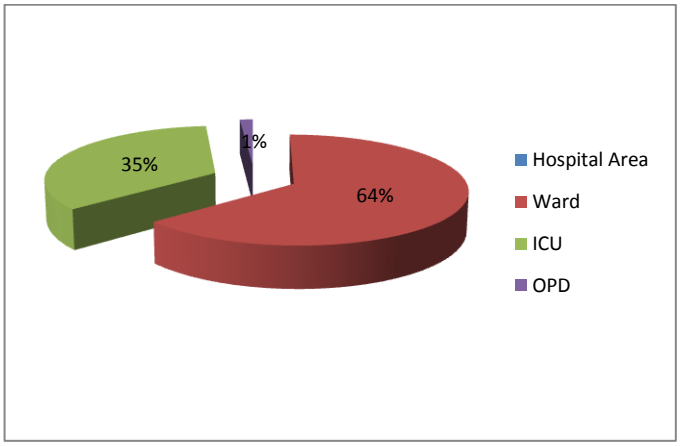
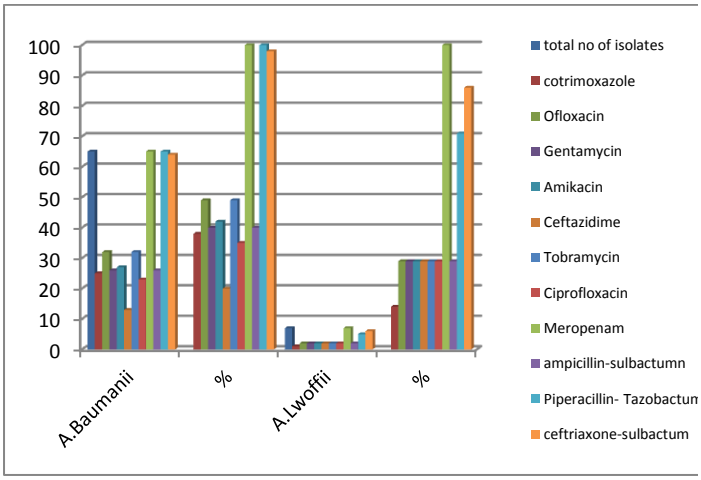


Figure – 7: Antibiotic sensitivity pattern of various clinical isolates of Acinetobacter



DISCUSSION

In present study 72 non duplicate, non Consecutive clinically diagnosed cases of Acinetobacter were studied during 20 months duration period from August 2014 to March 2016. Out of total 72 samples, 90% (65cases) is *A.baumannii* and 10%(7 cases) is of *A.Lwoffii*.

A.baumanii was the most common species isolated followed by *A.Lwoffii*. *A.baumannii* is among the most common of multidrug resistant clinical isolates in the Asia, Europe and India and is a major threat moving forward. It has already been notified by Infectious Disease Society of America as a "red alert" pathogen. Larson et al 1986[82] shows that *Acinetobacter* was most common gram negative organisms carried on the skin of hospital personnel.

Age wise distribution of Acinetobacter Cases :

In the present study, incidence was seen to be highest in age group 0-20 years with total 24 cases out of total 72 cases. In Which 16 isolates were in < 1 Month child group.

Study	Age group
Dr Rahul Kamble et al[102],Mumbai,India.	0-10
Mustaba Moosavian et al[88] , Iran	0-10
Dr Azinun nahar et al[86],Dhaka,Bangladesh	0-10
Dr Deveshni Reddy et al[103], Durban,South africa	0-10
Present study(2016)	0-10 year

Dr Rahul Kamble et al[102], Mumbai, 2015 also shows similar result of higher incidence in age 0-10 year (29.1%) patient. The next highest incidence was in group 20-30 years (20.4%). The lesser of age showed the Highest incidence of Acinetobacter infections. The findings are consistent with other studies. Other studies have reported Acinetobacter infection more common in pediatric age group.

Sex wise distribution of Acinetobacter cases

In the present study, Male to female ratio was 1:1.32 in our study. females (56.94%) were more commonly affected than males (43.05%). Review article was published in IJCM in 2015, by Dr Rahul Kamble et al[102], Mumbai,2015 also mentioned that chances of Acinetobacter infection are more common in Female (55.30%), is comparable to our study result 56.94%.

Most common species isolated:

A.baumannii was the commonest clinical type 65 (90.18%) .

This finding is consistent with other studies in India and abroad.

Studies like study Dr Rahul Kamble et al[102],Mumbai,India, ;Dr Namita Jaggi et al[104] , Gurgaon, Haryana,Dr Devanshi Reddy et al[103], Durban,South africa Showed *A.baumannii* being most common organism with incidence of 87.2%, 98% and 85%, respectively.

Duration of hospital stay:

As from our study, it is shown that longer duration period of hospital stay increase chance of colonization of bacteria because of instrumentation and hospitalization, which is more frequent in younger peoples. It is comparable to studies done by Dr Namita Jaggi et al[104] & Dr Rahul Kamble et al[102],Mumbai,India.

Antibiotic sensitivity pattern:

In present study acinetobacter spp. are highly sensitive to Meropanam, Pipracillin-tazobactum, and Ceftriaxone-Sulbactum, while more resistant to Ampicillin-Sulbactum, Ceftazidime, Amikacin, Gentamycin and tobramycin. The sensitivity pattern are similar to other studies like Dr Rahul Kamble et al[102] ;Dr Azinun nahar et al[108], Dhaka,Bangladesh ;Dr Namita Jaggi et al[104] , Gurgaon, Haryana, Dr Devanshi Reddy et al[103], Durban, South Africa, for higher drugs which are more sensitive. In present study susceptibility to

Ampicillin-Sulbactum, Clotrimoxazole,Amikacin,Ciprofloxacin,Tobramycin and Gentamycin is reduced, it may be because of geographical distribution of organism or other host immune response and bacterial virulence factors.

CONCLUSION

In developing countries like INDIA where increasing cases of and poor hygienic practices are prevalent, chances of Hospital aquired infection are high. The important clinical concerns of Acinetobacter in hospitalized individuals are its contribution to morbidity, either risk of developing a symptomatic infection or its more serious risks of developing other complications Prompt diagnosis is essential for the patient to reduce the morbidity.

I was found to be more positive in Female hospitalized patient. Most common age group was 0-20 year in both male and female. Increase chance of *Acinetobacter* is also correlate with increase in duration of hospital stay.

In present study *A.Baumanii* was isolated most commonly in both male and female. The high prevalence of Bacteremia of 59.72 % in this study is of major public health importance. The predominant pathogen was *A.Baumanii* and this organism is beginning to acquire resistance to some of the clinically used antibiotics. The level of resistance to antibiotics recorded in this study is of great concern.

SUMMARY

The present study was conducted for a period of 20 months from August 2014 to March 2016 in the Department of Microbiology, Government Medical college and Sir Takhtsihji General Hospital, Bhavnagar, Gujarat.

Blood, pus and sputum samples from the patients not having any type of drug consumption History were collected at In Patient Department at Sir Takhtsihji General Hospital, Bhavnagar, Gujarat. Patient From Outpatient Department are excluded.

The present study includes total 72 clinically diagnosed, non-duplicate and non consecutive cases of Acinetobacter Isolates.

Most common age group found positive for Acinetobacter was between 0-20 years with 24(33.33%)isolates & lowest in 46-55 years with 5(6.94%).

Females were more commonly affected than males with female to male ratio 1.32:1. Most commonly affected age group is 0-20 years.

Out of 72 positive cases *A.Baumanni* was most common species 65(90.18%) found. It was also most common in female 37 (39%) and male 12 (48%) patient. 2nd most common species was *A.Lwoffii*, 7 cases isolated (9.8%).

In my study amongst 72 cases 64% of isolates were coming from Ward area only 35% cases were comes from ICU & 1% case were come from OPD.

Amongst 72 samples, 45(62%) cases were from blood,23(32%) cases were from Pus discharge or wound infection , 2(3%) sample from sputum and 1(1%), 1(1%) sample from IJV Tip & LSCS wound gap were isolated.

In this study maximum number of cases were having 3-10 days duration of stay in hospital.

As far as antibiotic susceptibility was concerned, all isolates were tested for susceptibility to Ampicillin-Sulbactum, ceftriaxone-sulbactum, , ceftazidime, levofloxacin, pipracillin-tazobactum, ciprofloxacin, ofloxacin, gentamycin and tobramycin and meropenem. Acinetobacter spp. are more sensitive to meropenem, pipracillin-tazobactum and ceftriaxone-sulbactam and less sensitive to, ampicillin-sulbactum, ciprofloxacin, ofloxacin, gentamycin, amikacin, tobramycin and ceftazidime.

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