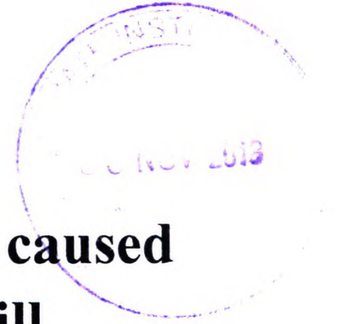

Risk factors and outcome of infections caused by *Acinetobacter* spp. among critically ill patients in a tertiary care hospital in Sri Lanka

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DISSERTATION FOR MD MICROBIOLOGY

POST GRADUATE INSTITUTE OF MEDICINE

UNIVERSITY OF COLOMBO

2017

004589

STATEMENT OF ORIGINALITY

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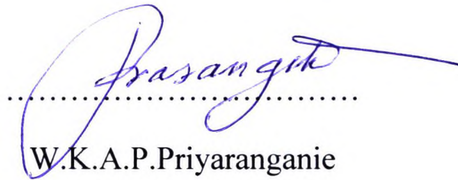

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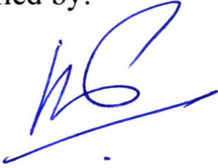

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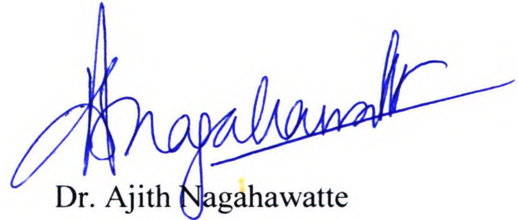

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ABSTRACT

Risk factors and outcome of infections caused by *Acinetobacter* among critically ill patients in a tertiary care hospital in Sri Lanka

Introduction

Acinetobacter are ubiquitous and often multi-drug resistant pathogens causing severe hospital acquired infections in intensive care units (ICU).

Objective

To study the significance of associated risk factors and the outcome of *Acinetobacter* infections

Methods

A prospective descriptive study was done including all patients who were staying for more than 48 hours in 3 ICUSs in a tertiary care hospital over a period of 4 months.

Screening cultures of urine, respiratory, and skin swabs were performed at the time of admission and repeated every 4th day and with new signs of infection.

Results

From 113 patients 78 (69%) were males. Age ranged from 2 months to 83 years with mean age of 67 years. 28% ICU admissions were due to sepsis.

The incidence of colonization and infection with *Acinetobacter* were 35 and 24 cases per 1000 patient days respectively. The incidence of, ventilator associated pneumonia, central venous line (CVL) related bacteraemia, and shunt infection with *Acinetobacter* were 24, 10, and 11 per 1000 device days respectively.

Colonization rates varied among respiratory (40%), skin (18%), CVL tips (4%), drains (2%), and urine (2%) specimens.

Endotracheal tube and CVL in situ for more than 12 days had 5 and 4 times the risk of developing *Acinetobacter* infections respectively. The presence of haemodialysis line had 3 times the risk. The presence of 3 or more such invasive devices in situ had 9 times the risk. ICU stay more than 12 days had 6 times the risk (p=0.000). Colonization index >0.33 carried 4 times risk (p=0.000, RR=4.053, 95%C.I. =1.949-8.431). Neurological illness, cardiopulmonary resuscitation, and use of inotropes were other risk factors.

The mortality rate (OR=3.43, 95% C.I= 1.188-6.797) and prolonged hospital stay (OR=15.109, 95% C.I. =5.658-40.350) of *Acinatobacter* infected patients were higher than that of non-infected patients.

Conclusions

Intubation and CVL in-situ more than 12 days, more than 3 invasive devices in situ, and colonization index >0.33 were the most significant risk factors for the development of *Acinetobacter* infections. Patients with *Acinetobacter* infections had higher rates of mortality and hospital stay than non-infected patients.

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CONTENTS

Preliminaries	Page No.
Title Page	i
Statement of Originality	ii
Abstract	iii-iv
Contents	v-vi
List of tables	vii
List of figures	viii
Key for abbreviations	ix-x
Acknowledgement	xi
Chapter 1 – Introduction	1-4
Chapter 2 – Literature review	
- 2.1.introduction	5
- 2.2.Epidemiology , Pathogenesis and risk factors	5-9
- 2.3.Infections caused by *Acinetobacter* spp.	10-15
- 2.4. Antibiotics sensitivity testing and associated resistance pattern	16-17
- 2.5.Outcome of the patients infected with *Acinetobacter* spp.	17
- 2.6. Prevention and control	18
Chapter 3- Materials and methodology	19-27
Chapter 4 – Results	28-38
Chapter 5 – Discussion	39-46
References	47-55

Annexure

Annexure 01-Instructions for specimen collection and transport for bacterial cultures

Annexure 02-Standard Operating Procedures for the processing of specimen

Annexure 03-Identification of Organisms

Annexure 04- Standard Operating Procedures for antibiotics sensitivity testing(CLSI guidelin es)

Annexure 05-Data Sheet

Annexure 06- Consent form

Annexure 07-Ethical approval

LISTS OF TABLES

Table **Page No.**

01: Age distribution of study population 29

02: Infections associated with *Acinetobacter* spp. 30

03: HAI rates caused by *Acinetobacter* spp. 31

04: Risk factors associated with *Acinetobacter* spp. 32-33

05: Antibiotic resistance pattern to carbapenem in screening & infections causing isolates 35

06: Comparison of MDR between screening & infections causing isolates 36

07: Outcome of patients infected and non-infected with *Acinetobacter* spp. 37

08: Logistic regression analysis for outcome of the patients infected with *Acinetobacter* spp. 37

09: Logistic regression for analysis of *Acinetobacter* infections and prolonged hospital stay 38

LIST OF FIGURES

Figure		Page No.
01:	Gender distribution of study population	28
02:	Age distribution	28
03:	Reasons for hospital admission	29
04:	Infections associated with *Acinetobacter* spp.	31
05:	Frequency distribution of colonization associated with *Acinetobacter* spp.	32
06:	Antibiotic sensitivity pattern of screening isolates	34
07:	Antibiotic sensitivity pattern of infections causing isolates	35
08:	Comparison of carbapenem sensitivity between screening & infections causing isolates	36
09:	Comparison of MDR between infections causing isolates and screening isolates	36

KEY FOR ABBREVIATIONS

ABST : Antibiotics Susceptibility Tests

AK : Amikacin

AMP : Ampicillin

AMC : Amoxycillin-Clavulanate

ARSP : Antbiotic Resisitance Surveillance Project

BAL : Bronchoalveolar lavage

CAR-S: Carbapenem Sensitive

CAR-R: Carbapenem Resistant

CAUTI: Catheter Associated Urinary Tract Infections

CAZ : Ceftazidime

CDC : Centers for Disease Control

CFU : Colony forming units

CIP : Ciprofloxacin

CLABSI: Central line associated blood stream infections

CLSI : Clinical Laboratory Standard Institute

CN : Gentamicin

CPIS : Clinical Pulmonary Infection Score

CRO : Ceftriaxone

CT : Colistin

CTX : Cefotaxime

CVC : Central venous catheter

DO : Doxycycline

ESBL : Extended spectrum of beta-lactamase

FEP : Cefepime

HAI : Hospital acquired infections

HAP : Hospital acquired pneumonia

HCW	:	Health care worker
ICU	:	Intensive Care Unit
IMI	:	Imipenem
LEV	:	Levofloxacin
MDR	:	Multi-Drug resistance
MIC	:	Minimum inhibitor concentration
MEM	:	Meropenem
PTZ	:	Piperacillin Tazobactam
SAM	:	Ampicillin Sulbactam
SCF	:	Cefeparazone Sulbactam
SJGH	:	Sri Jayawardanapura General Hospital
SOP	:	Standard Operating Procedure
SSI	:	Surgical Site Infections
SXT	:	Trimathoprim-Sulfamethoxazole'
TGC	:	Tigecycline
TIM	:	Ticarcillin Clavulanate
UTI	:	Urinary tract infections
VAP	:	Ventilator Associated Pneumonia

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CHAPTER 1

INTRODUCTION

Acinetobacter infection and colonization is becoming a great threat to the survival of compromised population in intensive care (ICU) and high dependency settings. This environment is ideal for the spread of these pathogens because vulnerable patients are managed by different categories of health care workers in a susceptible environment.

Acinetobacter spp. are ubiquitous pathogens capable of causing both community (Villegas and Hartstein, 2003) and health care associated infections, of which hospital acquired infections are the most common form. They are pleomorphic aerobic Gram negative cocobacilli commonly isolated from hospital environment and hospitalized patients.

They are catalase positive, oxidase negative, non-motile, encapsulated bacteria which usually grow on routine laboratory media. This organism was first described in 1911 as *Micrococcus calco-aceticus* eventually becoming known as *Acinetobacter* in the 1950s (Munoz-Price & Weinstein 2008). In the late 1980, *Acinetobacter* spp. was identified as an emerging pathogen with multi-drug resistantce.

Acinetobacter baumannii, Acinetobacter calcoaceticus and *Acinetobacter lwoffii* are species most commonly associated with human infections in the clinical literature. As differentiation of *Acinetobacter* spp. is difficult by phenotypic characteristics , the term *A.calcoaceticus-A.baumannii* complex is also mentioned in some studies (Munoz-Price & Weinstein 2008).

As this organism has the distinct ability to adhere to the epithelial cells in skin, perineum, groin, axillae, the mucous membranes and upper airways, they are more prone to colonize these areas (Munoz-Price & Weinstein 2008). They are capable of causing suppurative infections in almost every organ system of the body.

Acinetobacter spp. is commonly isolated in the hospital environment and hospitalized patients. So this ubiquitous nature of the pathogen, frequent inhabitant in ICU setting

and vulnerable population in the ICU settings are frequently associated with infections among critically ill patients in the ICUs. The risk of acquiring infections due to *Acinetobacter* spp. among this vulnerable population is compounded when patients are exposed to various invasive procedures in the ICU setting.

This organism also causes intermittent outbreaks in the ICU settings and can make the situation worsened with challenges to eradicate the organism. (Joshi et al 2006).

These outbreaks have been investigated to identify sources. Contaminated respiratory equipment ,ventilator parts and cross infections by the hands of health care workers were identified as common sources (Munoz-Price & Weinstein 2008).

Not only respiratory care equipment, wound care procedures, humidifiers and other patient care items but also environmental contamination on curtains, laryngoscope blades, patient lifting items, door handles, mops and key-boards have involved in these outbreaks (Maragakis & Perl 2008).

Once this organism is established in the hospital environment especially in the ICU setup, it can show an epidemiological pattern of serial or overlapping outbreaks with this resistant organism (Munoz-Price & Weinstein 2008).

During last two decades *Acinetobacter* spp. was the most commonly identified resistant organism associated with hospital acquired infections (Garcia-Gamendia JL *et al,* 2001: Munoz-Price & Weinstein 2008).

These outbreaks are much more common in a hot humid climate (Joshi *et al,* 2006). As Sri Lanka is a tropical country with a hot humid climate, there may be a tendency to have frequent outbreaks in ICU setting in Sri Lanka.

Initially this organism was susceptible to penicillin, cephalosporins, aminoglycosides and quinolones but later development of resistance to these antibiotics had been recognized. Subsequently the worldwide distribution of carbepenem resistant Acinetobacter spp. was identified. At this time many of these strains were susceptible to sulbactam and polymyxin.(Gilbert *et al.* 1998)

Ever increasing drug résistance has become a problem for most ICUs in the world. The ability to express diverse mechanisms of resistance, emergence of acquired resistance to available antibiotics and lack of new antibiotics in the pipeline are the

major threats posed by these organisms to health care system. Therefore prevention of these infections is better than treating with broad-spectrum antibiotics. Identification and elimination of common sources of these organisms is important to prevent associated high morbidity and mortality (Munoz-Price & Weinstein 2008).

These infections are not only an important cause of morbidity and mortality but also cause a mass economic burden to health-care system especially in a developing country.

The prediction of *Acinetobacter* infection and colonization in critically ill patients in the ICU set-up is important to plan effective infection control measures and empirical antibiotic treatment.(Arlanzón et al. 2015)

The risk factors for colonization and infection by multi-drug resistant *Acinetobacter* in the intensive care setting were identified as follows : 3 times greater risk in male gender and medical patients , 4 times in immunosuppressed or in trauma patints , 6 times risk in those admitted from a hospital ward, 12 times in those who from an other ICU or from a long term care facility and 14 times greater risk in those who have skin and soft tissue infection. (Arlanzón et al. 2015)

Prolonged ICU stay , exposure to ICU environment , mechanical ventilation, colonization pressure , previous exposure to antibiotics , recent surgery , invasive procedures and the underlying severity of illness are also considered as risk factors for colonization and infections (Playford E.G.2007:Founier 2006)Even though carbepenem resistant *Acinetobacter* spp. are isolated from all around the world, carbepenems (imipenem,meropenem,doripenem) are the mainstay of treatment. In addition to carbepenems , polymyxins E and B , sulbactam , piperacillin-tazobactam,tigecycline and aminoglycosides are also considered as effective for treatment of these infections (Michalopoulos et al. 2016)

In the literature, the outcome of infections caused by *Acnetobacter* spp. were identified as high morbidity , mortality and increased length of hospital stay which led to acquiring and up regulating resistance genes (Munoz-Price & Weinstein 2008).

Though it is hard to find separate studies on risk factors and the outcome of *Acinetobacter* infections in Sri Lanka, there are few published data on risk factors in several different studies.

The Teaching Hospital Karapitiya is the largest tertiary care hospital in the Southern province with paediatirc ICU, one neonatal ICU, 2 adult ICUs, one cardiothoracic and one oncology ICU. There are 64 wards with 1920 bed strength in this hospital.

This study was mainly focused on critically ill medical and surgical adult and paediatric patients (except cardiothoracic, neonatal, oncological patients). Out of these patients in ICUs this majority need mechanical ventilation, undergoing various invasive procedures and have multiple comorbid factors.

Routine laboratory surveillances carried out in T.H.Karapitiya revealed that the rate of multi-drug resistant *Acinetobacter* isolates is rising especially among ICU population.

According to laboratory surveillance carried out in T.H.Karapitiya, the number of patients with *Acinetobacter* spp. isolated in clinical samples was 630 during 2015. Out of these patients 465 (81%) patients were in ICUs and remaining 165 patients were in wards. During 2015 laboratory surveillance data revealed 966 clinical samples were positive for *Acinetobacter* spp. Out of these isolates 72 (7.4%) were from blood culture isolates, 27 isolates from CSF through external ventricular drains (EVD)/head drains/ventriculoperitoneal (VP) shunts and the rest from respiratory and other sites.

Further advances in the prevention and treatment of the infection will depend on the better understanding of the epidemiology of infection and colonization with *Acinetobacter* spp. This information will be useful not only in the infection control but also in better management of patients.

CHAPTER 2

LITERATURE REVIEW

INTRODUCTION

Acinetobacter spp. is pleomorphic Gram negative coccobacilli commonly isolated from the hospital environment and from hospitalized patients. They are ubiquitous in nature and can survive in the external environment for months, making it is difficult to eradicate. *Acinetobacter* spp has become one of the most important pathogens causing nosocomial infections, especially in ICU settings (Sheng, W. et al., 2010).

This organism was described as *Micrococcus calcoaceticus* in 1911 (Baumann, 1968). Since 1950 it has been known as *Acinetobacter* spp. This belongs to order Pseudomonadales and family Moraxellaceae. It is a strictly aerobic, non-motile, catalase positive, oxidase negative, non-fermenter with a coccobacilliary shape.

Acinetobacter can easily grow in standard culture media used in the microbiology laboratory but many biochemical tests used in diagnostic are non-reactive.

This organism was identified as the fifth cause of pneumonia and eighth most frequent isolate in blood cultures in ICUs in the United States. This showed increasing trend of infections caused by *Acinetobacter* (Gaynes R., Edward J.R., 2005). *Acinetobacter* was an emerging cause of infections in soldiers wounded during war in U.S. (Davi KA, Moran KA et al, 2005)

Acinetobacter was found as the third leading cause of ventilator-associated pneumonia in Spain. (Rodriguez-Bano J,Cisneros JM,2004)

EPIDEMIOLOGY

Acinetobacter can colonize the respiratory tract, skin, gastrointestinal tracts and wounds. Patil & Chopade in 2001 described *Acinetobacter* as being responsible for 35%-45% of skin colonizers. Chu,Leung et al in 1999 discussed the ability to

colonize other parts of the body such as the intestinal tract, the nares and the throat. *Acinetobacter* prefer a moist environment and colonization in damaged tissue is also common (Lahiri, Mani & Purai, 2004).

This organism is widely distributed in nature, can be found in water, soil and on vegetables. Chu, Leng et al in 1999 described seasonal variability in skin colonization by *Acinetobacter* . The study carried out in Hong Kong found seasonal variation of the organism. They found that 53% were colonized with *Acinetobacter* in summer and 32% in winter season.

The environmental bacterial load in ICU is greater than surgical wards. This may be due to the large number of high risk patients with high risk procedures and several other factors in ICUs. Their environmental bacterial load was heavier when comparing study done in Europe. They also showed that isolates from clinical samples were similar to environmental samples. *Acinetobacter* genomic species 3 was found in water, on vegetables and on human skin (Houang, Chu et al, 2001).

Acinetobacter can be carried in droplets from respiratory secretions, skin scales and dust (Gerner-Smidt. P, 1987).

The ecological distinction of *Acinetobacter* is due to nutritional versality i.e. their ability to grow in a vast variety of organic compounds, (Baumann, 1968).Colonization of hospitalized patients occurs within the first week of admission.

A hospital based study carried in Qatar revealed 64.3% (239/372) isolates were categorized as infections and 35.7 %(133/372) were classified as colonizers. The most common site of the *Acinetobacter* infections was the respiratory tract (48.9%), followed by blood (16.7%) and urinary tract (17.6%) (Saad et al. 2016a).

Even though members of this genus were considered as rare pathogens before 1970, recent decades have seen rapid increasing numbers of reported infections caused by this organism, particularly in ICU set-up. These infections occur in 4 groups of patients (i) Healthcare Associated Infections (HAI) in ICU setup (ii) HAI outside the ICUs (iii) infections associated with trauma, especially following natural disasters (iv)community acquired infections which usually affect the patients with comorbidity factors in subtropical and tropical countries. ICU infections caused by this organism

in Asian and European hospitals were higher than in USA hospitals (Falagas, Karveli & Kelesidis, 2007).

During last two decades, *Acinetobacter baumanni* emerged as an important nosocomial pathogen due to resistance to most of antibiotics. The ability to survive in environmental desiccation for weeks promotes fomite transmission in the hospital setting (Schreckenberger, Daesher *et al*, 2007). Bergogne-Berezin & Towner in 1996 showed that numerous outbreaks have occurred due to the ability of this organism to cause cross transmission especially in intensive care unit.

PATHOGENESIS

Green, Johnson *et al in* 1965 showed that *Acinetobacter sp.* act as an opportunistic pathogen with a highly variable degree of virulence. This organism can affect any organ in the body, causing bacteremia, pneumonia, endocarditis, intra-abdominal abscess, osteomyelitis, meningitis, soft tissue and surgical site infections and urinary tract infections (Gaynes R.et al, 2005). It also causes device associated secondary meningitis and peritonitis in patients with peritoneal dialysis catheters (Bergogne-Berezin & Towner, 1996).

Virulence factors

Acinetobacter have numerous virulence factors allowing survival in an adverse environment. This organism is encapsulated with a capsule and has lipopolysaccharide containing cell wall but the effect of cell wall in the pathogenesis is not well studied. The factors which contribute to transition from colonizer to invasive bacteria are important in pathogenesis.

One of the important virulence factors was exopolysaccharide produced by this organism giving protection against the host immune system (Joly-Guillou M, 2005)

In 2004 Dorsey, Tomaras *et al*, described an iron acquisition system mediated by siderophore. *Acinetobacter* sp. have the ability to survive in the iron –deficient environment of human by secreting ferric binding compounds such as siderophores. This organism has ability to form biofilms and which acts as a virulence factor (Tomaras , Dorsey *et al,*2003). Adherence and OMP functions (Lee, Koerten *et al,* 2006) and LPS (Knapp, Wieland *et al,* 2006) were also described as virulence factors.

Adherence and formation of biofilms on inanimate objects and surfaces lead to success as pathogens especially in the hospital environment.

Lee, Koerten *et al* in 2006 demonstrated that a pilus like structure of this organism aids in adherence of this organism to bronchial epithelial cells and erythrocytes. Its ability to adhere leads to the creation of biofilm on surfaces and human cells.

After adherence they can induce both catalase dependent and catalase independent apoptosis by using protein, OMP (Choi, Lee *et al*, 2005). Four different quorum sensing signals regulate the virulence mechanism of this organism. They can acquire foreign genetic materials which lead to enhance survival and the development of antibiotics resistance.

Acinerobacter is able to live in the hospital environment due to resistance to major antibiotics, resistance to disinfectants and resistance to desiccation. These features allow the organism prolonged survival in the hospital environment causing subsequent outbreaks, especially in ICU settings.

Catalano, Quelle *et al,* in 1999 found that dry vectors can act as a secondary reservoir where this organism can survive.

Risk factors

Risk factors for *Acinetobacter* colonization and infections have been identified in several studies done throughout the world. They can be categorized as follows

1) Host related factors – severity of illness, history of infections, extremes of age.

2) Related to the hospitalization – prolong stay in hospital or ICU

3) Related to treatment – previous use of antibiotics eg; 3rd generation cephalosporin, use of fluoroquinolones and carbapenems.

4) Various invasive procedures – ET tube in situ and mechanical ventilation, central lines and other devices such as EVD, VP shunts, indwelling urinary catheter insertion, enteral feeding.

5) Severe underlying disease – immunosuppression, burns, malignancy.

Previous therapy with a fluoroquinolone was identified as an independent risk factor for infections with *Acinetobacter* sp. This is due to indiscriminate use of these antibiotics leading to selection pressure (Villers Espaze *et al,* 1998).

Stainer , Palleroni, & Doudoroff in 1966 showed severe illness or disability , relative status of immunosuppression , and extremes of ages are further risk factors.

A hospital based study carried out in Qatar showed *Acinetobacter* infections were associated with mechanical ventilation (65.3%), invasive procedures (61.5%), and enteral feeding (61.9%) (Saad et al, 2016)

According to a prospective study carried out in Slovakia, neutropenia (p=0.0001), prior antibiotics therapy (p=0.0006) and antineoplastic chemotherapy (p=0.0001) were significant risk factors for bacteremia due to this organism in cancer patients. In children mechanical ventilation, surgery (p=0.0001) and prior antibiotic therapy were identified as risk factors. (Koprnova Svetlansky *et al,* 2001).

Duration of hospital stay before bacteremia (p=0.001), using invasive procedures such as mechanical ventilation (p=0.0010) central venous catheterization (p=0.0001) and isolation of carbepenem resistany organisms(p=0.005) were identified as significant risk factors in a Korean retrospective study (Park *et al*, 2013).

Acinetobacter acquisition may vary in different hospital set ups and cause endemic colonization and epidemic outbreaks. *Acinetobacter baumannii* is identified as the third most common cause of Gram negative sepsis in immunocomprmised patients with a high mortality (Koprnova Svetlansky *et al,* 2001).

Factors associated with an increased risk of pneumonia or colonization of the lower respiratory tract by *Acinetobacter* spp. have been identified, such as advanced age , prior use of antibiotics , presence of invasive devices such as ET, gastric tubes and other devices, chronic lung diseases, and immunosuppression (Lortholary, Fagon *et al* 1995).

Prior *A.baumannii* colonization, use of central venous catheters and mechanical ventilation, and cardiovascular organ failure were associated with blood stream infections due to this organism (Jang T.N, Lee S.H, *et al* ,2009).

Infections caused by *Acinetobacter spp.*

Hospital acquired pneumonia and ventilator associated pneumonia are the most frequent and most important infections caused by *Acinetobacter* spp, resulting high mortality and prolonged hospital stay. It is important to decide on the significance of this organism in cultures as many respiratory samples can be positive with the colonizing flora.

According to a prospective study carried out in 2000, this organism caused 35% of ventilator associated pneumonia (VAP) (Sofianou, Constandinidis, 2000).

The lower respiratory tract (32/77.41.6%) was identified as most frequent site of infections due to *Acinetobacter* followed by intravascular sites (13/77, 16.9%) (Jang TN, Lee SH, *et al* 2009).

1%-2% of blood stream infections (typically intravascular device associated infections) are caused by this organism (Gaynes R, Edward JR, 2005). *Acinetobacter* accounts for 1.6% of urinary tract infections (Gaynes R, Edward JR, 2005). Outbreaks of post-neurosurgical meningitis due to *Acinetobacter* has also been reported and community associated meningitis without surgical intervention was very rarely reported (Shunlan Ni, Shanshan Li, *et al, 2015*).

(1). Hospital Acquired Pneumonia (HAP) and Ventilator Associated (VAP)

HAP is defined as an inflammation of the lung parenchyma occurring 48 hours or more hours after hospital admission but not incubating at the time of admission VAP is defined as an inflammation of lung parenchyma 48 hours after intubation (Andre, C. K. Mark ,L.M. Michael, K. *et al*,2016).

According to the time of onset, these infections can be divided into two types. Early onset VAP/HAP with better prognosis occurs within first 4 days of hospital admission. These are likely to be caused by antimicrobial sensitive organisms.

Late-onset VAP/HAP occurs 5 days or more after hospital admission. These are frequently caused by MDR organisms and associated with high mortality and morbidity (Andre, C. K. Mark ,L.M. Michael, K. *et al*,2016).

Pathogenesis

Inhalation, aspiration and haemotogenous spread are the three main ways by which bacteria reach the lung. The primary route by which organism enter the lower airway is aspiration of oropharyngeal secretions.

The delicate balance between host defense mechanism and ability of the microorganism to colonize and invade should be shifted in favor of propensity of the microorganism to invade the lower respiratory tract to occur HAP. A number of factors are associated with pathogenesis of HAP and VAP. The host related and treatment related factors are: severity of the patient's underlying disease, prior surgery, use of invasive respiratory devices, and exposure to antibiotics (Kollef M.H., 1999).

After entry of this microorganism in to the respiratory tract, they can colonize. When these organisms overcome host mechanical, cellular and humoral defenses they can establish HAP in susceptible individuals.

Leakage of microorganisms around the ET cuff and aspiration of oropharyngeal bacteria are the primary route of entry of bacteria in to the lower respiratory tract. Formation of biofilm in the ET tube with subsequent embolization of microorganisms in to distal air ways may be important in pathogenesis of VAP.

Diagnosis

There is no universally accepted 'gold standard' for the accurate diagnosis of VAP which leads to over and under diagnosis of the condition (Andre, C. K. Mark ,L.M. Michael, K. *et al*,2016).

The Clinical Pulmonary Infection Score (CPIS) was developed to overcome the poor specificity of the clinical diagnosis and microbiological investigations. This score consists of 6 variables, scoring vary from 0 to 12. These variables are temperature, volume and purulence of tracheal secretions, leukocytosis, pulmonary radiography, oxygenation and semi-quantitative culture of tracheal aspiration (Pugin,J. ,Auckenthaler , Mili N.,*et al*, 1991).

An algorithm for the diagnosis of clinically defined pneumonia was developed by CDC/National Health Safety Network. (Horan, T.C. Dudeck, M.A., 2008).

Management

Initial empiric antibiotics should be guided by local epidemiological data and risk factors associated with those pathogens. Cost, availability and formulary restriction of the antibiotic also should be considered in initiating empiric antibiotics.

Pathogen specific therapy should be started as soon as microorganisms are isolated from clinical samples. According to IDSA guidelines, VAP/HAP due to *Acinetobacter* spp. can be treated with carbepenems or ampicillin/sulbactam if the isolate is susceptible to these agents. For infections caused by *Acinetobacter* spp. that is sensitive only to polymyxin, intravenous polymyxins (colistin or polymyxin B) with or without adjuvant inhaled colisin is recommended. Colistin with adjunctive rifampicin is also recommended in treating HAP/VAP due *Acinetobacter* spp. which is sensitive only to polymyxins (Andre, C. K. Mark ,L.M. Michael, K. *et al*,2016).

Prevention

1. General measures

i. Staff education

ii. Use of clinical guidelines and protocols

iii. Screening patients and their environment

iv. Hand hygiene and use of PPE

2. Infection control issues related to the use of equipment and maintenance of instrument – maintenance and sterilization or disinfection are important.

3. Patient procedures - such as use of non-invasive ventilation, suctioning, methods of ET intubation, prevention of aspiration, enteral feeding, stress ulcer prophylaxis.

4. Control of environmental issues

(Masterton, R.G. Galloway, A. *et al,* 2008)

Implementation of a VAP care bundle was described by the SARI working group (A strategy for the control of Antimicrobial Resistance in Ireland) in the Guidelines for prevention of VAP in adults in Ireland in 2011. This VAP care bundle comprises the following measures (Karen Burns, Edmund Carton *et al*,2011).

1. Sedation reviewed and, if appropriate stopped each day.

2. Patient assessed for weaning and extubation each day.

3. Avoid supine position. Aim to have the head of bed elevated to at least 30^0.

4. Use chlorhexidine as part of daily oral care (0.12-2.0% applied 6hourly)

5. Use of subglottic secretion drainage in patients likely to be ventilated for more than 48 hours.

(2). Catheter Related Blood Stream Infections (CRBSI)

Pathogenesis

Initially microbial adherence and formation of biofilm occurs after the entry of these organisms through the extra-luminal and intraluminal surface of the device. This results in colonization by microorganism and subsequent haematagenous dissemination (Marrie , T. Costerton, J.W,1984).

Four recognized routes of entering microorganisms have been identified. They are;

1. Migration of skin organisms.

2. Direct contamination of the catheter hub or catheter by direct contacts with hands or contaminated materials.

3. The Catheter can be haematogenously seeded from another focus of infection. (Less commonly)

4. Infusate contamination might occur. (Very rarely)

There are important pathogenic determinants leading to CRBSI. They are;

1. The material from which the device is made

2. Host factors- protein adhesions such as fibronectin and fibrin that forms a sheath surrounding the catheter.

3. Virulence factors of the organisms.

Diagnosis

Clinical findings have poor sensitivity and poor specificity and are not adequate for the diagnosis of CRBSI.According to IDSA guidelines, the definitive diagnosis of CRBSI can be made by growing the same organisms from 2 blood samples (1 from catheter hub, 1 from a peripheral vein) or catheter tip and peripheral blood culture.

Semi-quantitative (15 CFU per catheter segment) or quantitative (>10^2 CFU per catheter segment) methods are used to interpret the significance of a catheter tip culture.

The colony count of microbes grown from CVP line should be at least 3 fold or more greater than the colony count grown from blood culture drawn from peripheral vein for quantitative blood cultures.

Time of positivity is also important to interpret CRBSI. Growth of microorganisms from blood culture drawn from CVP line should be detected at least 2 hours before the growth of the same microorganism in the blood drawn from a peripheral vein.

Prevention

CDC guidelines for the prevention of intravascular catheter-related infections in 2011 recommended following the measures to prevent these infections:

Education regarding proper indication for CVP lines, aseptic procedures for insertion, & maintenance, infection control measures to prevent CRBSI and periodic assessment on knowledge and adherence to guidelines.

A well trained person should insert the line and in adults upper extremity site should be chosen for catheter insertion. Avoid using the femoral vein for central access in adults.

Inspect daily the catheter insertion site by palpation for tenderness or inspection through transparent dressings.

Remove catheters if the patient develops features of phlebitis or if the catheter is malfunctioning or if any intravascular catheters are no longer essential.

Aseptic procedures should be used for insertion and care of these devices.

Strict aseptic procedures should be employed by using maximal sterile barrier precautions (a cap, sterile gown and gloves) for insertion of CVP lines.

Advice to report any changes in catheter insertion sites.

Do not use prophylactic antibiotics before insertion and during the use of CVP line.

(3). Health – Care Associated meningitis

A different spectrum of microorganisms, including resistant Gram-negative bacilli and *Staphylococci* causes nosocomial meningitis.

Pathogenesis

Four mechanisms by which a shunt can be infected

1. Colonization of the shunt at the time of surgery- most frequent cause
2. Retrograde infections from the distal site of the shunt
3. Through the skin – insertion of the needle to the shunt or reservoir to take CSF for culture or assess the patency of the catheter, injection of drugs, erosion of catheter through the skin.
4. Haematogenous

Diagnosis

Contamination – define as isolated positive Gram stain or CSF culture with normal CSF full report and lack of clinical symptoms.

Colonization- is defined as multiple positive Gram stain and CSF culture with normal CSF full report and lack of clinical symptoms.

Infections – defined as single or multiple positive CSF culture with positive CSF full report and clinical symptoms suggestive of meningitis.

Management and prevention

Empirical treatment for shunt infections caused by *Acinetobacter* should be initiated with IV meropenem. Prolonged infusion of meropenem is effective in treating infections caused by resistant organisms (Allan, R.T. Rodrigo, H. *et al,* 2017).

A combination of intravenous and intraventricular colistimethate sodium or polymyxin B is recommended in infections with carbepenem resistant organisms (Kim, B.K. Peleg, A.Y. *et al,*2009).

According to the IDSA guidelines for health-care associated ventriculitis and meningitis in 2017, periprocedural antibiotics administration, use of antibiotic impregnated devices, and adherence to standard protocol for insertion are important measures to prevent device associated infections.

Antibiotics Sensitivity Testing and associated Resistance Pattern

This organism exerts antibiotics resistance through the production of beta-lactamases .Group 1 Ambler class C beta-lactamases hydrolyze penicillin, 2nd and 3rd generation cephalosporins including ceftriaxone, cefotaxime , ceftazidime but hydrolysis of 4th generation cephalosporins and carbapenem are low. Production of Ambler class A extended spectrum beta-lactamses cause hydrolysis of penicillin and all cephalosporin and subsequently lead to the use of carbapenem in treating infections caused by *Acinetobacter* spp. Emergence of Ambler class D oxacillinases results in resistance to carbapenems. Ambler class B, metallo-beta-lactamases also cause carbapenem resistance.

A hospital based study carried out in Qatar revealed 42.7% was susceptible and 57.3% were MDR. It also showed 1.4% colistin resistance and 3.6% tigecycline resistance. Most resistant antibiotic was cefotaxime (58.3%). Carbepenem resistant isolates were identified including 45.6% resistance to meropenem and 35.1% resistance to imipenem (Saad et al. 2016).

Among beta-lactamase inhibitors sulbactam is known to be more efficient than clavulanic acid against *Acinetobacter* spp (Vila, Marcos *et al,* 1993; Joly-Guillou & Decre , 1995).Resistance to beta-lactam antibiotics can be due to beta-lactamase production, reduced permeability of the cell wall and alteration of the target site (Quale, Bratu *et al, 2003*).Colistin resistance was observed in 4% of the isolates. Rising trend in carbepenem resistance was seen and 87% of carbepenem resistance was reported in Croatin according to EARS-Net survelliance data in 2016 (Anon 2016).This organism is intrinsically resistant to most of antibiotics due its selective ability to prevent penetration of antibiotics across the bacterial outer membrane(Munoz-Price & Weinstein 2008).

Various types of resistant mechanisms exist for resistance to antibiotics. They are

1. Decreased permeability
2. Enzymatic breakdown

a) Class D beta lactamases – most common

b) Intrinsic carbapenem-hydrolysing oxacillinase (OXA-51)

c) Most frequent acquired oxacillinases are OXA-23-like, OXA-24/40 like, OXA-58 like, OXA-143like, and OXA-235like

d) Metallo-beta-lactamases such as imipenemase or Verona integron-encoded metallo-beta-lactamase (VIM) and New Delhi metallobeta-lactamase (NDM)

e) Production of *K.pneumoniae* carbapenemase (KPC) and OXA-48 oxacillinase

f) Aminoglycoside-modifying enzyme

3. DNA topoisomerase mutations – Quinolones

4. Efflux – second most common method of acquiring antibiotic resistance in *Acinetobacter* causing resistance to beta-lactams, macrolides, chloramphenicol, tigecyclines, tetracyclines, aminoglycosides , and certain antiseptics.Their inherited ability of developing antibiotics resistance leads to difficulty in control and treatment of nosocomial infections. These resistance patterns differ among countries, among different centers and even among different units in the same hospitals. Therefore local surveillance is important in deciding empirical treatment.

Resistance to tetracycline can be due to an efflux pump of the drug subsequently leading to protection of ribosome (Fluit, Florijin *et al*,2005). Tigecycline is a modified tetracycline antibiotic with a broader cover.Colistin resistant *Acinetobacter* spp. has been reported (Urban, Mariano *et al*, 2001). Many hospitals in the world are facing the challenge of managing pan-drug resistant *Acinetobacter* spp. with limited therapeutic options such as colistin and tigecycline only.

Outcome for the patients infected with *Acinetobacter*

The crude mortality was 31%(65/239) in a study carried out in Qatar (Saad et al. 2016). A high mortality rate associated with *A.baumannii* bacteremia (ranging from 29%-46.9%) was identified in a study carried out in Korea (Park et al. 2013).

Acinetobacter infection was associated with high mortality and length of stay in ICUs leading to enhanced health care cost. Attributable mortality was identified as 25% with risk ratio for death 2.0 due to this organism (95% C.I. =1.11-3.61) and the duration of ICU stay was 10.3 days more than the control group (Lortholary, Fagon *et al* 1995). Nosocomial meningitis caused by *Acinetobacter baumannii* leads to a high rate mortality rate. Two different studies showed a mortality rate of 71.4% (Tuon FF, Penteado-Filho SR, Amarante D *et al*,2010) and 72.7% (Metan G, Alp E, Aygen B, Sumerkan B, 2007) respectively due to this nosocomial infection.

According to previous data, the mortality rate due to nosocomial *Acinetobacter* meningitis ranged from 30%-70%, especially higher among patients in developing countries (Huttova, M. Freybergh, P.F. *et al, 2007*).

Prevention and infection control

Hand hygiene among health care workers and meticulous aseptic handling of vascular devices were pointed out as most important measures to prevent *Acinetobacter* colonization and blood stream infections (Jang TN, Lee SH, *et al* 2009). Adherence to standard protocols and implementing antibiotic stewardship in the health care setting has been proved to reduce the burden of nosocomial infections.

CHAPTER 3

METHODOLOGY

OBJECTIVES

GENERAL OBJECTIVE

To determine the incidence, risk factors and outcome of critically ill patients who are infected with *Acinetobacter* spp. in a tertiary care hospital.

SPECIFIC OBJECTIVES

1. To determine the incidence of colonization and infection caused by *Acinetobacter* spp.

2. To identify the ABST pattern of *Acinetobacter* spp. isolated from colonized and infected patients

3. To identify risk factors for *Acinetobacter* infection

4. To describe the relationship between infections and disease outcome for the patients infected with *Acinetobacter*.

STUDY DESIGN AND SETTINGS

- Study type : A hospital based prospective descriptive study
- 2 adult ICUs and 1 paediatric ICU at Teaching Hospital Karapitiya

Study duration - From 1st of December 2015 to 31st of March 2016

Study population

- All patients who were admitted and staying more than 48 hours in 2 adult ICUs and 1 paediatric ICU at T.H.Karapitiya during the study.

Exclusion criteria

- Patients who were admitted before the study
- Patients already infected with *Acinetobacter* spp. prior to this admission
- Patients who stayed less than 48 hours
- Patients who did not give consent
- Co infections and mixed infections.

SAMPLE SIZE

- Sample size was calculated using the following formula. for studies estimating an incidence rate with specified relative precision

  $n = (z_1 - \alpha_2/\Sigma)^2$

(For incidence rates with relative precision of 0.2 with 95% confidence)

n = sample size

$Z_{1-\alpha 2}$ = Standard Normal Deviation (for 95% confidence interval)

Σ = Relative Precision = 0.2

- The required sample size (n) was 97
- Therefore all eligible patients during my study period was taken

  (Lwanga and Lemeshow , 1991)

METHODOLOGY

Patients were selected according to the inclusion criteria and exclusion criteria. Screening samples and specimens taken from patients with suspected infections for culture were collected to detect colonization and infections due to *Acinetobacter* spp. from the eligible patients.

Screening samples

Initial screening cultures were performed with urine, sputum/ endotracheal aspirate, and skin swabs (nasal, axillary, groin and perineal region) on admission to ICU. ET secretion or tracheal aspiration from ventilated patients , sputum samples from non-ventilated patients ,catheter urine sample from catheterized patients , voided urine samples from non-catheterized patients were collected on admission. Repeat screening cultures were performed every 4th day of ICU stay.

Specimens taken from patients with suspected infections

All patients included in the study were followed up and observed for clinical features suggestive of infection. Additional cultures were performed when newly developed signs of infections or clinical deterioration occurred. The results of other investigations such as WBC, CRP, Urine Full Rrport and Chest X-rays routinely performed in the ICUs to detect the presence of infections were noted.

Blood cultures were taken from the both central line (if available) and peripheral from the patients with following clinical signs and symptoms (Dellinger R., .Carlet, J.M. *et al* 2004)

- Body temperature ≥ 38°C or ≤36°C
- WBC ≥ 12000 or ≤ 4000cells/m^3
- Heart rate ≥ 90 beats/min
- Respiratory rate ≥ 20/min (in non-paralyzed patients) or P_aCO_2 < 32 mmHg in ventilated patients

Sputum from non-ventilated patients and ET secretion /tracheal aspirates from ventilated patients were collected from patients with the following clinical features (Koenig, S.M. Truwit J.D., 2006)

- Newly developed X-ray changes in the lung field or progressive changes
- Body temperature ≥ 38°C
- WBC ≥ 12000/m^3
- Purulent sputum or secretion

Urine for culture was collected from patients with fever ≥ 38°C and significant pyuria.

Specimen collection, transport and laboratory procedures

Collection, transport, processing of relevant specimens for microbiological cultures and identification of the isolate were done according to the standard operative procedures (SOPs) by The Sri Lanka College of Microbiologists (Annexure - 1 and Annexure - 2)

Identification of the organism was done by using culture characteristics and biochemical tests including Rapid ID commercial kits. (Annexure 3)

Typical colony morphology (1-2mm non-pigmented, domed shaped with entire margin and non-lactose fermenting with pink tinge appearance on Mac-Conkey plate was noticed.

Typical Gram stain of Gram negative coccobacilli/ Gram negative cocci and other biochemical tests routinely done in the laboratory were used to presumptive identification of *Acinetobacter* spp.

Other basic tests such as positive catalase, negative oxidase, and non-reactive Kligler-iron agar were noted. Motality test was done by using the hanging drop technique. Identification of each and every isolate was further confirmed as *Acinetobacter* spp. by using Remel Rap-ID one system.

Antibiotic susceptibility test (ABST)

ABST was done according to Clinical and Laboratory Standard Institute (2015) using the standard disc diffusion method and MIC (Annexure- 4)

Disc diffusion testing of tigecycline and cefoperazone sulbactam was interpreted by using methodology described by Jones, Ferraro *et al* in 2007 and Sulperazone manual for the Microbiologist in 1988 respectively (Barry et al. 1988).

Measurements of zones of inhibition using CLSI methods

ABST plates were observed after 16-18 hours of incubation for a confluent lawn of growth, satisfactory plating without any contamination. If those criteria were not fulfilled then tests were repeated. The diameter of the zones was measured by using ruler without opening the plates. Interpretation was done by using CLSI guidelines (2015). Colistin MIC was performed with HiComb ™ MIC Test Strips by using CLSI guidelines (2015) for cut off values.

Reading of MIC values

MIC values were read according to the edge of the inhibition ellipse intersecting the MIC stripes.

Quality control

Quality control (QC) in ABST was done according to CLSI guidelines (2015). Before starting and during the study QC was done to check the quality of the media, antibiotics potency and manual errors.

Clinical validation and interpretation of culture isolates were done according to SOPs, standard definitions and clinical criteria. Patients were followed up during ICU stay to detect colonization and infections due to *Acinetobacter* spp. using standard definitions and criteria. Infected patients with *Acinetobacter* spp. were followed up during ICU stay to determine the outcome.

Data collection

Details of selected patients were recorded in a data sheet containing the following information (Appendix 06)

- Demographic data
- Details on admission (underlying surgical medical conditions, previous antibiotics use, previous hospital admission)
- Assessing risk factors during ICU stay (invasive procedures, antibiotics usage)
- Details of culture isolates
- Outcome of the infection

Data analysis

Data analysis was done by using SPSS-20. Case definitions were determined according to standard definitions.Patients with colonization or infections with *Acinetobacter* spp. and type of the infections were assessed following the criteria proposed by the Center for Disease Control and Prevention (CDC/NHSN Surveillance Definitions for Specific Types of Infections, 2017: Terresa *et al,*2008)

In summary, infection with *Acinetobacter* spp. was defined as the presence of clinical symptoms or signs of infection and *Acinetobacter* spp. isolated from clinical specimen obtained from sterile sites such as blood, CSF, joint effusion and pus, tissue, or fluid obtained during surgery or needle aspiration.

Clinical definitions

Infections due to *Acinetobacter* spp. were defined by standard clinical definitions as follows.

1. ***Hospital Acquired Pneumonia (HAP) and Ventilator Associated Pneumonia (VAP)***

- VAP – pneumonia in a patient on mechanical ventilation for equal or more than 48 hours
- HAP-pneumonia that occurs equal or more than 48 hours after hospital admission

- LRTI is defined as the presence of new onset of purulent sputum, change in characteristics of sputum, presence of new or progressive infiltrates, consolidation, cavitation or pleural effusion on chest-X-rays with *Acinetobacter* spp. isolated from blood cultures or cultures of ET, bronchial brushing , or lung biopsy.
- Patients were screened for following features to diagnose VAP (Vishal B Shete ,*et al* ,2010)

  (1).New or persistent pulmonary infiltrates

  (2). Fever

  (3). Leukocytosis

  (4). Oxygenation P_aO_2/FiO_2

  (5). Purulent respiratory secretion

2. ***ICU acquired nosocomial bacteremia*** – isolation of microorganisms from blood culture of a patient with systemic inflammatory response syndrome (SIRS) 48 hours after admission to ICU

SIRS was defined according to following criteria (presence of two or more criteria)

- Body temperature > 38^0C or <36^0C

- Leukocytosis > $10000/mm^3$, leucopenia < $4000/mm^3$

- Heart rate > 90 bpm

- Respiratory rate > 24 /min

Primary blood stream infections are defined as in the presence of fever, chills or hypotension with *Acinetobacter* spp. isolated from blood cultures without concurrent isolation of the pathogen from other sites

3. ***Central Line Associated Blood Stream Infections (CLABSI)*** – diagnosis of CLABSI was made by isolating the same organism from the line or catheter tip and simultaneously drawn peripheral blood culture

4. ***Catheter Associated Urinary Tract Infections (CAUTI)***

Urinary tract infections in a currently catheterized patients or one who has been catheterized within the previous 48 hours

UTI is defined as the presence of urinary urgency, frequency, dysuria, supra-pubic tenderness, or pyuria with *Acinetobacter* spp.in pure culture with colony count $>10^5$ CFU/ml.

5. ***Device associated meningitis*** – as discussed in literature review (see page 12-13)

6. ***Surgical sites infections*** - were defined as fever ($>38^0$C) and localized pain or tenderness with *Acinetobacter* spp. isolated from the culture of wound discharge or pus/tissue obtained from the incision wound or closed drainage

Colonization

Colonization was defined according to the CDC /National Healthcare Safety Network as the presence of microorganism without showing signs and symptoms of infections i.e. isolation of *Acinetobacter* spp. from the skin, mucous membrane, open wounds or excretions or secretions in the absence of relevant signs and symptoms in the patients. (Horan TC, Andus M, Dudeck MA, 2008).

The colonization index was calculated using following formula (Giuseppina, C., *et al,*2011)

$$= \frac{\text{No of sites colonized}}{\text{No of sites cultured}}$$

Prior antibiotics use

Was defined as the use of systemic antibiotics for at least 72 hours within 2 weeks preceding the date of the positive cultures

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Definition of Multi-drug resistant (MDR), pan-drug resistant (PDR) and extensive drug resistant (XDR) *Acinetobacter* spp. (Magiorakos A.P., Srinivasan A., *et al* , 2012) Antimicrobial categories and agents used to define MDR, XDR, and PDR

Antimicrobial category	Antimicrobial agent
1.Aminoglycosides	• Gentamicin
	• Tobramycin
	• Amikacin
	• Netilmicin
2.Antipseudomonal carbapenems	• Imipenem
	• Meropenem
	• Doripenem
3. Antipseudomonal fluoroquinilones	• Ciprofloxacin
	• Levofloxacin
4.Antipseudomonal-penicill +β-lactamase inhibitor	• Piperacillin-tazobactam
	• Ticarcillin-clavulanic acid
5.Extended-spectrum cephalosporins	• Cefotaxime
	• Ceftriaxone
	• Ceftazidime
	• cefepime
6. Folate pathway inhibitors	Trimethoprim-sulphamethoxazole
7.Penicillin+β-lactamase inhibitor	• Ampillin-sulbactam
8. Polymyxins	• Colistin
	• Polymyxin B
9. Tetracyclines	• Tetracyclin
	• Doxycycline
	• Minocycline

Multi-drug resistance (MDR)

Defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories

Pan- drug resistance (PDR)

Non-susceptibility to all agents listed above

Extensive-drug resistant (XDR)

Non-susceptible to seven or more categories in the above table. (i.e. bacterial isolate remain susceptible to only one or two catergories)

Statistical analysis

The incidence of colonization and infections were calculated. The incidence of Ventilator associated pneumonia, bacteremia, CRBSI, device associated meningitis were calculated.

Relative risks for dependent variables were calculated to describe the association of risk factors and *Acinetobacter* infections. Logistic regression was used to identify factors independently associated with *Acinetobacter* infections in this sample of patients.

Ethical consideration

Ethical clearance was obtained by the ethical review committee of Medical Research Institute and Faculty of Medicine, University of Ruhuna. Permission was taken from the Director and consultant in-charge in each ICU in T.H.Karapitiya.

Informed consent was obtained from each and every patient who were able to give consent. Others who were unable to give consent, informed consent was obtained from close relative of the patient. Confidentiality of data was maintained throughout the study using code numbers to identify the participants.

CHAPTER 4

RESULTS

A total of 113 patients out of the 276 patients admitted to 3 ICUs at T.H. Karapitiya during the period of 1st of December 2015 to 30th of March 2016 were included in the study.

Socio-demographic characteristics of the population

Out of these patients 78(69%) were males and 35(31%) were female. Among the study group the minimum age was 2 months and the maximum age was 83 years and mean age was 67 years.

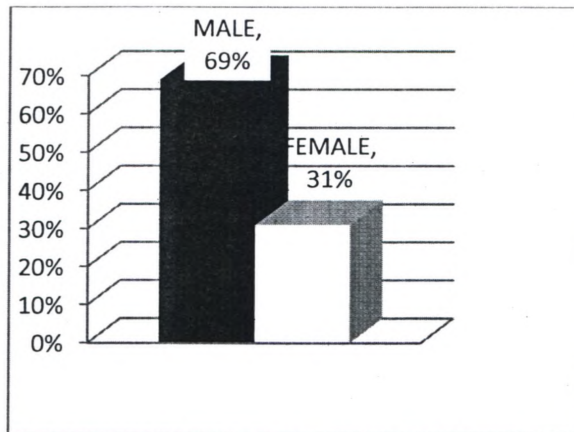

Figure 1: Gender distribution of study population

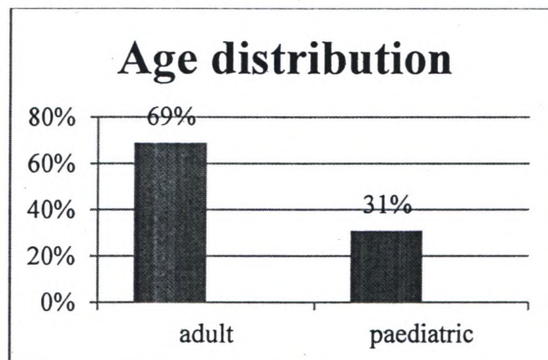

Figure 2: Age Distribution

The majority of the study population were adults (n=78, 69%) and the remaining 31% were in paediatric age group (2 months to 12 years)

Table 1: Age distribution of the study population

Age	Number(n=113)	Percentage (%)
2/12 to 20 years	**42**	37.2
20 years to 40 years	**14**	12.4
40 years to 60 years	**30**	26.5
> 60 years	**27**	23.9
Total	**113**	100

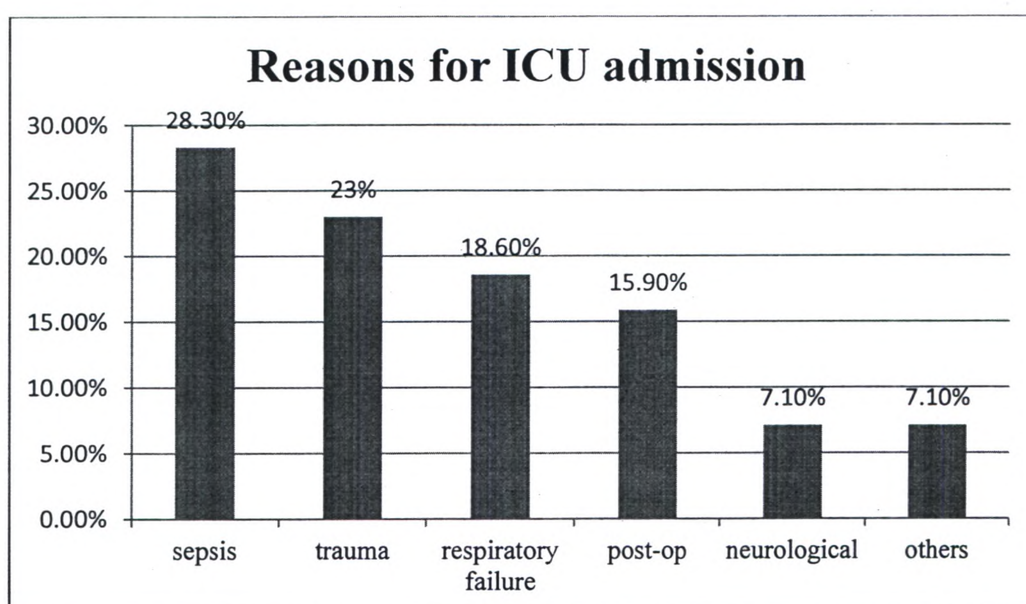

Figure 3: Reasons for hospital admission

Out of the various reasons for ICU admission, most the common reason was sepsis (28.3%).

During the four months of study period 127 samples were positive for *Acinetobacter* spp in screening samples and samples from infected patients. These samples were collected from 113 patients who stayed for 1536 patient-days on the ICUs.

They acquired 38 infections caused by *Acinetobacter* spp. including 26 Device Associated Infections (DAIs).

Infection incidence =

Total number of patients infected with *Acinetobacter* spp during study period

Total patients days during study period

38/1536= 24.74 = 25 cases per 1000 patients-days

Colonization incidence =

Total number of patients colonized with *Acinetobacter* spp.during study period

Total number of patients- days during study period

54/1536=35 cases per 1000 patients-days

Table 2: Infections associated with *Acinetobacter* spp.

INFECTIONS	NUMBER(n)	PERCENTAGE (%) OF PATIENTS INFECTED AT THIS SITES
VAP	20	17.7
Bacteremia	12	10.6
CRBSI	4	3.5
Shunt infections	2	1.8

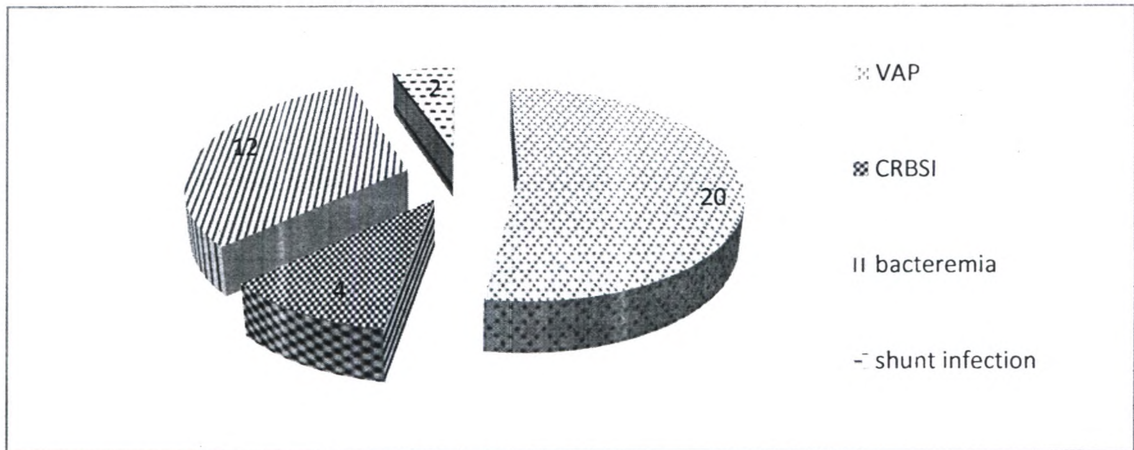

Figure 4: Infections associated with *Acinetobacter* spp.

VAP was the commonest infection caused by *Acinetobacter* spp.

Table 3: HAI rates caused by *Acinetobacter* spp.

INFECTIONS	RATES PER 1000 DEVICE DAYS *	RATES PER 1000 ICU DAYS**
VAP	24.69	13.02
Bacteremia		7.81
CRBSI	10.17	2.60
Shunt Infections	11.23	1.30

*(Number of infections caused by *Acinetobacter* spp. / Number of device days) X 1000

** (Number of infections caused by *Acinetobacter* spp/Number of ICU days) X 1000

Out of 127 positive samples for *Acinetobacter* spp. 84 – Screening isolates, 43- isolates from infected patients

ET colonizer = 39.8% (45) ,Skin colonizer = 18.6% (21) ,CVP tip colonizer= 4.4 %(5), Drain colonizer = 1.8 %(2),Urine colonizer = 1.8 %(2)

Commonest site of the colonization of *Acinetobacter* spp. is the respiratory tract. Skin is the second most common site of colonization

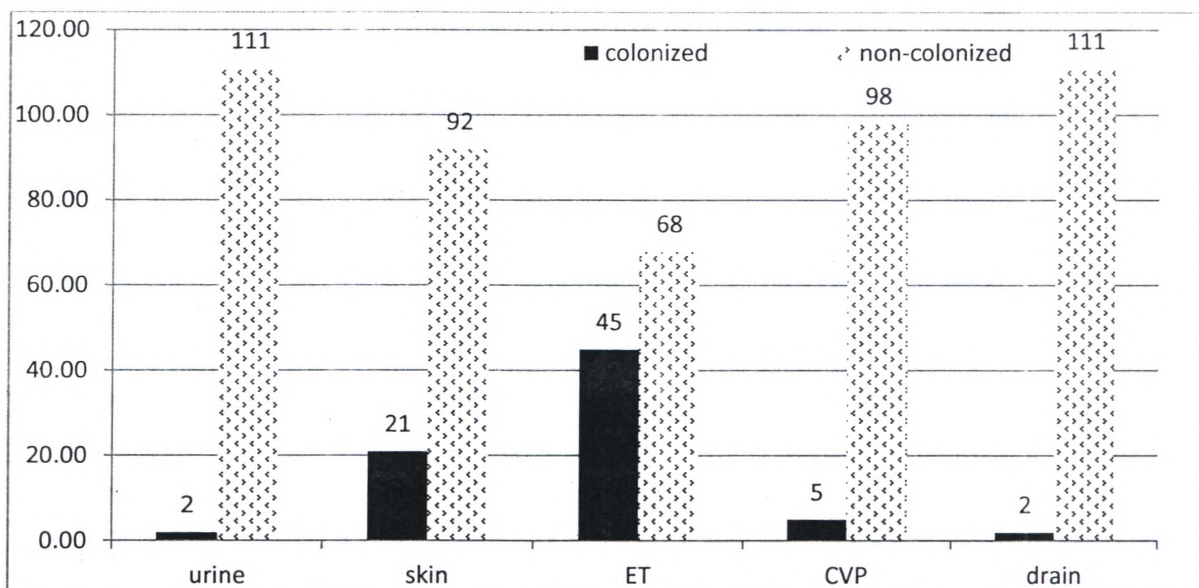

Figure 5: Frequency distribution of colonization associated with *Acinetobacter* spp

Risk factors

Table 4: Risk factors associated with *Acinetobacter* infections

Risk factors	Relative Risk	95% Confidence Interval	P value
Invasive procedures			
1.ET tube duration >12 days	5.470	3.073-9.737	0.000
2.CVL duration >12 days	4.535	2.713-7.578	0.000
3.Presence of HD line	3.153	2.089-4.758	0.000
4.Total invasive procedures ≥3	9.733	4.453-21.277	0.000
Colonization	4.053	1.949-8.431	0.000
1.Skin colonization	2.278	1.418-3.659	0.000
2.ET colonization	2.906	1.664-5.059	0.000
3.Colonisation sites>1	4.053	1.949-8.431	0.000

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4.Colonisation Index (0.33-1.00)	4.053	1.949-8.431	0.000
Long term ICU stay (more than 12 days)	5.997	2.889-12.450	0.000
Neurological illness	2.333	1.450-3.754	0.004
Cardiopulmonary resuscitation	2.151	1.328-3.483	0.005
Inotropes use	2.141	1.314-3.488	0.003
Prior hospital admission	1.643	0.989-2.726	0.0058
Other medical illnesses COPD HTN DM			0.230 1.660. 0.055
Sex - male gender			0.069

The P value for an ET tube in situ more than 12 days, CV line in situ more than 12 days, presence of HD line and use of more than 3 invasive procedures was less than 0.05.

Therefore these factors are significantly associated with infections due to *Acinetobacter* spp. The relative risk of these factors was also within 95% confidence interval.

The presence of an ET tube in-situ more than 12 days gave five times the risk of getting infections due to *Acinetobacter* spp.

The presence of a CVL in situ for more than 12 days had 4 times the risk of developing infections. A HD line in situ also had three times the risk developing *Acinetobacter* infections. Use of more than three invasive procedures per patient had almost ten times the risk of developing *Acinetobacter* infections

Long term ICU stay (more than 12 days) had six times the risk of developing *Acinetobacter* infections. Neurological illness, giving CPR and the use of inotropes had two times the risk of developing infections.

According to this study prior hospital admission, other medical illnesses such as DM, hypertension, COPD and gender were not identified as significant risk factors in developing *Acinetobacter* infections.

The P values of neurological illness, cardiopulmonary resuscitation, and use of inotropes was less than 0.05. So they were also identified as significant risk factors for *Acinetobacter* infections. The relative risks of these factors were also within the 95% confidence interval.

Antibiotic sensitivity pattern

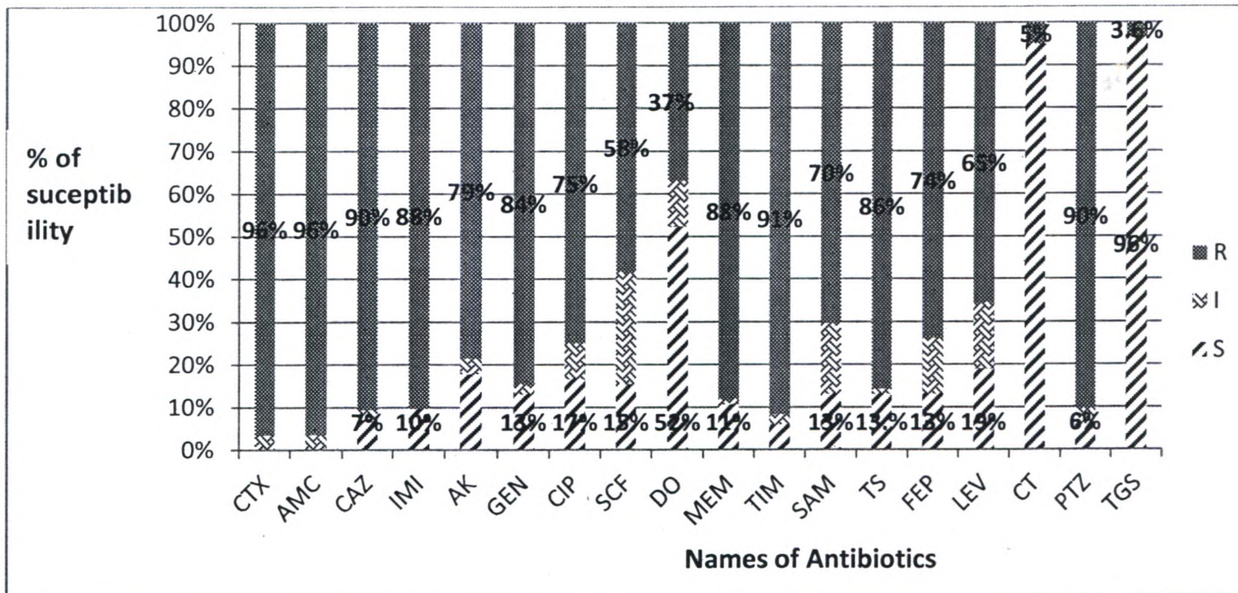

Figure 6: Antibiotic Sensitivity Pattern of Screening Isolates

R= Resistant, I=Intermediate, S=Sensitive

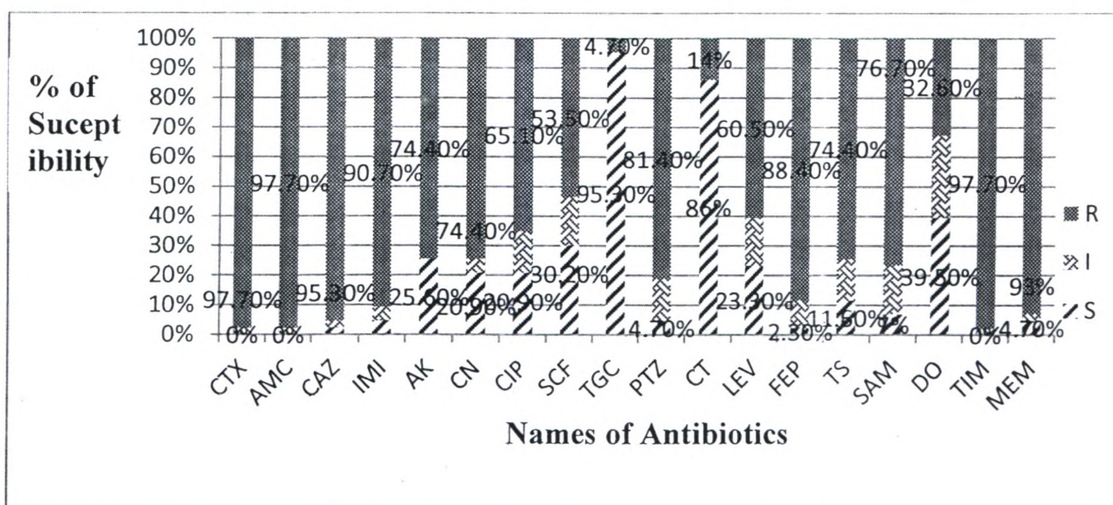

Figure 7: Antibiotics Sensitivity Pattern of Isolates causing infections

R=Resistant, I=Intermediate, S=Sensitive

Both infection causing and screening isolates show high levels of resistance to beta-lactam plus beta- lactamase inhibitors (>80% resistance in pipreracillin tazobactam). Resistance to 3rd generation cephalosporins in both types of isolates was more than 95%. Resistance to 4th generation cephalosporins was also high (resistance rate of cefipime – 73% in screening isolates and 88% in infected isolates).

When considering aminoglycosides, both amikacin and gentamicin showed similar resistant rates in infected isolates. But in screening isolates there was more resistance to gentamicin than amikacin. Fluoroquinolones such as ciprofloxacin and levofloxacin in both screening and infected isolates had more than 60%resistance.

Table 5: Antibiotic resistantance pattern to carbapenems in screening and infection causing isolates of *Acinetobacter* spp

	INFECTION CAUSING ISOLATES	**SCREENING ISOLATES**
CAR - R	95.3%	75%
CAR-S	4.7%	25%

CAR-R – Carbapenem resistant

CAR-S – Carbapenem sensitive

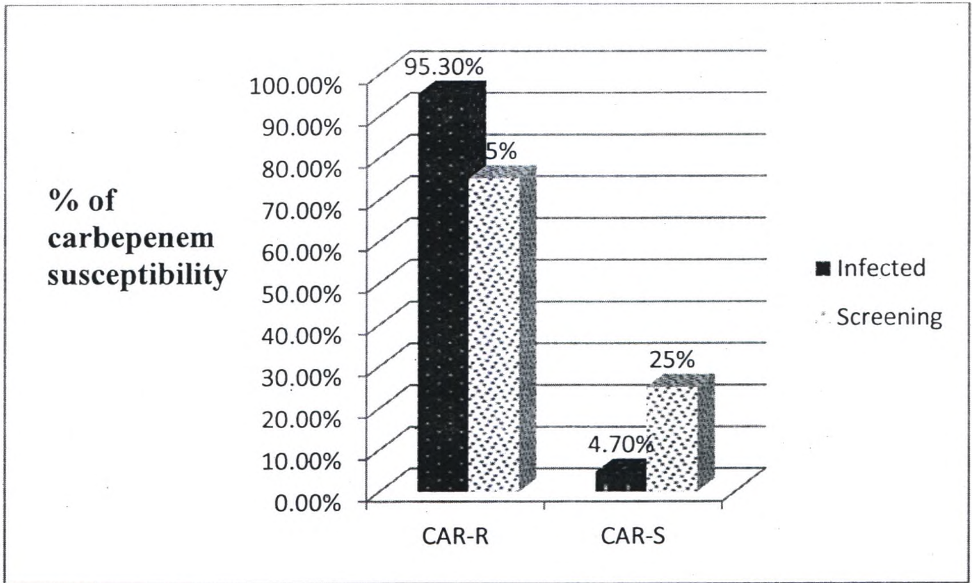

Figure 8: Comparison of carbapenem sensitivity between infections causing isolates and screening isolates

Table 6: Comparison of MDR between infection causing isolates and screening isolates

	INFECTED	**SCREENING**
MDR	74.7%	77.4%
Non-MDR	25.6%	22.6%

MDR – Multi-drug Resistant

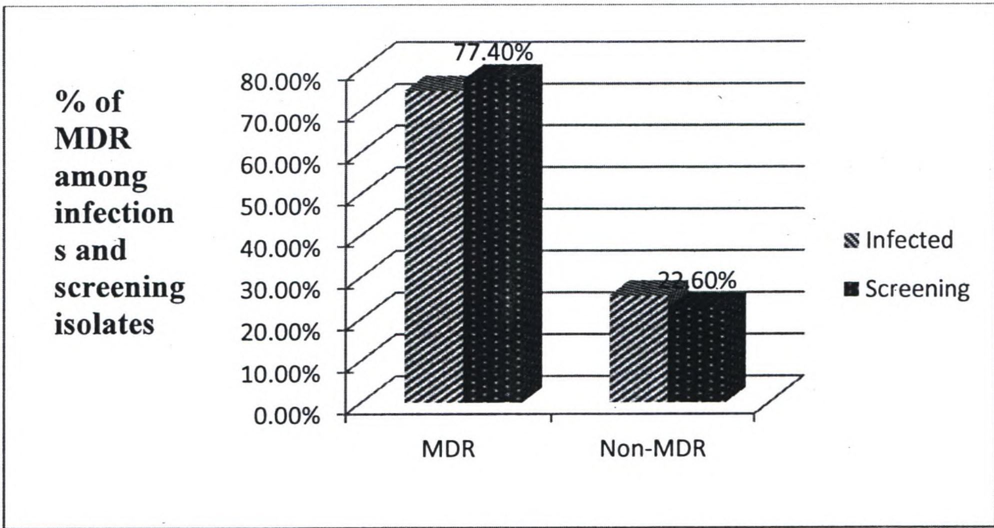

Figure 09 - comparison of MDR between infection causing isolates and screening isolates

The percentage of multidrug resistance infection causing isolates and screening isolates was similar.

Outcome of the patients with infected with *Acinetobacter* spp.

Table 7: Outcome of the patients infected and non-infected with *Acinetobacter* spp.

outcome	death	discharge	total
infected	15	23	38
Non-infected	14	61	75
total	29	84	113

Risk Estimation			
	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for outcome (discharge / death)	2.842	1.188	6.797

OR of the mortality rate of the patients infected with *Acinetobacter* spp. was 2.842 and lay within 95% C.I. of 1.188-6.797. Therefore mortality rate was higher among infected patients with *Acinetobacter* spp. than among non-infected patients.

Logistic regression was performed using outcome (death or discharge) as the dependent variables with infection status and hospital stay as independent variables.

Table 8: Logistic regression for analysis of *Acinetobacter* infections and outcome of the patients

		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
Step 1[a]	infected	1.044	.445	5.510	1	.019	3.432	1.188	6.797
	Constant	.427	.332	1.659	1	.198	1.533		

OR for infections status was 3.43 (95% CI is 1.188-6.797,p=0.19). There was significant relationship between infection status and mortality rate.

Logistic regression analysis was done with prolonged hospital stay (>12 days of hospital stay) as dependent variable as outcome measure and infections with *Acinetobacter* as independent variable.

Table 9: Logistic regression for analysis of *Acinetobacter* infections and long hospital stay

		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
Step 1[a]	infected	2.715	.501	29.353	1	.000	15.109	5.658	40.350
	Constant	-1.488	.418	12.645	1	.000	.226		

OR for infectious status were 15.109 when considering prolonged hospital stay as dependent variable. This OR within 95% CI (5,658-40.350), p value-0.000.

There was statistically significant difference between prolonged hospital stay among *Acinetobatcer* infected patients than non-infected patients.

CHAPTER 05

DISCUSSION

Acinetobacter spp. plays a major role in nosocomial infections especially among critically ill patients in the intensive care unit. This study was carried out over a 4 months period of time in the ICU setup of a tertiary care hospital in Southern province of Sri Lanka. As a developing country these special units are functioning amidst great problems such as financial restrictions, inadequate staff, poor quality equipment, drugs and sub optimal infrastructure which concomitantly lead to greater health care costs and high mortality and morbidity among critically ill patients.

Out of 276 admissions to these ICUs, 113 of patients were included in this study. 40 of these patients were discharged from ICU within 48 hours. 38 patients died within 48 hours due to the underlying disease condition of these patients. 40 of these patients were known to have infections other than *Acinetobacter* spp. 30 patients were lost to follow up. Other patients were excluded in the study due to difficulty in taking informed consents.

The age of the patients who were included in this study varied between 2 months to 83 years old. As well as majority in this study were male (69%). In hospital based study carried out in Qatar also showed that male predominance(76.2%) in *Acinetobacter* infections. (Saad et al. 2016)

The diversity of this special group of patients was varied due to their comorbidity factors. There were various reasons for admission to ICUs. Out of these reasons, sepsis was the most common cause for ICU admission (28.3 %). According to an Indian study done on *Acinetobacter* infections in a tertiary level intensive care unit, the most common reason to admission to ICUs was medical sepsis (38.3%). (Sara et al. 2012). Other than this factor there were several other factors which caused for ICU admission.

Infection rates caused by *Acinetobacter* spp. in ICUs

"Patients days in ICU" during the study period were 1536. Mechanical ventilator days and CVC days were 810 and 393 respectively. The number of External Ventricular device days was 178. Almost all patients were catheterized with urinary catheters.

During this period 38 of hospital acquired infections (HAI) due to *Acinetobacter* spp. were identified. Out of these HAI, 26 were device associated infections (DAIs). Our hospital acquired infection rate was 33% and device associated infection rate was 23%. Our infection rates were higher when comparing with the previous study done in ICUs in TH Karapitiya in 2010 on incidence of hospital acquired infections among intensive care unit patients at a tertiary care hospital in the southern province of Sri Lanka (Lewkebandara, 2010).

The percentage distribution of various hospital acquired infections due to *Acinetobacter* infection in this study were, VAP- 17.7% (24.69 cases per 1000 device days , 13.02 cases per 1000 ICUs days), bacteremia 10.6% (7.81 cases per 1000 ICU days) , CRBSI 3.5% (10.17 cases per 1000 device days, 2.60 cases per 1000 ICU days) and shunt infections 1.8% (11.23 cases per 1000 device days). We didn't detect catheter associated urinary tract infection due to *Acinetobacter* spp. in this study. The most common site of infection was the respiratory tract followed by blood stream infections in our study. A study done in Qatar also found the most common sites of infection due to *Acinetobacter* was respiratory tract followed by urinary tract. Blood stream infections was 3rd most common infection due to *Acinetobacter* spp in that study (Saad et al. 2016b).

Our incidence density rate due to *Acinetobacter* spp. is 24 cases per 1000 patient's days. This is higher than that found in developed countries and more or less similar to other developing countries'data. In a Korean study done on controlling an endemic multidrug-resistant *Acinetobacter baumannii* in Intensive Care Units using antimicrobial stewardship and infection control, found 22.82 cases per 1000 patient-days in a baseline investigation phase in 2013 and dramatic reduction of incidence density rate down to 2.68 cases per 1000 patient-days in 2014 after the interventions implemented (Cheon et al. 2016).

Infections due to *Acinetobacter* in any site of the body may lead to blood stream infections. The most common source of blood stream infection is the central line. The pneumonia, UTI, skin and soft tissue infections are other sites of infections subsequently lead to blood stream infection in susceptible individuals. (Rungruanghiranya S.*et al,*2005) The mortality rate due to blood stream infections with *Acinetobacter* spp was found to be 15 to 46 percent (Rungruanghiranya & Hon, 2005).The study done in Southeastern Brazil described the overall incidence rate of hospital acquired blood stream infections due to *Acinetobacter* was 15.8 per 10000 patient-days. This incidence of HA-BSI was less than our study (Lastoria et al. 2014). During the last few years the incidence of *Acinetobacter* pneumonia in ICU set-up has increased 6-30 percent (Rungruanghiranya & Hon 2005). Our incidence rate of VAP (17.7%) is within this range. According to the study done by Fagon and co-workers, mortality rate was 87% due to VAP caused by MDR *Acinetobacter* compared with mortality rate due to VAP caused by other organisms which was 55%.The study on multi-drug resistant *Acinetobacter* ventilator-associated pneumonia done in India recorded an incidence of 11.6% of VAP due to *Acinetobacter* spp. (Vishal B Shete, *et al,*2010)

The colonization incidence in our study was 35 cases per 1000 patients' days. As a percentage, colonization due to *Acinetobacter* was 47% and infection due to *Acinetobacter* was 33%. . The study done in Qatar found 64.3% (239/372) of infections due to *Acinetobacter* and 35.7 % (133/372) were considered as colonizers. In that study, the percentage of infections due to *Acinetobacter* was higher than in our study. But colonization due to *Acinetobacter* was similar when comparing data with our study (Saad et al. 2016).

A prospective study done in Spain revealed the overall incidence of *Acinetobacter baumannii* infection and/or colonization was 0.39 cases per 1000 patient-days ,ranging from 0 to 1.17 cases per 1000 patient-days depending on health care facility. This range varied from 0.14 to 4.55 cases per 1000 patient-days in ICUs (Rodriguez-Bano J,Cisneros JM,2004).

Risk factors associated with infections due to Acinetobacter spp.

An ET tube in situ more than 12 days, CVL in situ more than 12 days, presence of HD lines , use of more than three invasive procedures, colonization with *Acinetobacter* spp. , long term ICU stay , neurological illnesses , cardiopulmonary resuscitation, inotropes use were identified as significant factors associated with *Acinetobacter* infections in our study. According to the current study prior hospital admission, other medical illness such as DM, COPD, and HTN were not identified as significant risk factors associated with *Acinetobacter* infections.

A Korean study on risk factors for mortality in patients with *Acinetobacter baumannii* bacteremia revealed invasive procedures such as mechanical ventilation, CVL insertion , ICU stay as risk factors (Park et al. 2013).

1-2% blood stream infections due to *Acinetobacter* spp. associated with central lines had been reported (Gaynes R, Edward JR, 2005). Our catheter related blood stream infection rate was higher (3.5%). Even though local protocols on vascular lines and EVD have been established, staff adherence to recommendations is sometimes not satisfactory due to heavy work load.

Prior hospitalization, colonization with *Acinetobacter* spp, longer hospital stay, history of ICU stay in the recent past were identified as risk factors associated with *Acinetobacter* infections in a Taiwan study (Sheng et al. 2010).

Length of stay in ICU , mechanical ventilation, prior colonization and colonization pressure were described as risk factors for MDR *Acinetobacter baumannii* bacteremia in patients with colonization in the intensive care unit in a South Korean study (Jung et al. 2010).

Recent surgery, central vascular catheterization, tracheostomy , mechanical ventilation, enteral feeding and treatment with third-generation cephalosporins, fluoroquinolones or carbapenems were identified as risk factors for colonization and infections due to *Acinetobacter* spp.(Munoz-Price & Weinstein 2008)

Head injury, cerebral haemorrahage and chronic obstructive disease were found to be associated with VAP due to *Acinetobacter* in an Indian study carried out in 2010 (Vishal B Shete, *et al, 2010*)

Acinetobacter had been reported as the predominant cause of pneumonia (26%) in patients with more than 5 days of mechanical ventilation. In our study, the ventilator associated pneumonia rate was 17.7% having an ET tube in situ more than 12 days had five times at the risk in developing VAP due to *Acinetobacter* spp.

Colonization and colonization index as risk factor for *Acinetobacter* infections

In our study, skin and respiratory tract colonization were significantly associated with *Acinetobacter* infections. This skin and respiratory tract colonization had 2 times the risk of developing *Acinetobacter* infections in our study group. Colonization of more than 1 site of the body also had significant association with developing *Acinetobacter* infections having 4 times increased risk.

A colonization index of *Acinetobacter* spp. within the range of 0.33-1.00 had 4 times the risk of getting infections (p=0.000, RR= 4.053, 95% C.I. =1.949-8.431).

In our study, *Acinetobacter* colonization of the respiratory tract was 39.8%, which is five times more than a study which was carried out in the same institute 5 years back. That study revealed 7.89% of *Acinatobacter* respiratory tract colonisation. During that time period the most common respiratory tract colonizer was coliform spp. while no *Acinetobacter* spp was found as urinary catheter colonizers (Lewkwbandara R.H ,2012). But in our study 1.8% of urinary tract colonization was found.

Antibiotic resistance pattern

Multi-drug resistant or pan-drug resistant *Acinetobacter* spp. may be selected by the use of antibiotics especially fluoroquinolones. These resistant organisms can be passed from person to person most frequently via healthcare workers in hospital set-up or via environmental contamination (Paterson DL, 2006). In our study, among screening isolates more than 90% was resistant to 3rd generation cephalosporin and beta-lactam and beta-lactamase inhibitors. Infecting isolates also showed a similar pattern of antibiotics sensitivity with higher rates of resistance. Resistance to cefoperazone-sulbactam was 47.6% among screening isolates and 53.5% among infecting isolates. Resistance to doxycycline in screening isolates was 36.9%, while in clinical isolates it was 32.6%. Carbapenem resistance among screening isolates in our study was 75%. But carbapenam resistance in infected isolates was 95.3%. These

differences in antibiotic resistance pattern may be due to frequent treatment with these antibiotics in these patients.

A previous local study done in 2010 in the same institute, revealed increasing resistance to 3rd generation cephalosporins and beta-lactam and beta-lactamase inhibitors. Carbapenem resistance was more or less similar in both studies .Resistance to imipenem and cefoperazone sulbactam was more in our study than in the previous study. But aminoglycosides and ciprofloxacin had more sensitive rates in our study when comparing with the previous study carried out in the same institute 6 years back . These differences may be most probably due to over use of carbapenem and cefoperazon-sulbactam. Multi-Drug resistance among screening and clinical isolates was more than 70% in our study.

The study done in TH.Anuradhapura identified majority of *Acinetobacter* isolates were MDR (70%), comparing with our study which was more than 70% in both screening and clinical isolates. In this study sensitivity to tigecycline and colistin were 85.7% and 100% respectively, while carbapenem and cephalosporins had 100% resistance and more than 80% resistance to other antibiotics. In our study carbapenem resistance and cephalosporin resistance was more than 90% in clinical isolates. But sensitivity to tigecycline in clinical isolates was more than 90% and sensitivity to colistin was more than 85%(Wickramasingha, D. , 2012)

The study done at TH.Anuradhapura showed fairly high sensitivity for cefoperazone-sulbactam(S-46.6% , I-33,3% ,R-20%). Even though our study also showed fairly high sensitivity towards cefoperazone-sulbactam, sensitivity has been reduced compaired previous values (S-30.2%, R-53.5%,I-16.3%). In the present study, carbapenem resistance was more prevalent among *Acinetobacter* infected patients than among colonizing screening isolates. The study done in 15 hospitals in Brooklyn, New York revealed 53% isolates were resistant to one or both carbapenems (David Landman MD, John H, 2002).

When comparing our data (2016) with the Antimicrobial Resistance Surveillance Project(ARSP -2009/2010) data and WHO-NET data analyzed at Sri Jayawardanapura General Hospital(SJGH) in 2013, our data showed more resistance to commonly used antibiotics than the two other previous studies carried out 3-6 years

back in Sri Lanka. This indicates a dramatic increase in antibiotics resistance among *Acinetobacter* spp. With limited treatment options, this trend may lead to huge health care problems in a developing country. Therefore immediate actions should be taken to combat antibiotic resistance with a combination of strengthening of surveillance, initiation of antibiotics stewardship at local and national level and effective infection prevention programs.

Outcome of the patents infected with *Acinetobacter* spp.

A high mortality rate associated with *Acinetobacter* infected patients. Prolonged hospital stay was the significant consequence of the patients infected with *Acinetobacter* spp.

A study done in Spanish hospitals on clinical features and epidemiology of *Ainetobacter baumannii* colonization and infection revealed high mortality rate and longer hospital stay in patients with infections than colonization (Rodriguez-Bano J,Cisneros JM,2004).

Limitations

1. This study was performed in a single institute therefore results may not be generalized to other hospitals/other countries or may not represent the general population.

2. Molecular analysis was not done for the isolates so that the source/s of the pathogen or cross infection could not be established.

3. 30 days mortality rate was not included in this study due to limited time period to carry out the study.

4. CLSI break points for disc diffusion methods for cefoperazone-sulbactam and tigecycline were not available. Therefore different references were used to interpret these antibiotics sensitivity.

Recommendations for current practices

1. Implementation of infection control measures such as strict adherence to hand hygiene, and following aseptic techniques in invasive procedures.

2. Antibiotics stewardship

- restricted use of 3rd generation cephalosporins , carbapenems

- Implementation of guidelines for prescribing antibiotics.

3. Adherence to standard cleaning and disinfection protocols

4. Continuous education and awareness

- Programs to all categories of health care workers in ICUs

5. Adequate ICU staff – one to one care

6. Isolation facilities and dedicated instruments for patients with MDR pathogens.

Suggested further studies

Molecular analysis of the isolates from the infected patients with the environmental/staff sampling to establish common source/s and cross infection which will show the deficit in infection control

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ANNEXURE – 1

Instruction for specimen collection and transport for cultures (Laboratory Manual in Microbiology , The Sri Lanka College of Microbiologist, 2011)

1. ***Blood culture***

Types of specimens

i. Blood by venipuncture ii) Blood through intravascular catheters

Collection of peripheral blood

- Select the vein to be used for venipuncture.
- Clean with 70% alcohol followed by 7.5% povidone iodine and allow to dry.
- Label the bottle with patient identification details, date,time and site of collection
- Wipe top of the bottle with 70% ethyl alcohol and allow to dry.
- Wash hands with soap and water and wear sterile gloves.
- Volume of blood – 6-10ml for adults and 3-5ml for children.
- Inoculate into the blood culture bottle carefully.
- Thoroughly mix bottles to avoid clotting.
- Collection of blood from intravascular catheters
- Clean the catheter hub with alcohol or povidone iodine and allow adequate drying.
- Attach syringe to the hub, draw and discard some blood (3ml in adults).
- Using a sterile syringe,collect recommended volume of blood for culture through hub taking aseptic precautions and quickly reconnect tubing .
- Inoculate into the blood culture bottles as mentioned above.

Specimen transport and storage

- Do not refrigerate blood cultures.
- Specimens should be sent to laboratory with accompanying request form with patient identification details, clinical details, date ,time and site of collection.
- If immediate transport is not possible, blood cultures can be stored at room temperature.

Rejection criteria

- Specimen leaking from container
- Broken or cracked bottles
- Patient details on request form do not match on those on bottle
- Unlabeled specimen

2. ***Central venous catheter tips***

Specimen collection

- Clean skin at the insertion site using 70% alcohol and allow surface to dry.
- Remove catheter aseptically using sterile forceps and avoid contact of tip of the catheter with skin.
- Using a sterile pair of scissors, cut the distal 5-6cm of the catheter.
- Place the cut portion in a dry sterile screw capped bottle.

Specimen transport and storage

- Specimen should be sent to lab immediately with accompanying request form.
- Process catheter tip immediately.

Rejection criteria

- Specimen sent in non-sterile container
- Major delay to receipt of samples (>1 days)

3. ***Skin swab culture***

Sample collection

- Cotton swabs were used to collect samples from each patient.
- Swabs wet with sterile saline were rubbed each with a new swab , in a rotating manner from nostrils , axilla, groin .

Specimen transport and storage

- Specimen was processed immediately after collection.

4. ***Urine for culture***

Types of specimens

1. Midstream urine
2. Catheter specimens

Specimen collection

Midstream sample

- Instruct the patient to clean the genital area with soap and water.
- Collect the mid part of the urine flow into a wide mouthed sterile , dry, screw capped container.

Indwelling catheter sample

- Clamp the catheter and collect the urine aseptically using a sterile needle and syringe.
- Insert the needle in head to toe direction.

Specimen transport and storage

- Specimen should be transported to the laboratory as soon as possible.
- If there is a delay in transport keep the specimen at 4^0C. refrigeration should be for less than 24 hours.

Rejection criteria

- Unlabeled specimens
- Specimens refrigerated for >24h
- Catheter tips
- Leaking specimens
- Specimen kept at room temperature for >2h
- Specimen taken from a receptacle
- Unsterile container

5. *__Lower respiratory tract specimens__*

Types of specimens

1. Expectorated sputum

2. Endotracheal aspiration

Specimen collection and transport

Expectorated sputum

- Ask the patient gargle throat and rinse mouth with water (without antiseptics) and then collect at least 1 ml of sputum by deep coughing.
- Postural drainage with a physiotherapist would be helpful when expectoration is poor.

Induced sputum

- With the aid of a nebulizer, collect sputum into a sterile container.

Endotracheal aspirates

- Blunt aspiration through ET tube done under sterile conditions.

Specimen transport and storage

- Specimens should be transported and processed in the laboratory without delay, ideally within 2 hours of collection to maximize the recovery.

Rejection criteria

- Salivary sample
- Tips of endotracheal tubes
- Samples in unsterile containers
- Swabs of ET secretions
- Samples taken 24 hours ago
- Repeat sample taken on the same day.

6. *CSF obtained from EVD or shunt*

Sample collection

- Clean site of puncture with 70% alcohol.
- Using sterile needle/syringe aspirate CSF
- Collect 2-2.5ml of CSF into 4 bottles – 1. Fluoride containing bottle for CSF sugar estimation

  2. 1ml into sterile bottle for Microbiology

  3. for protein and cell count detection

  4. for additional testing

Specimen transport and storage

- Sent to lab immediately with properly filled request form
- Do not refrigerate CSF samples
- Process CSF immediately.

ANNEXURE-2

Standard operating procedures for the processing of specimens

(Laboratory Manual in Microbiology, The Sri Lanka College of Microbiologists 2011)

1. ***Processing of blood culture***

- Use BACTEC automated blood culture system for blood cultures.
- After indicating as positive ,withdraw 1ml of blood culture broth using a sterile needle and syringe.
- Inoculate blood agar and MacConkey agar
- Incubate at 35^0C overnight
- Place a drop of broth onto a clean glass slide
- Gram stain the smear and examine
- Perform direct ABST.
- Following day after identification of the organism ,follow the proper ABST
- In addition to biochemical tests , Rapid ID – one system for confirmation of identity

2. ***Central venous catheter tip***

Specimen processing – semi-quantitive method (Maki method)

- Roll the catheter tip 4-5 times on a blood agar plate using whole without touching the edge with use of a sterile pair of forceps.
- Incubate 35^0C overnight.

Readings

- Note presence/absence of growth
- If growth present count the number of colonies for each colony type.
- <15 colonies – not significant
- $\geq$ 15 colonies –significant
- Mixed growth of < 15 colonies of each types- not significant
- Identify and perform ABST isolate of $\geq$ 15 colonies

- Compare with simultaneously drawn peripheral blood culture if available for rule out Central Line Associated Blood Stream Infection (CLABSI).

3. **Processing of respiratory samples**

Sputum specimen processing

- Macroscopic appearance – mucoid, muco-purulent, muco-salivary.salivary,blood

  stained , purulent
- Microscopy – Gram stain
- Select purulent or mucopurulent portion of the sample to do Gram stain
- Observe under low power (10X10) for cells
- Under oil immersion , look for organisms
- Assess the quality of the specimen according to Murray and Washington's grading system.

Grade	Epithelial cells per low power field (X10)	Pus cells per low power field (X10)
Group 1	≥ 25	< 10
Group 2	≥ 25	10-25
Group 3	≥ 25	≥ 25
Group 4	10-25	≥ 25
Group 5	< 10	≥ 25

Murray and Washington's grading system for assessing the quality of sputum

- This classification is aid to differentiate infected sample with the clinical history from screening samples
- Inoculate on culture media and incubate overnight

4. ***Urine***

Specimen processing

- Urine samples should ideally be processed on arrival to the laboratory. If a delay is unavoidable sample should be refrigerated until processed.
- Note the appearance – clear, turbid , blood stained
- Mix the urine sample well.

- Flame calibrated wire loop and allow cooling.
- Insert the wire loop vertically into urine, insert only the loop part.
- Inoculate ¼ plate (9cm) or 1/8 plate (14cm plate) in an inverted cone shaped manner and incubate overnight.
- Interpretation and reporting according to the laboratory manual.

5. ***CSF samples obtained from EVD or shunt***

- If CSF sample > 1ml ,centrifuge the sample.
- Inoculate centrifuged deposits on culture media and incubate overnight.
- Microscopy – prepare the smear on a new slide , do not spread and allow to air dry and after

  that gentle fix the smear and Gram stain.
- If no growth after overnight incubation, reincubate for a further 24 hours.
- If there is no growth enrich the CSF samples by using BHI broth.

ANNEXURE- 3

Identification of the organism

Preparation of media

Blood agar, CLED and MacConkey agar were prepared according to the manufacturers 'instructions and poured into 9cm petri-dishes to a depth of 4mm. prepared plates were stored at 2-8^{0}C in a refrigerator and used within a week of preparation.

All plates were dried in an incubator at 35^{0}C for 15 min prior to inoculation.

Blood agar was used because *Acinetobacter* spp. grows well on blood agar. MacConkey agar was chosen because *Acinetobacter* spp. non lactose fermenters and give typical colony appearance.

Gram stain of isolates

A single colony was touched with a sterile wire loop and emulsified in a drop of normal saline on a clean glass slide to make a circle of 1cm in diameter. Smears were fixed by gentle heat over a flame.

Gram staining was done by using Ammonium Crystal violet, Lugol's Iodine, Iodine acetone and Carbol Fuchsin respectively. The stained smear was allowed to air dry and examined under oil immersion(X 100).

Hanging drop techniques

A circle was drawn with a grease pencil on the surface of a cover slip. A drop of an inoculum of suspected colonies in normal saline was placed on the cover slip. Grease was applied with use of a glass rod on four corner of the cover slip. A cavity slide was inverted over the coverslip. This slide was examined under microscope.(initially X10 power then X40 power) to detect motility.

Catalase test procedure

Presumptive single colonies of *Acinetobacter* from isolate on MacConkey agar were carefully immersed in the 3% hydrogen peroxide. Bubbling within 20 seconds was considered as positive reaction.

Oxidase test procedure

The wet filter paper method was used. A strip of filter paper was soaked with freshly prepared 1% solution of the oxidase reagent (Tetramethyl-p-phenylenediamine dihydrochloride). The wooden applicator which was touched with suspected colonies of *Acinetobacter* was rubbed on it. A colour change to blur purple within 5-10 seconds was recorded as positive and this organism was identified as belonging to the Pseudomonas genus. No colour change or colour change >60 seconds was taken as oxidase negative. A known isolate of *Pseudomonas aeruginosa* was used as a positive control.

RapID ONE System (remel) → by using manufacturers' instructions

RapID ONE system is a qualitative micromethod employing conventional and chromogenic substrate for the identification of medically important *Enterobactericeae* and other selected oxidase-negative, Gram negative bacilli isolated from human clinical specimens.

Procedure

Inoculum preparation

- Test organisms must be grown in pure culture and examined by Gram stain and oxidase prior to use in the system.
- Test organisms may be removed from a variety of selective and nonselective agar growth media, preferably isolate should be 18-24 hours old.
- Prepare inoculum by using RapID inoculation fluid (2ml) to achieve visual turbidity equal to #2 McFarland turbidity standards.
- Suspension should be mixed thoroughly and vortexed if required.
- Suspension should be used within 15 minutes of preparation.

Inoculation of RapID ONE panels

- Using a sterile pipette , gently transfer the entire contents of the inoculation fluid .
- Test cavities should appear bubble-free and uniformly filled.
- Slight irregularities in test cavity fills are acceptable and will not affect test performances.
- If the panel is grossly misfiled a new panel should be inoculated.

Incubation of RapID ONE Panels

- Incubate inoculated panels at 35-37^{0}C in a non CO_2 incubator for 4 hours.

Scoring of RapID ONE Panels

- Add 2 drops of RapID Reagents and RapID Spot Indole Reagents to relevant panels and allow at least 10 seconds but no more than 2 minutes for colour development.
- Record and score test cavity and obtain microcode.
- Compare this microcode with reference microcode in ERIC for the identification.

Quality control

- Testing of control organisms should be performed

ANNEXURE 4

CLSI susceptibility testing procedures

i) *Preparation of Mueller-Hinton Agar (MHA)*

MHA was prepared from a commercially available dehydrated base according to the manufacturer's instructions. Plates were used within three days after preparation. A representative sample of each batch of plates examined for sterility by incubating at 30 to 35^0C for 24 hours or longer. Plates were dried in an incubator at 35^0C for 15 minutes before carrying out the antibiotic sensitivity testing.

ii) *Inoculum*

Turbidity standard for inoculum preparation

0.5 McFarland standards (a $BaSO_4$ solution) were used to standardize the inoculum density for a susceptibility test.

Inoculum Preparation

An overnight pure culture of the test organism on blood or MacConkey agar was used to prepare an inoculum of an approximately standard number of bacteria. Three to five colonies were inoculated into sterile tube which containing 4-5ml of normal saline. Turbidity of the test organism was compared with the 0.5 McFarland standards. The density of the test suspension was adjusted by adding more organisms or more sterile saline.

iii) *Method of inoculation*

This inoculum should be used within 15 minutes after adjusting the turbidity. A sterile swab was dipped into prepared inoculum and excess inoculum was removed. This swab was streaked all over the medium three times by rotating the plate through an angle of 60^0 after each application. Finally the swab was passed round the edge of the agar surface.

iv)*Disc/ MIC strips application*

Antibiotics discs of standard strengths and MIC strips were used for the determination of antibiotic sensitivity. The antibiotic disc and MIC strips were placed on inoculated plates by using sterile forceps and pressed gently over the surface to ensure even

contact with the medium. Use a template to place the discs uniformly and place only 5 discs on a 9cm plate.

v) *Incubation*

Inverted plates were incubated in 35^{0}C within 15 minutes of preparation overnight.

vi) *Reading and measurement of zones of inhibition*

- Read after overnight incubation (16-18 hours)
- Measure the diameter of each zone (including diameter of the disc)
- MIC values were read according to the edge of the inhibition ellipse intersect the MIC stripes.