Report on

Isolation of Pathogens from Tracheal Infection of Intensive Care Unit Admitted Patients and Analysis of Antibiotic Susceptibility

By

Sabrina Haque
16226008

A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree of
B.Sc in Microbiology

Department of Mathematics and Natural Sciences

BRAC University

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
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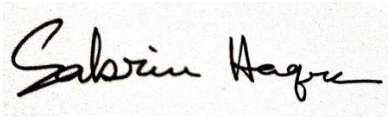

Sabrina Haque
16226008

Approval:

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[Sabrina Haque (16226008)]

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Examining Committee:

Supervisor:	_____
(Member)	Fahim Kabir Monjurul Haque, PhD Assistant Professor, Department of Mathematics and Natural Sciences BRAC University
Program Coordinator:	_____
(Member)	Mahbubul Hasan Siddiqee, PhD Assistant Professor, Department of Mathematics and Natural Sciences BRAC University
Departmental Head:	_____
(Chair)	A F M Yusuf Haider, PhD Professor and Chairperson, Department of Mathematics and Natural Sciences BRAC University

Ethics statement:

The departmental review board of BRAC University in Dhaka, Bangladesh, provided ethical clearance. IBN SINA Diagnostic and Imaging Center gave permission to complete my thesis in their microbiology laboratory. The respondent’s privacy and the data's confidentiality were strictly protected during data collection.

Abstract/ Executive Summary

Being a highly vulnerable group, ICU patients are prone to be affected by infectious diseases due to various invasive, operation procedures as well as their critical health conditions. Tracheal specimens were tested to determine the prevalence, types and susceptibility of microorganism against regular antibiotic including the 4th generation drugs. Prevalence of gram-negative bacteria outnumbered (81%) other microorganism, where *Acinetobacter spp.* & *Klebsiella spp.* contributed 56% of the total organisms. *Staphylococcus aureus* & *Candida spp.* were, however, the most prevalent gram-positive bacteria and fungi respectively. Among the five most prevalent bacteria other than *Staphylococcus aureus,* 90% or more were found to be resistant to multiple drugs. Whereas, around 60% of *Acinetobacter spp*. and *Pseudomonas spp.* were extensively drug resistant. Imprudent use of antibiotics, inattention of caregivers, inadequate disinfection of equipments, lacking protocol has been remaining the principal cause of this scenario. However, proper training, monitoring and motivation can combat the rapid spread of infectious microbes.

Acknowledgement

First of all, I would like to express my gratitude to the Almighty for giving me the strength to complete the thesis.

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Sabrina Haque

ID: 16226008

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Chapter - 1

Introduction

1. Introduction:

Since the invention of antimicrobials, antibacterial have been considered as an effective pathological solution. Antibiotics remains the widely used most reliable antibacterial medicine. Different types of antibiotics are effective against different group of microorganisms. Health care facilities, hospitals, nursing homes, and large animal farm are the primary places which uses large amount of antibiotics. As we know antibiotics works on bacteria's cytoplasm, chromosome, and cell membrane, on ribosome or on cell wall. Different types of antibiotic work better against different types of microorganisms. For instance, those antibiotic works better against gram-negative bacteria might not work as good against gram-positive bacteria due to its cell wall structure difference. As living cell microorganisms especially, bacteria can develop antimicrobial resistance in several ways. These antibiotic resistants' can be categories as intrinsic, adaptive and acquired antibiotic resistance (**Figure 1**). Intrinsic resistance is the innate response of an organism, whereas adaptive and acquired resistance depends on exposure to antimicrobials and genetic modification (Garner, n.d.). Excretion of incompletely metabolized or partially metabolized antibiotics from human and animal, disposal of unused drugs and pharmaceutical manufacturing process are the main sources of antibiotic contamination in environment (Grehs et al., 2020; Kumar & Pal, 2018). However, super availability, low cost, imprudent use – misuse or over use – of antibiotics are often considered as the main cause of increasing multi drug resistant bacteria (Zilahi et al., 2016). International health care organizations like, European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC), have used terms such as "crisis," "catastrophic consequences" and "nightmare scenario" to highlight the rapid emergence and spread of antibiotic resistance (Drinka et al., 2011; Martin-Loeches et

al., 2015; Michael et al., 2014; Singh et al., 2002; Viswanathan, 2014). Antibiotic resistance is a serious problem over the world including Asia–Pacific, Latin America, Middle East, Europe and North America regions (Zilahi et al., 2016). However, two regions contributing high burden of antimicrobial resistant are South-East Asia and the Middle East where antibiotics can be easily bought over the counter (Heddini et al., 2009; Zilahi et al., 2016). Considering an immense threat to public health WHO has also categorized spread of antibiotic resistant as one of the most three serious threats in the twenty-first century (WHO, 2014).

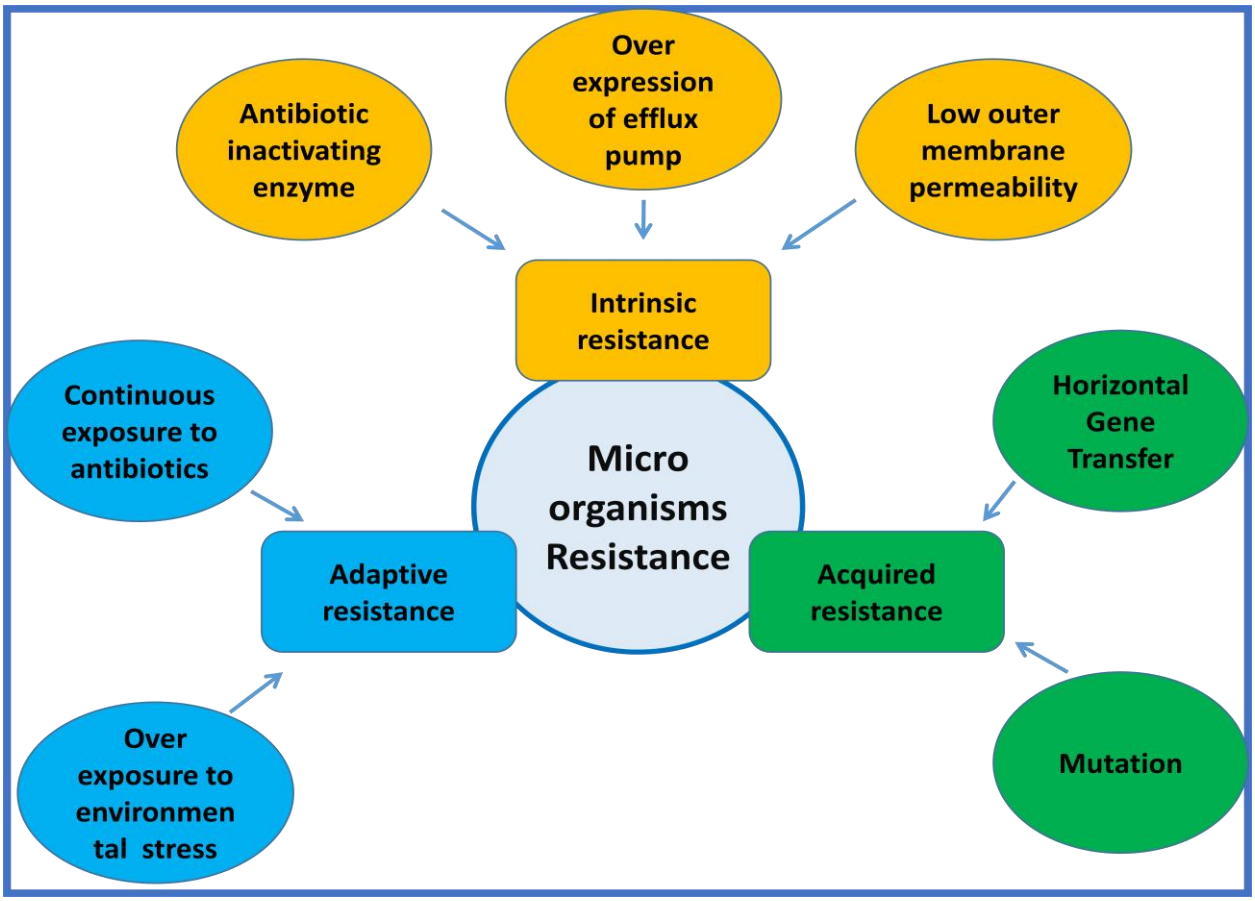

Figure 1: Antibiotic resistant mechanism.

Incidence of drug resistance organism is increasing day by day in health care settings. Though antibiotic resistant microorganisms are ubiquitous, recent research showed that increase is exponential in Intensive Care Unit (ICU) (Pachori et al., 2019). ICUs are the major facilitators in Creation, dissemination, and magnification of drug resistant organisms at health care facility (Pachori et al., 2019). Usually critically ill patient admitted in ICUs have the higher risk of acquiring nosocomial infections by resistant strains (Jamil et al., 2016; Singh et al., 2002).The rate of nosocomial infection is about 2 -5 times higher in the intensive care unit (ICU) than general in-patient hospital population (Pachori et al., 2019). It has been observed that ICUs are the major source of both gram-positive and gram negative bacteria which considered as one of the major causes of infectious diseases (Vincent et al., 2009). Since the last decade *Pseudomonas spp., Acinetobacter spp., Klebsiella spp., Citrobacter spp., Escherichia coli* and *Candida spp*. have been found consistent at ICUs (Bhandari et al., 2015; Gonlugur et al., 2004; Jamil et al., 2016; Japoni et al., 2009; Jesmin et al., 2021; Lagacé-Wiens et al., 2008; Mukhopadhyay et al., 2003; Singh et al., 2002). Three categories of Gram negative bacteria (GNB), namely (Extend spectrum beta-lactamase) ESBL producing *Escherichia coli*, and *Klebsiella spp.,* Multi drug resistant (MDR) *Pseudomonas spp.,* and carbapenem resistant *Acinetobacter spp.* were addressed as high priority bacterial pathogen by Infectious Disease Society of America due to their ubiquitous presence at healthcare facilities (Talbot et al., 2006).

Most common diseases respiratory, catheter-related bacteremia, non-catheter related bacteremia, secondary peritonitis, surgical wound infections and a few urinary tract infections are most common at ICU (Papia et al., 1999; Vosylius et al., 2003). Patients have an increased risk of acquiring infection is due to the diminished host defense, suppressive immune condition with the use of multiple procedures and invasive

devices such as mechanical ventilations, central venous catheterization (CVC), and urinary tract catheterization (Jesmin et al., 2021; Ranjan et al., 2014). Likewise, critically ill ICU patient usually go through several aspects that makes them highly vulnerable to various microbes. Firstly, due to their critically health condition suppress their natural immunity and leads them to acquire nosocomial infections (Lim & Webb, 2005). Secondly, lines and tubes usually used to monitor the condition of patient can facilitate opportunistic pathogens bypassing the natural barriers of the host (Lim & Webb, 2005). And finally, critically ill patients need a consistent care from the hospital staffs providing opportunities for cross contamination from other potential patients or the environment (Chastre & Trouillet, 2000; Reuter et al., 2002). The common sources of the pathogens could be patients own flora, visitors, ICU environment like water, air, foods, and equipments, health care workers, other patients, or inanimate objects that are in close surrounding area of patients works as a potential reservoir and sometimes work as facilitator (Bhandari et al., 2015).

Among the frequently occurred diseases at intensive care unit lower respiratory tract infections are more common in ICUs; around 10-25% ICU patient acquired these infections and results a huge toll of mortality ranges from 22-71% (Jamil et al., 2016). Most common causes of acquiring such infections are multidrug resistant microorganisms. Recent studies have reported most of the frequently isolated microorganisms from ICU are multidrug resistant and these are the common phenomena all around the globe (Bhandari et al., 2015; Frattari et al., 2019; Jamil et al., 2016; Japoni et al., 2009; Jesmin et al., 2021; Lim & Webb, 2005; Magiorakos et al., 2012; Nagarjuna et al., 2018). A study of Nepal ICUs reported 83.1% of the isolated organisms from patient tracheal aspirates were Multidrug Resistant (Bhandari et al., 2015). A review predicted anti-microbial resistant might cost around 10 million

death worldwide within next 30 years (Meyer et al., 2010). Antibiotic resistant not only shrinking the choice of available antibiotic, but also increasing hospital stay (S. Blot et al., 2003; S. I. Blot et al., 2003; Heyland et al., 1999) along with huge amount of healthcare cost (Dimick et al., 2001; Heyland et al., 1999). Loss of working hour along with out-of-pocket health care cost due to extended hospital stay forces people below the poverty-line. Antibiotic resistance varies over time, geographic area, availability, local production and uses it can even happened in different health care setting within a certain area (Jamil et al., 2016; Khan et al., 2014). For instance, carbapenem resistance remains at relatively low levels in northern Europe whereas constitute a serious threat worldwide as a consequence of acquisition of carbapenems genes with a higher prevalence in southern Europe and Asia (Zilahi et al., 2016). Time to time monitoring of the local health care settings specially the ICUs is required to prevent or reduce the infection of multidrug resistant opportunistic pathogens. Thus, the aim of this study was to know the most prevalent microorganisms and their susceptibility against frequently used antimicrobials in a local hospital's ICU.

Chapter - 2

Objectives

2. Objective:

2.1 General objective:

- To identify the microorganisms and their susceptibility against widely used antibiotics from the tracheal specimen of ICU patients.

2.2 Specific objectives:

- To identify the common microorganism at patient's tracheal specimen.
- To identify the pathogens that causes tracheal or respiratory infection at ICU patients.
- To determine the susceptibility pattern of the organisms against widely used antibiotics.
- To recommend essential steps to mitigate the multi drug resistant infection.

Chapter - 3

Methodology

3. Methodology

3.1 Sample collection site:

Study area of this study was a renowned diagnostic center with hospital, namely “Ibn Sina Diagnostic and Imaging Center”, Dhaka, Bangladesh. It has been operating as a diagnostic center with hospital and ICU facilities. As a diagnostic center with hospital, it remains one of the popular and busiest ICU facilities of Dhaka city. In this study 200 specimen were collected from the participant’s tracheal aspiration.

3.2 Sample collection technique:

Sample was collected from tracheal infection area of ICU patients.

Tracheal aspiration samples were collected from Endotracheal tube connectors of ICU patients by following the guideline by Irwine & Pratter (Irwin & Pratter, 1981). Collected specimens were then handled carefully by following the same protocol and after that specimen were cultured into the designated media.

3.3 Bacterial Culture:

After careful transportation of the specimens were streaking to three different agar media namely Blood agar, MacConkey agar & Chocolate agar. After streaking they left to incubate for the next 24 hours. The first check for colony formation was taken after 24 hours if the media won’t show the colony growth; they leave for 24 more hours to incubate. After incubating for a total of 24 hours the media were checked for the second time, if they won’t provide satisfactory results, they will leave for further 24 hours before the last check. After gating the growth of microorganism an inspection of colony morphology was take place for primary identification of microorganisms and their gram staining characters. This identification process

followed the bellow table for primary determination of microorganisms and their probable type.

Table 1: Expected colony morphology for microorganisms

Organism	Color Appeared						
	Colony Shape	Colony color			Media color		
		MacConkey	Blood	Chocolate	MacConkey	Blood	Chocolate
E. coli	Circular	Pink	Colorless	White	Yellowish or pink	Red	brown
Klebsiella spp.	Mucoid	Deep pink	White	Off-white	Pinkish	Red	brown
Pseudomonas spp.	Circular	Greenish white	White	Colorless	Greenish pink	Red	Greenish brown
Staphylococcus aureus	Circular	X	Hemolytic	Cream	X	Orange	brown
Acinetobacter spp.	Circular	Bright pink	White	White	Purple	Orange	Light brown

A detail biochemical test was performed to identify the organisms correctly. Among the several biochemical testes TSI, MIU, Citrate utilization test, Coagulase test, Catalase test, and lastly Oxidase test were performed for this study.

Table 2: Biochemical Test

Organisms	TSI Slant / butt / gas	MIU Motility / Indole / Urease	Citrate Utilization Test	Coagulase Test	Catalase Test	Oxidase Test
E. coli	**A / A / +**	**+ / + / -**	**-**	**-**	**+**	**-**
Klebsiella spp.	**A / A / +**	**- / - / +**	**+**	**X**	**+**	**-**
Pseudomonas spp.	**A / A / +**	**+ / - / -**	**+**	**+**	**+**	**+**
Staphylococcus aureus	**K / K / -**	**- / - / -**	**-**	**+**	**+**	**-**
Acinetobacter spp.	**A / A / -**	**- / + / +**	**+**	**+**	**+**	**-**

*A= Acidic; K=Alkaline.

After observing the growth at the first phase in different media microorganisms other than bacteria were inoculate in Sabouraud's Dextrose Agar (SDA) for better growth. These media were left for another 21 days to form colony.

A cotton stick was used to inoculate the identified microorganisms into a nutrient ager plate with a known antibiotic disc to observe the resistant pattern of those microbes against the antibiotic. These plates were left for 24 hours to incubate, after incubation these plates were observed to identify if the organisms were resistant, sensitive or intermediate. Table below was used to identify the status:

Table 3: Antibiotics and Diameters

Antibiotics	Symbol	Disc content	sensitive	intermediate	Resistance
Amikacin	AK	30mcg	17	15-16	14
Amoxyclav	AMC	mcg	13	14-17	18-20
Amoxycillin	AUG	20/10mcg	18-20	14-17	13
Ampicillin	AMP	10mcg	17	14-16	13
Aztreonam	AT	30mcg	22	16-21	15
Cefepime	CPM	30mcg	18-26	15-17	14
Cefixime	CFM	5mcg	18	15-17	17
Cefotaxime	CTX	30mcg	23	15-22	14
Ceftazidime	CAZ	30mcg	18	15-17	14
Cefuroxime	CXM	30mcg	18	15-17	14
Ceftriaxone	CTR	30mcg	21	14-20	13
Cephalexin	CN	30mcg	18	15-17	14
Ciprofloxacin	CIP	5mcg	21	16-20	15
Cloxacillin	COX	10mcg	15	11-12	10
Colistin	CL	50mcg	11	-	10
Cotrimoxazole	COT	25mcg	16	11-15	10
Levofloxacin	LE	5mcg	19	16-18	15

Antibiotics	Symbol	Disc content	sensitive	intermediate	Resistance
Gentamycin	GEM	10mcg	15	13-14	12
Imipenem	IMP	10mcg	16	14-15	13
Netilmicin	NET	30mcg	15	13-14	12
Meropenem	MEC	20mcg	16	14-15	13
Linezolid	LZ	30mcg	21	18-19	20
Fusidic Acid	FC	10mcg	13	11-12	10
Tazobac/Piperacillin	PIT	10/100mcg	21	18-20	17
Tigecycline	TGC	30mcg	19	15-18	14
Vancomycin	VA	30mcg	15	-	14

3.4 Evaluation of antibiotic resistance:

Resistant:

Antibiotic resistant was defined as, the organisms were previously susceptible but now it shows resistant against exposed antimicrobials.

Criterion for Multidrug Resistance:

Multidrug resistance was defined according to the standardized international terminology to describe acquired resistance profiles in multi drug resistant organisms (MDROs). This terminology was created by a group of international experts brought together by a joint initiative between the ECDC (European Center for Disease control) and the CDC (Center for Diseases Control). MDROs have been divided into three categories depending on their resistance profile: 1. MDROs—non-susceptible to at least 1 agent in 3 antimicrobial categories; 2. extensively drug-resistant (XDR) organisms non-susceptible to at least 1 agent in all but 2 or fewer antimicrobial categories; and 3. pan-drug-resistant (PDR) organisms—non-susceptible to all agents

in all antimicrobial categories (Magiorakos et al., 2012; Zilahi et al., 2016). In this study if an organism non susceptible to at least 1 agent in less than 3 antimicrobial categories has considered as Not-MDRO and named as single drug resistant (SDR).

Chapter - 4

Results

4. Results

4.1 Demographic characteristic of the participant:

Majority of the participants of these studies are old adult aged 60 or more (64%), however around 11% of the participants are representing the age group of young adults (**Table 4**).

Table 4: Participants Age

Age of the respondent	Frequency	Percent
≤35	22	11%
36 – 59	51	25%
≥60	127	64%

Among the 38% female study participants 22% were aged 60 years or more, 12% of female ranges between 36 – 59 years of age whereas 42% of male represent the age group grater or equal 60 years (**Figure 2**).

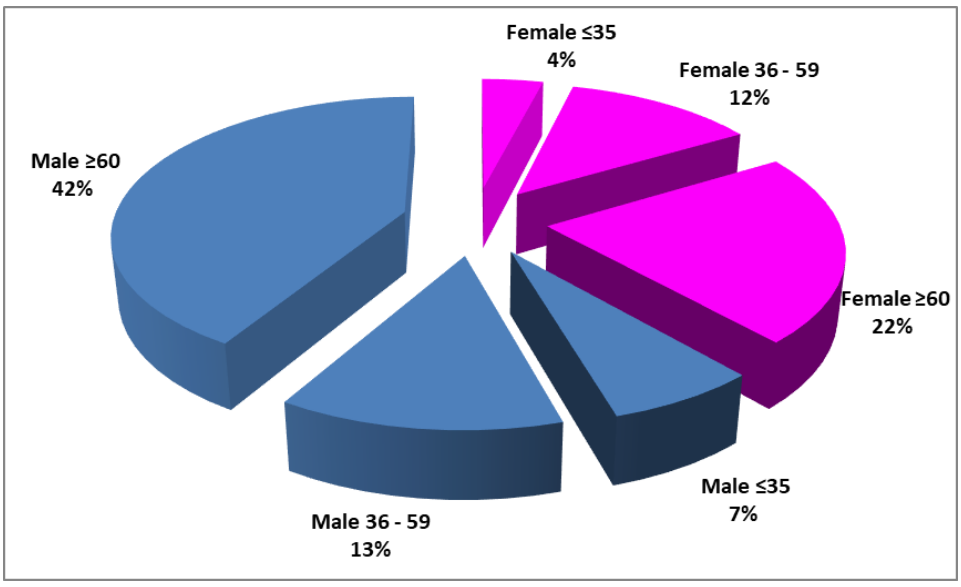

Figure 2: Sex wise age category of participants.

4.2 Isolated Organisms:

At first colony characteristics of isolated organisms were observed on agar plates and identify the gram positive and gram- negative bacteria through gram staining. Sometimes the media changes the actual color because of the growth of bacteria. (**Table 2**)

Figure 3: Growth of isolated organisms in different media.

Gram positive isolates were further identified by using catalase, oxidase, coagulase, and optochin sensitivity tests while for identification of Gram- negative isolates different biochemical tests like catalase, oxidase, motility, H_2S and indole production, citrate utilization, MR- VP, urea hydrolysis, and triple sugar iron utilization were done and then identified based on their results. (**Table 2**)

This study found Gram-negative bacteria (81%) was the most prevalent of isolated organism, whereas only 10% of the organism was Gram-positive (**Figure 4**).

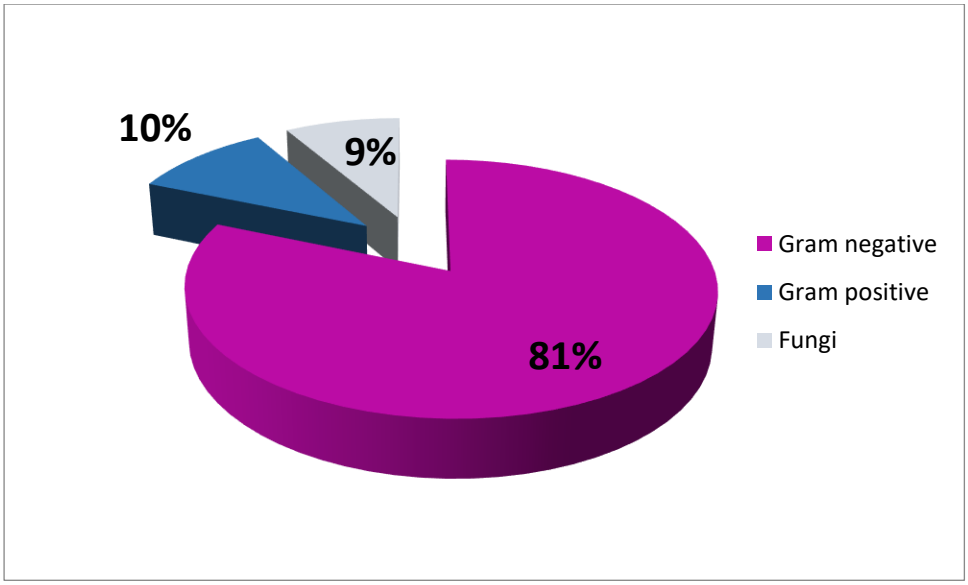

Figure 4: Isolated organisms

Among the gram-negative microorganism *Acinetobacter spp.* & *Klebsiella spp.* are consist of more than 56% of the all organisms, whereas *Enterobacter spp., Moraxella spp., Proteus spp., Saprophytic fungi* & *Staphylococcus spp*. were the most less prevalent organism altogether added up to 3.65% of all organisms (**Table 5**).

Table 5: Types of Microorganisms

Gram stain type	Isolated organism	n (%)	Total
Gram-negative	*E. coli* *Pseudomonas spp.* *Klebsiella spp.* *Moraxella spp.* *Enterobacter spp.* *Acinetobacter spp.* *Proteus spp.*	25 (9.16) 38 (13.92) 60 (21.98) 2 (0.73) 2 (0.73) 93(34.07) 2 (0.73)	222 (81.32)
Gram-positive	*Staphylococcus aureus* *Staphylococcus spp.* *Streptococcus spp.* *Enterococcus spp.*	16 (5.86) 2 (0.73) 6 (2.20) 4 (1.47)	28 (10.26)
Fungi	Candida spp. Other Fungi	20 (7.33) 3 (1.1)	23 (8.43)

This study found that around 66% of the participants were infected by single microorganism whereas around 34% respondents were infected with multiple microorganisms (**Table 6**)

Table 6: Infection status of participants

Type of infection	**Frequency**	**Percent**
Single microorganism	132	66%
At least two microorganisms	62	31%
At least three microorganisms	6	3%
Total	200	100%

4.2.1 E. coli:

This study found isolated *E.coli* strains are highly resistant to penicillin drugs (Ampicillin 100%, Amoxicillin, Cloxacillin, Amoxicillin individually the same 96%), Cephalosporin (Cefixime 100%, Cefepime 92%, 96% *E. coli* were found resistant to other drugs of this group namely cefotaxime, Ceftazidime, Cefuroxime, Ceftriaxone, Cephalexin). A concerned level of resistant showed by the organism against Linezolid 96%, Vancomycin 96%, Fusidic acid 96%, and Aztreonam 96%. However, Colistin 92%, Tigecycline 72% showed a high level of sensitivity against the isolated *E. coli*. Whereas Aminoglycosides (Amikacin 32%, Netilmicin 44%, Gentamycin 20%), Carbapenem (Imipenem 44% & Meropenem 24%), Tazobactam and Piperacillin 24% showed a moderate level of sensitivity against the organisms (**see figure-5**).

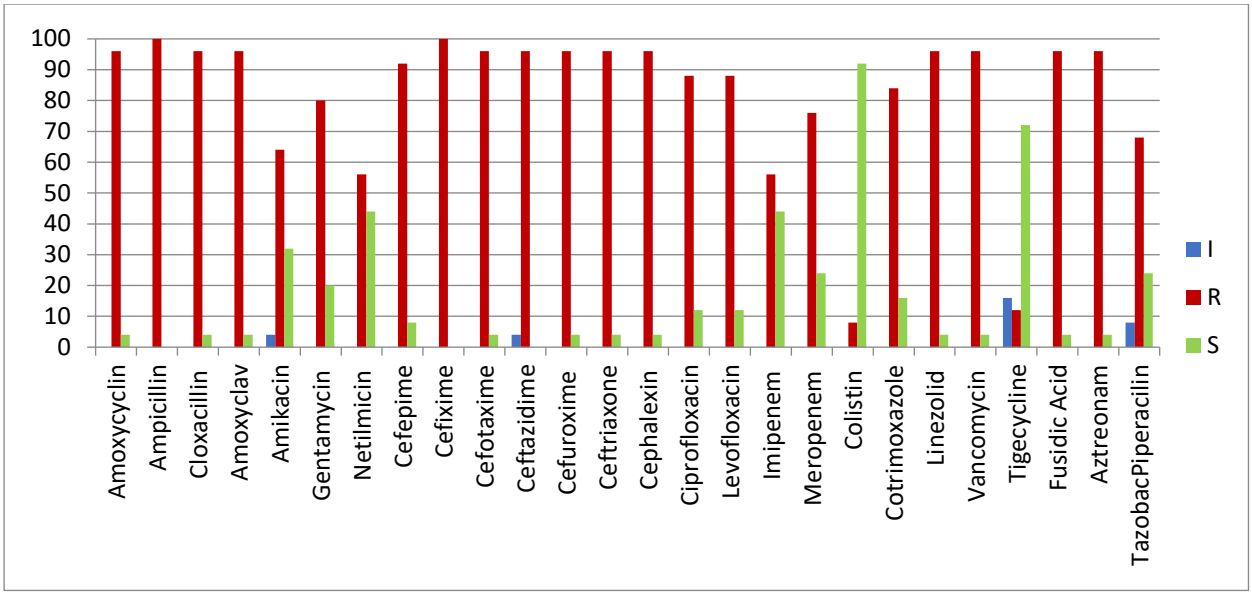

Figure 5: Antibiotic resistance pattern of *E.coli spp.*

4.2.2 Acinetobacter spp.:

Although sensitivity against Peptide (Colistin 88%), Tigecycline 72%, and sulfonamide 34% looks promising (**Figure 6**), an alarming percentage 99% of the organisms were resistant against most of the drug groups -Penicillin, Cephalosporine, Carbapenem, Oxazolidinone, Glycopeptide, and Fusidane - were tested in this study have made the *Acinetobacter spp.* a great matter of concern.

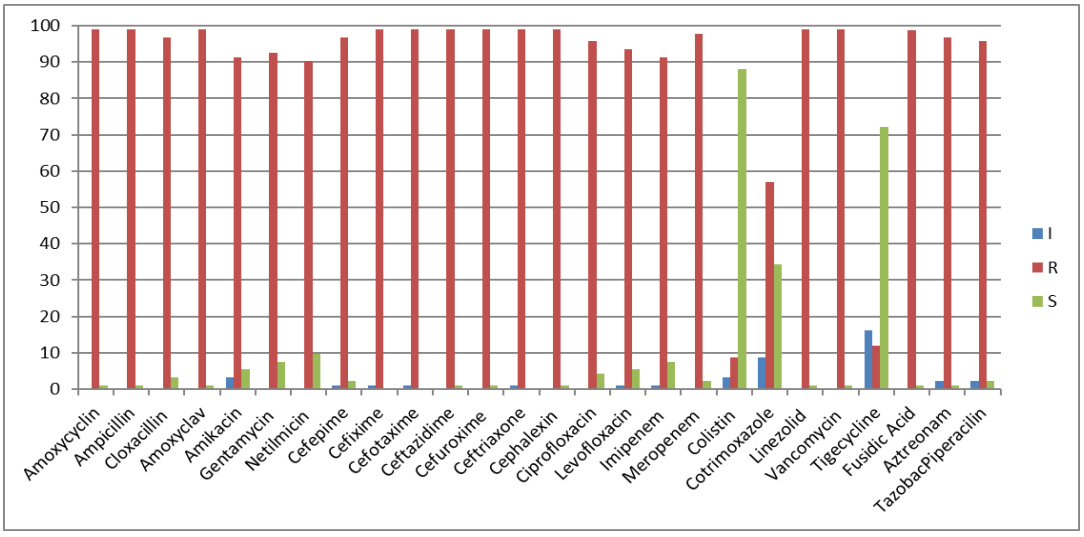

Figure 6: Antibiotic resistance pattern of *Acinetobacter spp.*

4.2.3 Klebsiella spp.:

Antibiotics have shown mixed reaction against *Klebsiella spp.* Antibiotics like Colistin, Tigecycline, Gentamicin & Imipenem can be a potential solution against *klebsiella spp.* as it shows a high to moderate sensitivity against those. However, these organism species showed a high level of resistances against some of the fine antibiotic groups namely Penicillin, Cephalosporine, Glycopeptide, Fusidane and Monobactam (**Figure 7**).

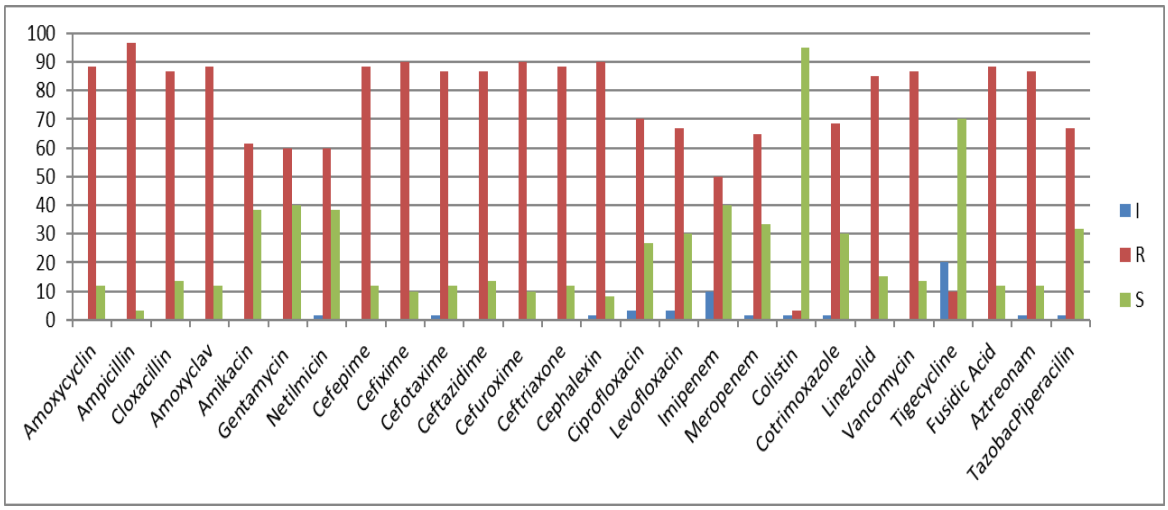

Figure 7: Antibiotic resistance pattern of *Klebsiella spp.*

4.2.4 Pseudomonas spp.:

As a potential antibiotic Colistin (84%, TazobacPiperacilin (61%) produces a high level of sensitivity against *Pseudomonas spp.* (**Figure 8**). Whereas Pseudomonas spp. were moderately sensitive to Aminoglycoside drugs, Monobactam, Carbapenem, and Cefepime. Most of the drugs from penicillin, Cephalosporine, Fluroquinolone, Fusidane, Glycopeptide and Oxazolidinone were highly resistant against this species.

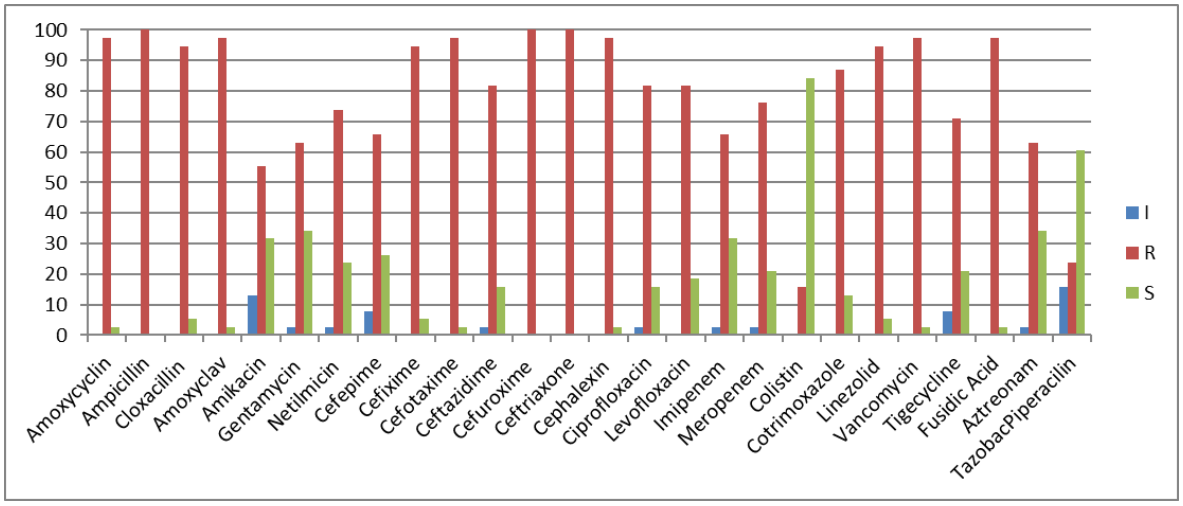

Figure 8: Antibiotic resistance pattern of *Pseudomonas spp.*

4.2.5 Staphylococcus aureus:

Staphylococcus aureus seems to be susceptible to various drugs tested in this study. This species was found highly sescetive to Vancomycine (100%), Netlmicin (81%), and Linezolid (81%). It was moderately sencetive to Fusidic acid(75%), Tigecycline(75%), Cotrimoxazole(69%), Gentamycin (63%), Imipenem (56%), Amikacin (56%), and Meropenem (50%) (**Figure 9**).

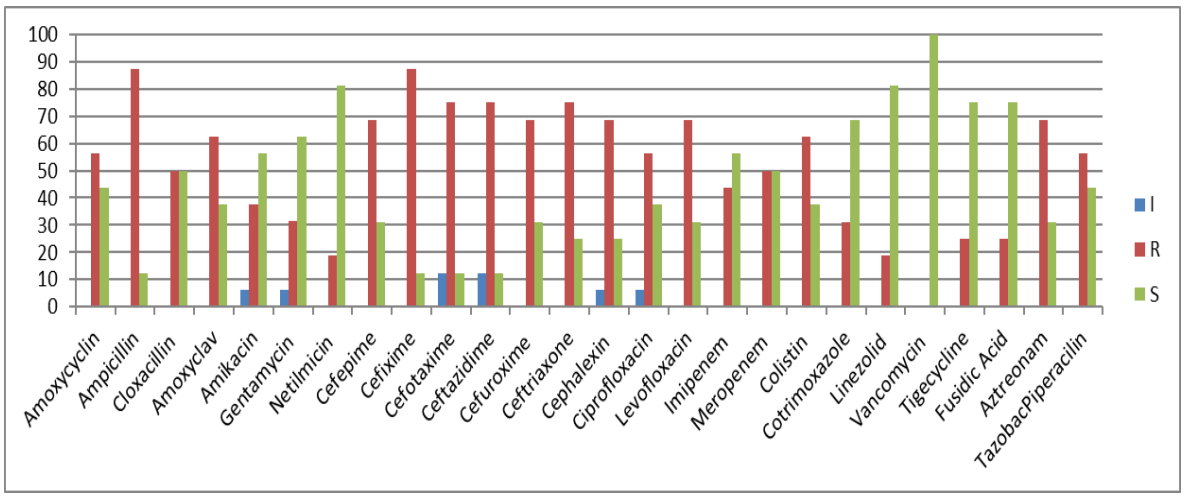

Figure 9: Antibiotic resistance pattern of *Staphylococcus aureus.*

However, this study found that isolated *staphylococcus aureous*were highly resistant to Ampicilin (88%),Cefexime (88%), cefotaxime(75%), Ceftazidime(75%), Ceftriaxone(75%) and were moderately resistant to Cefepime(69%), Cefuroxime(69%), Cephalexin(69%), Levofloxacin(69%), and Amoxyclav(63%).

Table 7: Isolated organisms and antibiotic Resistantance.

Group name	**Drug name**	**Gram Negative**				**Gram Positive**
		Acinetobacter spp.	***Klebsiella spp.***	***E. coli***	***Pseudomonas spp.***	***Staphylococcus aureus***
		%	**%**	**%**	**%**	**%**
Penicillin	Amoxicillin	98.92	88.33	96	97.37	56.25
	Ampicillin	98.92	96.67	100	100	87.5
	Cloxacillin	96.77	86.67	96	94.74	50
	Amoxyclav	98.92	88.33	96	97.37	62.5
Aminoglycoside	Amikacin	91.4	61.67	64	55.26	37.5
	Gentamycin	92.47	60	80	63.16	31.5
	Netilmicin	90.32	60	56	73.68	18.75
Cephalosporine	Cefepime	96.77	88.33	92	65.79	68.75
	Cefixime	98.92	90	100	94.74	87.5
	Cefotaxime	98.92	86.67	96	97.37	75
	Ceftazidime	98.92	86.67	96	81.58	75
	Cefuroxime	98.92	90	96	100	68.75
	Ceftriaxone	98.92	88.33	96	100	75
	Cephalexin	98.92	90	96	97.37	68.75
Fluoroquinolones	Ciprofloxacin	95.7	70	88	81.58	56.25
	Levofloxacin	93.55	66.67	88	81.58	68.75
Carbapenem	Imipenem	91.4	50	56	65.79	43.75
	Meropenem	97.85	65	76	76.32	50
Peptide	Colistin	8.6	3.33	8	15.79	62.5
Sulfonamide	Cotrimoxazole	56.99	68.33	84	86.84	31.25
Oxazolidinone	Linezolid	98.92	85	96	94.74	18.75
Glycopeptide	Vancomycin	98.92	86.67	96	97.37	0
Tigecycline	Tigecycline	11.83	10	12	71.05	25
Fusidane	Fusidic Acid	98.91	88.33	96	97.37	25
Monobactam	Aztreonam	96.77	86.67	96	63.16	68.75
Tazobactam + Piperacillin	TazobacPiperacilin	95.7	66.67	68	23.68	56.25

Among the isolated microorganisms this study found around 99% of the *Acinetobacter spp.*& 92% of *Pseudomonas spp.* were multidrug resistant whereas about 60% *Acinetobacter spp.* and 58% *Pseudomonas spp.* were extensively drug resistant. However, only 31% of *Staphylococcus aureus were multidrug resistant* (**Table 8**).

Table 8: Resistancy status of the isolated organisms.

Isolated organism	SDR n (%)	MDR n (%)	XDR n (%)	PDR n (%)
Acinetobacter spp.	1 (1.08)	92 (98.92)	56 (60.22)	NA*
Pseudomonas spp.	3 (7.89)	35 (92.09)	22 (57.89)	NA*
Klebsiella spp.	6 (9.99)	54 (90.01)	NA*	NA*
Staphylococcus aureus	11 (68.75)	5(31.25)	NA*	NA*
E. coli	1 (4.00)	24 (96.00)	NA*	NA*

* Didn't test all the antibiotics required to declare XDR and PDR.

4.3 Comparing Candida and fungi with other microorganisms:

In comparison to other microorganism species prevalence of *Candida spp.* was very low around 7% and this study found only one case of other fungi in the isolated samples (**Table 9**).

Table 9: Prevalence of Fungi.

Isolated organisms	Frequency	Percent
Candida spp.	20	7.33
Other fungi	1	0.37
Other organism	252	92.3

Chapter - 5

Discussion

5. Discussion

This study found that *Acinetobacter spp., Klebsiella spp., Pseudomonas spp., E. coli* and *Candida spp.*, and *Staphylococcus aureus* are the most prevalent microorganism spreads nosocomial infection at urban hospital ICU settings. However, in comparison of *Candida spp.* the presence of other fungi was minimal. High presence of infectious microorganisms in the participant throat is due to ventilation mediated transfer, poor management and Compromised disinfection of ICU equipments. A retrospective study done by Dereli et al. and studies from bangladesh , Jamil et al., and Jesmin et al. also (Dereli et al., 2013; Jamil et al., 2016; Jesmin et al., 2021) reported similar prevelence of microorganisms as this study. In recent time infection with *Acinetobacter spp.* has become a great threat to ICU admitted patients because of its frequent presence and highly drug resistant properties. Result of this research showed *Acinetobacter spp.* was the most prevalent (34.07%) and mostly multidrug resistant organism (98.92%). Among the isolated *Acinetobacter spp.* almost every microbes were resistant to Penicillin (Amoxicillin, Ampicillin, Cloxacillin, Amoxyclav). Same characteristics were also observed against third generation of Cephalosporine (Cephalexin, Ceftriaxone, Cefuroxime, Ceftazidime, Cefotaxime, Cefixime, Cefepime), Oxazolidinone (Linezolid), Glycopeptide (vancomycin) and Fusidane (Fusidic acid). More than 90% of the isolated *Acinetobacter spp.* were resistant to Aminoglycoside (Amikacin, Gentamycin, netilmicin), Fluroquinolones (Ciprofloxacin, Levofloxacin), Carbapenem (Imipenem, Meropenem), Monobactam (Aztreonam), and TazobacPipracillin. Several previous studies from Bangladesh, India, Nepal and other countries also reported the similar findings (Bhandari et al., 2015; Islam et al., 2015; Jesmin et al., 2021; Kumari et al., 2007; Moolchandani et al., 2017). One of the prospective studies from Morocco also reported resistance of

Acinetobacter baumannii - one of the microbes from *Acinetobacter spp.* – to Imipenem 100% (Lachhab et al., 2017) which is quite higher than my findings. Indicating that antibiotic resistant has some geographical differences, as well as the maintenance status of ICU, although antibiotic resistant organisms are spreading rapidly around the world. The increasing trend of carbapenem resistant in *Acinetobacter spp*. is due to naturally producing β-lactamases, acquired β-lactamases like metallo-β-lactamases, carbapenem hydrolyzing oxacillinases (CHDLs) enzyme, loss of outer membrane porin protein, and occasionally modification in penicillin-binding protein (Bhandari et al., 2015; Poirel & Nordmann, 2006). These adaptive capabilities have made *Acinetobacter spp*. highest concerns as it limits the therapeutic options for ICU patients (Bhandari et al., 2015). High percentages of Isolated *Acinetobacter spp.*, however, were susceptible to Colistin – a drug of peptide group and Tigecycline (Tigecycline drugs). Moderate levels of sensitivity were found against cotrimoxazole. These results are consistent with some previous studies from Bangladesh (Islam et al., 2015; Jamil et al., 2016; Jesmin et al., 2021).

This study found Presence of gram-negative *Klebsiella spp*. and *E. coli* was high among the Enterobacteriaceae. Previous study also found *Klebsiella Pneumoniae*, one of the important gram negative bacteria of *Klebsiella spp.* remains the most prevalent opportunistic bacterium causes hospitalized individuals (Ferreira et al., 2019) and specially immune compromised people at intensive care unit (ICU) (Saharman et al., 2020). Studies from various country proved that *Klebsiella spp.* and *E.coli* were found at ICU ubiquitously and all around the globe (Anupurba & Sen, 2005; CARA, 2007; Islam et al., 2015; Lagacé-Wiens et al., 2008; Singh et al., 2002).

Finding of this study shows more than 90% of the isolates were Resistant to multiple drug groups. However, drugs like Colistin (Peptide) and Tigecycline, have showed

high sensitivity against isolated *Klebsiella spp.* Nearly two fifth of the isolated *Klebsiella spp.* were sensitive to Imipenem and Aminoglycosides such as Amikacin, Gentamycin and Netilmicin. Almost one third of the organisms showed sensitivity against Levofloxacin, Meropenem & TazobacPipracillin.

Carbapenems are the preferred antibiotic for the treatment against the infection of Multidrug resistant (MDR) Gram-negative bacilli, however, worldwide emergence and rapid spread of carbapenem resistant *Klebsiella spp.* especially in ICU producing a great challenge (Saharman et al., 2020). Moreover, previous literature suggested the non- susceptibility may be due to production of Ambler class A β-lactamases (e.g. KPC), class B metallo- β-lactamases (MBLs, e.g. VIM, IMP, NDM) or class D oxacillinases (e.g. OXA-48 like enzymes) in *Klebsiella Pneumoniae* (Endimiani et al., 2016; Munoz-Price et al., 2013; Queenan & Bush, 2007). Although there are some geological differences among the *Klebsiella spp.*, spread of resistant species or resistant genes around the earth is rapid (Endimiani et al., 2016; Nordmann, 2014; Saharman et al., 2020; Tängdén & Giske, 2015).

Though the isolated *Klebsiella spp.* were susceptible to Colistin and Tigecycline, previous studies from Egypt and Brazil showed imprudent use of these antibiotic can increase resistance against *klebsiella spp.* (Biberg et al., 2015; Elgendy et al., 2018; Ferreira et al., 2019; Wang et al., 2015). Penicillin (Amoxicillin, Ampicillin, Cloxacillin, Amoxaclav), Cephalosporine (Cefepime, Cefixime, Cefotaxime, Ceftazidime, Cefuroxime, Ceftriaxone, Cephalexin), Glycopeptide (Vancomycin), Fusidane (Fusidic Acid), & Monobactam (Aztreonam) were the drugs, nevertheless, a high percentage of the microorganisms were resistant to previous studies from a tertiary health facility of Bangladesh also found a high resistance against third generation cephalosporin and penicillin (Jamil et al., 2016). These multi drug resistant

(MDR) *Klebsiella spp.* increases not only the chances of mortality but also morbidity due to infections delayed hospitalization (Ling et al., 2015; Saharman et al., 2020) and therefore increases health care cost.

Another Enterobacteriaceae *E. coli* was the highly frequent pathogenic organisms at ICU and most of which, more than 95%, were multi drug resistant in nature. Among the isolated *E. coli* this study found almost all the organisms were resistant to tested penicillin and third generation of cephalosporin drugs. Similarly, the number of resistant bacteria was high for Oxazolidinone (Linezolid), Glycopeptide (Vancomycin), Fusidane (Fusidic acid), Monobactam (Aztreonam). Moreover, we observed almost four fifth of the organisms were resistant against TazobacPipracillin, Sulfonamide (cotrimoxazole), fluroquinolones (ciprofloxacin, levofloxacin) drugs. Similar studies also previously found high level of resistant *E.coli* against carbapenem, cephalosporin, piperacillin+ tazobactum (Jamil et al., 2016). However, in the case of carbapenem and amino glycoside drugs, more than three fourth of the total isolated *E. coli* was resistant to some of the drugs (gentamycin, meropenem, and amikacin) of these groups. On the other hand, number of resistant *E. coli* was close to fifty to slightly above fifty percent against other drugs (netilmicin & imipenem) of these two groups (**Figure 5**). In Bangladesh resistant against Amino glycosides and carbapenem were reported have a great variety at ICU (Jamil et al., 2016; Jesmin et al., 2021) so did this study (56% -80%).

By the examination of isolated *E. coli* this study reviled that Colistin and Tigecycline can be an effective antibiotic against the organisms. A high sensitivity against these two drugs shows the light to fight against the nosocomial spread of *E. coli* at ICU setting in an urban hospital. Previous study (Jamil et al., 2016) also found Colistin are

the most effective anti- bacterial against isolated multidrug resistant *E.coli* organisms.

This study found Colistin (peptide) was the only drug to which most of the isolated (84.21%) *Pseudomonas spp.* showed high sensitivity, similarly almost two third of the organism were sensitive to Tazobacpipracillin. Previous study by Jamil ei al. and Jesmin at al. had the similar findings (Jamil et al., 2016; Jesmin et al., 2021) for Tazobacpipracillin. However, in the case of sensitivity against Colistin this study findings were higher than Jesmin et al. (Jesmin et al., 2021) but similar to Jamil at al. (Jamil et al., 2016). This study observed a mixed resistance and sensitive status against Aminoglycoside (Amikacin, Gentamycin, Netilmicin), Imipenem and Aztreonam where 30% to 35% of the organism were sensitive to these drugs. These results of this study are in line with previous study from Nepal, Egypt and Iran (Azzab et al., 2016; Tehrani et al., 2019; Yadav et al., 2020).

However, almost all of the organisms were resistant to penicillin (Amoxicillin, Ampicillin, Cloxacillin, Amoxyclav), Cephalosporine (Cefuroxime, Ceftriaxone, Cephalexin, Cefotaxime, Cefixime), Vancomycin & Fusidic Acid. High resistant of *Pseudomonas spp.* is due to its innate presence of over expressed efflux pump, low permeability of outer membrane (Santajit & Indrawattana, 2016), and acquisition of resistance gene or mutation in gene encoding porins, penicillin-binding proteins, chromosomal β-lactamase all contribute to acquired resistance against β-lactamase, carbapenems, aminoglycosides, and fluroquinolones drugs (Oie et al., 2009; Pachori et al., 2019).

Staphylococcus aureus was the most prevalent gram- positive organism isolated from ICU among them 31.25 % were Multi Drug Resistant (MDR). Previous study also

reported high prevalence of this gram positive cocci (Bhandari et al., 2015). This study found all isolated *Staphylococcus aureus* were sensitive to vancomycin. Likewise, almost three fourth of the organism were sensitive to Netilmicin, Linezolid, Tigecycline and Fusidic acid. Previous studies from Nepal also reported 100% sensitivity of *staphylococcus aureus* to vancomycin (Bhandari et al., 2015). A study from Egypt reported sensitivity against Tigecycline (100%) in 2016 which is noticeably higher than this study (75%) (Azzab et al., 2016). This phenomena show antibiotic resistant varies with time, geographical location, socioeconomic status, study design and study participants (Deyno et al., 2017; Falagas et al., 2013; Mogus et al., 2015; WHO, 2014). However, *staphylococcus aureus* isolates showed mixed susceptibility to Aminoglycoside (Amikacin, Gentamycin), Carbapenem (Imipenem, Meropenem) and tazobacpipracillin, where around half of the organisms were resistant and half were sensitive to those drugs. Resistance against Aminoglycoside (Amikacin (37.5%) and Gentamycin (31.5%)) were closed to a previous review article of Ethiopia (Deyno et al., 2017) whereas sensitivity against Gentamicin (62.5%) was slightly higher than previous study (54.55%) by Jesmin et al. (Jesmin et al., 2021) .

Beside other Gram-negative and Gram-positive organisms this study also found a significant number of isolated fungi most of which were *Candida spp.* (7.33%) of total isolated organisms. This high prevalence of *Candida spp.* may be due to presence of underlying conditions of the admitted patient like poor nutritional status, diabetes mellitus and the use of steroids and broad-spectrum antibiotics. Findings of this study aligned with previous study (Jamil et al., 2016).

Chapter - 6

Recommendations

6. Recommendations

After closely observed the urban hospital ICU some of points were identified to improve the situation of fight against the multidrug resistant microorganism.

- Regular screening of microorganisms at ICU is helpful to determine the prevalence, susceptibility patterns and the trend of microbial resistant against frequently used antimicrobials, which will allow the authority to manage and regulate such pathogens at intensive care unit.
- As the bacterial resistant varies over time and place a regular surveillance both nationally and locally is needed to reduce nationwide nosocomial infections empirically and effectively.
- Despite an enormous demand a strict and effective infection management protocol needs to apply while admitting and releasing patient.
- ICU equipments specially the invasive machineries need to be checked twice for opportunistic pathogens before administered.
- Proper training of health care providers on hygiene and infection control is an urgent need.
- A regulatory committee is required to enforce a strict and effective law against unauthorized (without prescribe or self- treatment) and prudent use of antibiotics.
- Some special drugs need to be preserved as the new generations of effective drugs are yet to develop.

Chapter - 7

Limitations

7. Limitations

This study has some limitations. The sample size is relatively small, and is a single-center study. So, the findings may not be generalizable. Anaerobic cultures and cultures suitable for isolating the fastidious organisms were not done. Some antibiotics which are less commonly used but having increasing importance in concurrent patient management might have been omitted from the sensitivity study. Also, the disc diffusion method, not the broth dilution method was used to determine the antibiotic sensitivity, accordingly, information regarding the minimum inhibitory concentration of antibiotics is lacking.

Chapter - 8

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Chapter - 9

Appendix

9. Appendix:

9.1 Culture media & Media preparation

- **Nutrient agar**

The nutrient broth is basically the nutrient agar, which lacks the agar powder, the solidifying agent. They remain at room temperature in liquid form and are typically used to conserve micro-organism stocks. They are usually used to make fastidious organisms evolve.

Table 10: Composition of Nutrient agar

Ingredients	Amount
'Lab-Lemco' powder	1.0
Yeast extract	2.0
Peptone	5.0
Sodium chloride	5.0
Agar	15.0

pH 7.4 ± 0.2 @ 25°C

Media preparation

i. Using 1L of distilled water, add 13g of nutrient broth powder (CM0001B).
ii. Fully blend and dissolve them.
iii. In the final tanks, pour them into (eg. conical flask)
iv. Autoclave for 15 minutes at 121 ° C. Sterilize.

- **MacConkey agar**

A selective and differential culture medium for bacteria is MacConkey agar. It is designed to selectively isolate and distinguish Gram-negative and enteric bacilli (normally located in the intestinal tract) based on lactose fermentation.

Table 11: Composition of MacConkey agar

Ingredients	Amount
Peptone (Pancreatic digest of gelatin)	17 gm
Proteose peptone (meat and casein)	3 gm
Lactose monohydrate	10 gm
Bile salts	1.5 gm
Sodium chloride	5 gm
Neutral red	0.03 gm
Crystal Violet	0.001 g
Agar	13.5 gm
Distilled Water	Add to make 1 Liter

Final pH 7.1 +/- 0.2 at 25 degrees C.

Preparation of MacConkey Agar

i. Using 1000 ml of purified/distilled water to suspend 49.53 grams of dehydrated medium.
ii. Heat to a boil to completely dissolve the medium.
iii. Sterilize for 15 minutes at 15 lbs of pressure (121°C) by autoclaving.
iv. To 45-50 ° C, cool.
v. Until pouring onto sterile Petri dishes, mix well.

Uses of MacConkey agar

- ✓ For the isolation of gram-negative enteric bacteria, MacConkey agar is used.
- ✓ It is used to distinguish between lactose fermenting and gram-negative lactose non-fermenting bacteria.
- ✓ It is used in water, dairy products and biological specimens to separate coliforms and intestinal pathogens.

- **Blood agar**

Blood Agar is an enriched, multi-nutrient supplied medium that typically comes as a basal medium for blood agar preparation by blood supplementation.

Table 12: Composition of blood agar

Ingredients	Gram/liter
Peptone	10.0
Tryptase	10.0
Sodium chloride	5.0
Agar	15.0

Final pH at 25°C: 7.3 ±0.2

Media preparation

i. For 1 liter of distilled water, suspend 28 g of nutrient agar powder.
ii. To completely dissolve all elements, heat this mixture while stirring.
iii. Dissolve the autoclave mixture for 15 minutes at 121 degrees Celsius.
iv. Enable the nutrient agar to cool but not solidify once it has been autoclaved.

v. Add 5 percent (vol/vol) of sterile defibrinated blood that has been heated to room temperature when the agar has cooled to 45-50 °C and mix it gently but well.

vi. Stop bubbles in the air.

vii. Dispense with liquid on clean plates.

Uses of blood agar

- ✓ Blood Agar is an enriched medium for general purposes, often used to cultivate fastidious species.
- ✓ Bacteria should be distinguished on the basis of their hemolytic properties (β-hemolysis, alpha-hemolysis and γ-hemolysis (or non-hemolytic)).

- **Salmonella Shigella (SS) agar**

Salmonella Shigella (SS) Agar is selective and differential medium for the isolation, cultivation and differentiation of Salmonella spp. and some strains of Shigella spp.

Table 13: Composition of Salmonella Shigella agar

Ingredients	Grams / Liters
Beef Extract	5.00
Enzymatic Digest of Casein	2.50
Enzymatic Digest of Animal Tissue	2.50
Lactose	10.00
Bile Salts	8.50
Brilliant Green	0.00033
Neutral Red	0.025
Agar	13.50

Sodium Citrate	8.50
Ferric Citrate	1.00
Sodium Thiosulfate	8.50

Distilled Water = 1000 ml

pH (at 25°C) 7.0 ± 0.2

Salmonella Shigella Agar preparation

i. Suspend 60.0 grams of Shigella Agar Salmonella with 1000 ml of distilled water.
ii. Heat to a boil to completely dissolve the medium.
iii. Do not operate autoclave.
iv. Mix thoroughly and pour into sterile Petri dishes.

Uses of Salmonella Shigella Agar

- ✓ It is used for the isolation of Salmonella and some Shigella species from clinical and non-clinical specimens as a selective and differential medium.
- ✓ For primary Shigella isolation, this medium is not recommended.
- ✓ It has also been developed to help differentiate lactose and non-lactose fermenters from clinical specimens, suspected foods and other samples of this type.

- **Xylose Lysine Deoxycholate (XLD) agar**

Xylose Lysine Deoxycholate (XLD) agar is a selective growth medium used for the isolation of Salmonella and Shigella species from clinical samples and from food.

Table 14: Composition of Xylose Lysine Deoxycholate agar

Ingredients	Gms/Litre
Yeast extract	3.0
L- Lysine	5.0
Lactose	7.5
Sucrose	7.5
Xylose	3.5
Sodium chloride	5.0
Sodium thiosulphate	6.8
Ferric ammonium citrate	0.8
Phenol red	0.08
Agar	15.0
Sodium deoxycholate	2.5

Final pH (at 25°C) 7.4±0.2

XLD Agar preparation

i. Using 1000 ml of filtered or distilled water to suspend 55 grams of dehydrated medium.
ii. Heat until the medium boils with frequent agitation.
iii. At 50°C, switch immediately to a water bath.
iv. Pour into sterile Petri plates after cooling.

Uses of Agar XLD

- ✓ XLD Agar is a selective differential medium for the isolation of fecal specimens and other clinical material from Gram-negative enteric pathogens.
- ✓ It is particularly suitable for isolating species of Shigella and Salmonella.

- ✓ Food, water and dairy products microbiological testing.

- **Chocolate agar**

The Lysed Blood Agar is Chocolate Agar (CAP). The name itself is derived from the fact that a chocolate-brown color is provided to the medium by red blood cell (RBC) lysis. When incubated at 35-37°C in a 5 percent CO2 atmosphere, chocolate agar is used for the isolation of fastidious species, such as Hemophilus influenzae.

Preparation of Chocolate agar

i. 5% horse or sheep blood heat-lyse is prepared very slowly in a water bath at 56 ° C.
ii. Dispense 20 ml into Petri dishes of 15 to 100 mm.
iii. Enable the media to solidify and to dry with condensation.
iv. In sterile plastic bags, place the plates and store at 4°C until used.
v. Incubate an uninoculated plate at 35-37 ° C with ~5 percent CO2 for 48 hours as a sterility measure (or in a candle-jar).

Uses of chocolate agar

- ✓ It is used to isolate and cultivate fastidious microorganisms, mainly species of Hemophilus and Neisseria.
- ✓ It is used to isolate Neisseria gonorrhoeae from chronic cases of gonococcal infections and acute cases.

- **Sabouraud's dextrose agar (SDA)**

Sabouraud's agar is a type of peptone-containing agar growth medium, or Sabouraud dextrose agar (SDA). It is used to grow dermatophytes and other forms of fungi and can develop filamentous bacteria as well.

Table 15: Composition of Sabouraud's dextrose agar

Ingredients	In gm/L
Dextrose (Glucose)	40 gm
Peptone	10 gm
Agar	15 gm
Distilled Water	1000 ml

Final pH 5.6 +/- 0.2 at 25ºC.

Preparation of SDA

i. Using ~900 ml of deionized water to mix all ingredients.
ii. Adjust to pH 5.6 with hydrochloric acid and adjust to 1 liter of final volume.
iii. Heat to a boil to completely dissolve the medium.
iv. 121oC autoclave for 15 minutes.
v. Cool to 45°C to 50°C and pour for slants in petri dishes or tubes.

Uses of SDA

- ✓ SDA is mainly used for the selective cultivation of acidic bacteria, molds and yeasts.
- ✓ The medium is also used to separate pathogenic fungi from a material containing large numbers of other fungi or bacteria with antibiotics.
- ✓ This medium is often used in food, cosmetics, and clinical specimens to assess microbial contamination.

9.2 Biochemical tests

- Motility Indole Urea (MIU) Test

  Urea is a diamide of carbonic acid. It is hydrolyzed with the release of ammonia and carbon dioxide. Many organisms especially those that infect the urinary tract, have a urease enzyme which is able to split urea in the presence of water to release ammonia and carbon dioxide. The ammonia combines with carbon dioxide and water to form ammonium carbonate which turns the medium alkaline, turning the indicator phenol red from its original orange yellow color to bright pink.

- The Triple Sugar Iron (TSI) Test

  Triple sugar iron agar test is used to determine whether gram negative bacilli utilize glucose and lactose or sucrose fermentative and produce hydrogen sulfide (H2S). It contains 10 parts of lactose: 10 parts of sucrose: 1 part of glucose and peptone. Phenol red and ferrous sulphate serves as an indicator for acidification of medium and H2S production respectively.

  Glucose is utilized first by a fermentative organism and the entire medium becomes acidic (yellow) in 8 to 12 hours. Butt remains acidic even after 24 hours incubation period because of the presence of organic acids resulting from the fermentation of glucose under anaerobic conditions in the butt of the tube. The slant reverts to alkaline state that is indicated by red color as the fermentation products gets oxidized to carbon dioxide (CO2) and water (H2O) and peptone in aerobic condition the slant undergoes oxidation releasing alkaline amines (Phenol red in alkaline pH turns red while in acidic pH turns yellow).

- Citrate test

  When an organic acid such as citrate (remember Krebs cycle) is used as a carbon and energy source, alkaline carbonates and bicarbonates are produced ultimately. In addition, ammonium hydroxide is produced when the ammonium salts in the medium are used as the sole nitrogen source. Utilization of exogenous citrate requires the presence of citrate transport proteins (permeases). Upon uptake by the cell, citrate is cleaved by citrate lyase to oxaloacetate and acetate. The oxaloacetate is then metabolized to pyruvate and CO2.

- Bile esculin test

  The bile esculin test relies on a microorganism that produces the enzyme esculinase to hydrolyze esculin into glucose and esculetin (6, 7-dihydroxy-coumarin). Esculetin forms a phenolic iron complex when it reacts with an iron salt (ferric citrate) in the medium, resulting in a dark brown or black appearance.

- Coagulase test

  The bacterial cell wall binds bound coagulase (clumping factor), which interacts directly with fibrinogen. When a bacterial suspension is combined with plasma, this causes fibrinogen to alternate and precipitate on the staphylococcal cell, allowing the cells to clump. This does not necessitate the use of coagulase-reacting factor. The activation of plasma coagulase-reacting factor (CRP), which is a modified or derived thrombin molecule, to form a

coagulase-CRP complex is known as free coagulase. The fibrin clot is formed as this complex interacts with fibrinogen.

- Catalase test

  The enzyme catalase mediates the breakdown of hydrogen peroxide into oxygen and water. The presence of the enzyme in a bacterial isolate is evident when a small inoculum is introduced into hydrogen peroxide, and the rapid elaboration of oxygen bubbles occurs. The lack of catalase is evident by a lack of or weak bubble production. The culture should not be more than 24 hours old. Bacteria thereby protect themselves from the lethal effect of Hydrogen peroxide which is accumulated as an end product of aerobic carbohydrate metabolism.

- Oxidase test

  Cytochrome containing organisms produce an intracellular oxidase enzyme. This oxidase enzyme catalyzes the oxidation of cytochrome c. Organisms which contain cytochrome c as part of their respiratory chain are oxidase-positive and turn the reagent blue/purple. Organisms lacking cytochrome c as part of their respiratory chain do not oxidize the reagent, leaving it colorless within the limits of the test, and are oxidase-negative.