

**Identification and Antibigram Profiling of Bacteria  
Isolated from Healthcare Associated Medical Devices and  
Inanimate Objects Used for Pediatric Patients**



**Master's Thesis**

**A Thesis Submitted to the Biotechnology and Genetic Engineering  
Discipline, Khulna University in Partial Fulfillment of the Requirements  
for the degree of of Masters of Science (M.S.) in  
Biotechnology and Genetic Engineering**

**Submitted by  
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Examination Roll No: MS-220715  
Session: 2021-2022**

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KHULNA-9208, BANGLADESH**

**June, 2024**

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BANGLADESH  
June, 2024**

## DECLARATION

This thesis paper is the original research work of the undersigned author except as otherwise appropriately cited. The materials presented have not been submitted either in whole or in part for a degree for this or any other university. The methods used were designed and implemented according to institutional and established international biosafety and bioethical guidelines. I also declare that the findings of the research have not been/will not be published anywhere or disseminated otherwise without the concurrence of the concerned supervisor/s.

Bijon Kumar Saha  
.....  
23.12.2024

**Bijon Kumar Saha**  
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**DEDICATED TO  
ALL OF MY RESPECTED  
TEACHERS.....**



## ACKNOWLEDGEMENT

Very beginning the author would like to express his heartiest gratitude to his creator Lord Krishna, the exalted and the most gracious & merciful that has enabled him to do such a work.

The author feels great sense of gratitude, deepest sense of appreciation and immense pleasure to his supervisor Shaila Siddiqua, Assistant professor and Co-supervisor Dr. Ayesha Ashraf, Professor, Biotechnology and Genetic Engineering Discipline, Khulna University, for their proper guidance, innovative suggestion, encouragement and constant supervision. Their valuable and constructive instruction helped him to complete this thesis paper.

The author is very much grateful to The Ministry of Education for getting National Science and Technology (NST) Scholarship which support him to conduct the research work. The author is very thankful to all the faculty members of the Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna for all sorts of co-operation.

The author is truly indebted to Dr. Saheduzzaman for his guidance and help for sample collection from Ad-Din Akij Medical College Hospital, Khulna. The author also expresses his gratitude towards Dr. Ariful Kamal Rasel and Dr. Forrukh Ahmed for sample collection from Khulna Shishu Hospital and Khulna Medical College Hospital respectively.

The author is also very much thankful to her senior Sabrina Islam and Pronob Majumdar who helped him for proper planning and useful guidance in conducting his thesis work.

The author would like to express his indebted thanks to his friend and lab partner Anisha Tashruba Riya for his inspiration and help. Besides, the author would like to express special thanks to his juniors (Nazmul, Barsha, Zisan, Ananna, Fahria).

Finally, the author expresses his greatest & deepest gratitude from the core of his heart to his parents, brother Rudro & beloved Pritha for their moral support, love & encouragement that brings him up to this level and supporting him all the time.

The Author

## ABSTRACT

Medical equipments and inanimate objects used in the Pediatric wards are heavily contaminated with different species of bacterial pathogens. Nosocomial infections are a subset of hospital acquired infections, can lead to serious problems like sepsis and even death. Due to the ubiquitous and unauthorized uses of antibiotic by general people, different species of bacteria are becoming resistant against antibiotic day by day. Thus, antibiotic resistance has become an emerging issue in the developing countries like Bangladesh. This study aimed to characterise bacteria and antibiogram profiling of isolated bacteria from inanimate objects and body fluids from healthcare associated medical devices used in Pediatric ward. A total of 105 swabs from inanimate objects and 130 body fluids from discarded healthcare associated medical devices were collected from 3 hospitals in Khulna city namely Ad-Din Akij Medical College Hospital (AAMCH), Khulna Shishu Hospital (KSH) and Khulna Medical College Hospital (KMCH). Of them, 51% and 62% samples of inanimate objects and body fluids from healthcare associated medical devices showed bacterial growth respectively. Colonies from pure culture were used to identify isolates morphologically and were characterized by gram staining. Nine (9) different bacterial strains were found namely *Staphylococcus aureus* (36%), *Klebsiella pneumoniae* (30%), *Pseudomonas aeruginosa* (11%), *E.coli* (11%), *Salmonella typhimurium* (6%), *Corynebacterium spp.* (5%), *Streptococcus pneumoniae* (4%), *Acinetobacter spp.* (3%) and *Shigella spp* (1%) based on various biochemical tests. Antibiogram profiling through Kirby-Baur disk diffusion method showed that out of commonly prescribed 10 antibiotics, identified all 9 bacterial strains were resistant to Penicillin (88%), Cefixime (75%) and Colistin (72%), whereas Amikacin (58%) and Gentamicin (50%) showed more sensitivity. Therefore, it is necessary to decrease the abundant use of antibiotics, as well as good clinical practices and techniques can reduce the potential risk of pathogenic bacteria to become antibiotic resistant.

**Keywords:** Pediatric ward, Antibiogram, Healthcare associated medical devices , Pathogenic bacteria, Antibiotic resistance



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## LIST OF ABBREVIATION

|                               |                                             |
|-------------------------------|---------------------------------------------|
| %                             | Percent                                     |
| °C                            | Degree Celsius                              |
| µg                            | Microgram                                   |
| Spp.                          | Species                                     |
| et. al                        | and others                                  |
| H <sub>2</sub> O <sub>2</sub> | Hydrogen Peroxide                           |
| OG                            | Orogastric                                  |
| NG                            | Nasogastric                                 |
| ICU                           | Intensive care unit                         |
| UV                            | Ultraviolet                                 |
| MR                            | Methyl Red                                  |
| AMR                           | Antimicrobial resistance                    |
| MDR                           | Multi-drug resistant                        |
| WHO                           | World Health Organisation                   |
| HAI                           | Hospital Acquired Infection                 |
| HCWs                          | Health Care Workers                         |
| HCAIs                         | Healthcare-Associated Infections            |
| AAMCH                         | AD-din Akij Medical College Hospital        |
| KSH                           | Khulna Shishu Hospital                      |
| KMCH                          | Khulna Medical College Hospital             |
| CLSI                          | Clinical and Laboratory Standards Institute |





# **Chapter One**

## **Introduction**

## 1. Introduction

Medical equipments and inanimate objects in the medical wards are heavily contaminated with different species of bacterial pathogens, followed by health-care-associated infections. Different types of bacteria are responsible for the contamination of patient-care equipments and inanimate objects.(Birru et al., 2021).

Antimicrobial resistance (AMR) has become an emerging issue in the developing countries as well as in Bangladesh. Antibiotic resistance in Bangladesh has increased by 11% over the past five years, with the effectiveness of several commonly used antibiotics decreasing by up to 82% this year, compared to 71% in last five years. As per the report, MDR resistance, which was at 71 percent in 2017, had risen to 82 percent by June in 2023. The Institute of Epidemiology Disease Control and Research (IEDCR) revealed statistics at an event at its auditorium on 21 November, 2023 in marking the release of the National Antibiotic Resistance Survey results . The National AMR Surveillance 2023 published that *Acinetobacter*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* are among the bacteria that are highly antibiotic-resistant. The southeast Asia is considered to have the highest risk of AMR among all the WHO regions . AMR is aggravated by irrational use of antimicrobials in a largely unregulated pluralistic health system (Tarin-Pelló et al., 2022). It reveals widespread availability of antimicrobials without prescription in the country including rise in its irrational use across sectors and consequent contamination of environment and spread of resistance. Antibiotic resistance is one of the biggest public health threats worldwide. Currently, antibiotic-resistant bacteria kill 700,000 people every year (Tarin-Pelló et al., 2022). A high prevalence of resistance was detected in most tested pathogens, and many of the common first-line drugs were mostly ineffective. Antibiotic resistance is a worldwide problem and Bangladesh is a major contributor to this owing to its poor healthcare standards, along with the misuse and overuse of antibiotics (Ahmed et al., 2019).

Since the discovery of antibiotics in the early 20th century, significant changes have occurred in their usage principles. Initially, there was great optimism about this new class of medications. However, it became evident that the uncontrolled use of antibiotics in humans, veterinary medicine, and agriculture led to antimicrobial resistance resulting in a global health emergency (Radoslaw et al.,2024).



The emergence of multi-drug resistant (MDR) strains in a hospital environment that is an obstacle for the management of HCAs. In Ethiopia, studies reported a high prevalence of HCAs mainly due to pathogens including the country's largest tertiary referral Hospitals, which warrants the role played by the inanimate environment in the transmission of nosocomial infections (Sebre et al., 2020). In recent year, introduction and transmission of antibiotic resistance has become a global public health crisis with significant burden on healthcare systems (Chen et al., 2023).

It results in high mortality and economic costs. In 2019, almost five million deaths were associated with drug-resistant infections, the global economy could lose \$100 trillion by 2050 (Chen et al., 2023). In 2021, WHO has declared that antimicrobial resistance is a global and developmental threat which is one of the top 10 global public health threats facing humanity. The 2022 GRAM Global Burden Report, calculated bacterial antimicrobial resistance to be associated with 4.95 million deaths in 2019, including 1.27 million attributable deaths, placing antimicrobial resistance as a leading cause of mortality globally (Mogasale et al., 2021). Antimicrobial resistance (AMR) has been prioritized by the World Health Organization (WHO) as one of the top 10 global public health threats facing humanity. It is estimated that global deaths attributable to AMR could reach 10 million annually by 2050 (Walsh et al., 2023).

Comprehensive data on the antibiogram for surveillance of antimicrobial use and resistance thus improving decision making and assessing the effect of interventions at the local, national and international level (Aika et al., 2022). Antimicrobial-resistant bacteria are limited, resulting in significant morbidity and mortality with high financial impact. The paucity in discovery and supply of new novel antimicrobials to treat life-threatening infections by resistant pathogens stands in sharp contrast to demand (Salam et al., 2023)

Therefore, clinicians should practice rational choice of antibiotics and treatment should be guided by antimicrobial susceptibility testing (Yitayeh et al., 2021). The emergence of antimicrobial resistance is multifactorial, and tackling its development is challenging. Consequently, infections caused by resistant bacteria are unresponsive to conventional drugs, resulting in prolonged and severe illnesses, higher mortality rates, and considerable healthcare costs (Tafa et al., 2023). Antibiotics significantly impact diversity and composition of the infant gut microbiome in Low and middle-income countries (LMICs), while selecting for resistance genes which can persist



for prolonged periods of time (Aika et al.,2022). Inappropriate antimicrobial usage is a key driver of antimicrobial resistance. Low and middle-income countries are disproportionately burdened by AMR and young children are especially vulnerable to infections with AMR-bearing pathogens. The impact of antibiotics on the microbiome, selection, persistence, and horizontal spread of AMR genes is insufficiently characterized and understood in children (Luchen et al., 2023).

Numerous studies have employed molecular typing to determine the clonal relationship between inanimate surface and clinical strains, suggesting that the source of nosocomial infections was linked to the hospital environments, health care workers (HCWs) hands as well as clinical specimens from admitted patients. It has also been reported that patients admitted to rooms previously occupied by individuals infected or colonized with MDR strain are up to a three-fold higher risk of acquiring HCAs (healthcare-associated infections) from contaminated environmental surfaces or equipment if terminal cleaning is not effective. The occurrence of antimicrobial resistance (AMR) is also increasing and resulting in higher morbidity and mortality associated with HCAs. (Genovese et al., 2020). Studies investigating hospital environments reported that pathogens were ubiquitous in all hospital units but the interest was usually focused on intensive care and operation unit, especially due to the vulnerability of patients in these units. There is also high antibiotic usage and invasive procedure from these units. Bacterial cross-contamination plays an important role in healthcare-associated infections (HCAs) and resistant strain dissemination (Sebre et al., 2020).

Several studies report that a simple and straightforward process, taking only a few seconds to clean hands with an alcohol-based hand rub helps prevent HCAs and save lives, reduce morbidity, and minimize health care costs. However, factors such as the availability of alcohol-based hand rubs and up-to-date knowledge of the importance of hand washing hinder good practice in hand hygiene. For example, an Australian observational study of community nurses highlighted poor practices of hand hygiene in comparison with a standard protocol (Rosenberg et al.,2016). Resistant bacteria may be transferred to humans and are capable of transferring their resistance elements to opportunistic human pathogens. Colonization by multidrug resistant bacteria is considered one of the predisposing factors for hospital acquired infections and is a daily challenge for the clinicians, particularly dealing with critically ill patients (Chaoui et al.,



2019). Several outbreaks associated with different types of medical devices have been linked to improper sterilization and reprocessing which could have been imposed to antibiotic drug resistance bacteria (Josephs-Spauldin J et al., 2021).

The majority of the HCAs are believed to be transmitted directly from patient to patient, but increasing evidence demonstrates that also the medical personnel, as well as the clinical environment (ie, surfaces and equipment), often are a source of infections. Hospital design and hygienic practices have been largely directed at controlling nosocomial pathogens and resistant strains contaminating air, hands, equipment, and surfaces.-(Sebre et al., 2020). The most critical organisms in this catalog includes multidrug resistant bacteria that pose a particular threat in hospitals, nursing homes, and among patients whose care requires devices such as Canula and blood catheters. Overuse or misuse of antibiotics causing such an increasing prevalence of antibiotic resistance is detrimental not only to human health but also to the ecosystem and environment (Aika et al.,2022).

Several studies suggest that simple infection-control procedures such as cleaning hands with an alcohol-based hand rub can help prevent HCAs and save lives, reduce morbidity, and minimize health care costs. Routine educational interventions for health care professionals can help change their hand-washing practices to prevent the spread of infection. In support of this, the WHO has produced guidelines to promote hand-washing practices among member countries (Haque et al.,2018). Infections with multidrug-resistant bacteria are hard to treat since fewer or no treatment options remain. In some cases, health care providers have to use antibiotics that are more toxic for the patient resulting in increased duration of illness and prolonged hospital stay, excess costs, number of complications, and deaths. Data from U.S intensive care unit studies and surveillance studies estimate that by 2050, 10 million deaths are projected to occur due to antimicrobial resistance (AMR), while another study projected AMR to cost the global economy US\$100 trillion, in the same period. (Sebre et al., 2020)

As we struggle with the aftermath of the COVID-19 pandemic, we should welcome and agree that a refocus on the value of hygiene and other non-medical interventions should be encouraged as an integral part of global health security. Such a refocussing is, in turn, dependent on a better understanding of the transmission pathways of pathogens (Warmbrod et al., 2021).

Bacterial cross-contamination plays an important role in healthcare-associated infections (HCAs) and resistant strain dissemination.-The majority of the HCAs are believed to be transmitted



# **Chapter Two**

## **Literature Review**

directly from patient to patient, but increasing evidence demonstrates that also the medical personnel, as well as the clinical environment (ie, surfaces and equipment), often are a source of infections. Hospital design and hygienic practices have been largely directed at controlling nosocomial pathogens and resistant strains contaminating air, hands, equipment, and surfaces (Sebre et al., 2020)

Despite the development of many hi-tech methods, hand washing with soap and water or alcohol rub is still the most important means of maintaining personal hygiene and preventing HCAs. However, due to the rise of antibiotic-resistant bacteria and a reluctance of some HCWs to implement best practice infection control, HCAs remain one of the biggest causes of death in most countries. Therefore, it is essential that strategic, policy, and education initiatives continue to focus on managing and controlling such (predominantly needless) infections (Haque et al., 2018). AMR is a One-Health problem, and can spread via humans, animals (domestic and wild), and the environment (water and air). Inadequate access to water, sanitation, and hygiene as well as inadequate access to healthcare services (e.g., cost-effective diagnostics) and affordable, appropriate antibiotics have served to accelerate the spread of AMR (Walsh et al., 2023). This Special Issue consolidates multidisciplinary investigations on antimicrobial stewardship in pediatrics. Given the overarching nature of this predicament, a comprehensive approach becomes imperative, aligning with the WHO's global initiative to enhance proper antibiotic usage. This is necessary to avert an impending post-antibiotic era, wherein common infections may once again pose life-threatening risks (Radoslaw et al., 2024).

### **Objectives:**

- I) Isolation of the anonymous bacteria from inanimate objects and clinical devices used for pediatric patient
- II) Morphological & biochemical characterisation of isolated bacteria
- III) Antimicrobial Susceptibility profile of isolated bacteria

## 2. Literature Review:

### i) Pediatric Patients

Etymologically, “pediatric” (or “paediatric” in Europe and elsewhere) comes from *paedo*, English for “children” and *iatros*, Greek for “physician.” Traditionally, “pediatrics” refers to the health care of children. Classically, “childhood” is defined as being between birth and puberty. Adolescent medicine, however, is still within the realm of pediatrics. Many children with congenital and acquired disease processes are cared for in the pediatric environment into adulthood ( Andrew et al., 2022).

The age band(s) of the studied patients: fetuses (not yet born), newborns (first day of life), neonates (less than 1 month old), infants (up to 1 year old), children (birth to 17 years old), adolescents (13–17 years old), young adults or adults (18 years and older). Further, definitions of “pediatric” among governmental and other organizations are not uniform. The United States National Institutes of Health, the United Nations and its Children’s Fund (UNICEF), and the European Union define “children” as persons younger than 18 years (Strouse et al., 2022)

### ii) Clinical devices

Handle patient care equipment soiled with blood, body fluids secretions or excretions with care in order to prevent exposure to skin and mucous membranes, clothing and the environment.

Ensure

all reusable equipment is cleaned and reprocessed appropriately before being used on another patient. It is also including the Clinicals devices eg. Central venus catheter, Ventilator, Used syringe, IV Drip Tube, Nasal Canula, Nasogastric Tube, Intubation Endotracheal Tube, Urinary Catheter, Needle aspiration, Suction pump ( Sartelli et al., 2018). They can be used to detect illnesses, monitor vital signs, deliver oxygen, manage the delivery of medications and many other important elements of patient care. Devices are considered a fundamental component of Health Systems; the benefits they can provide continue to increase as they’re essential to prevent, diagnose, treat and rehabilitate illnesses and diseases in a safe and effective way. The steps that involve Medical Devices manufacturing, regulation, planning, assessment, acquisition, and management are complex but essential to guarantee their quality, safety and compatibility with



the settings in which they are used. It means instrument, apparatus or machine that is used in the prevention, diagnosis or treatment of illness or disease, or for detecting, measuring, restoring, correcting or modifying the structure or function of the body for some health purpose (Heneghan et al., 2020).

The regulation of medical devices is less well developed than the regulation of medicinal products, which it could follow more closely. Devices that incorporate medicines should be required to meet the same regulatory standards as medicinal products. This would remove the anomaly that some delivery systems that incorporate medicines are classified as devices while other similar systems that deliver the same medicines are classified as medicinal products eg. Catheters, Contact Lenses, Suction tube, Stethoscope, Oxygen nebulizer, Canula etc.(Aronson et al., 2020).

Results revealed that the total 99 inanimate objects and items of patient-care equipment examined, showed contamination 66.6% from the pediatric ward. In the case of Gram-positive bacteria, coagulase-negative staphylococci (CoNS; 52.2%) were predominant, followed by *Staphylococcus aureus* (47.7%), whereas common Gram-negative counterparts were *Acinetobacter* spp. (28.5%) and *Klebsiella* spp. (23.8%) (birru et al.,2021).

### iii) Inanimate Objects

For the prevention of infectious diseases, knowledge about transmission routes is essential. In addition to respiratory, fecal–oral, and sexual transmission, the transfer of pathogens via surfaces plays a vital role for human pathogenic infections—especially nosocomial pathogens. Therefore, information about the survival of pathogens on surfaces can have direct implications on clinical measures, including hygiene guidelines and disinfection strategies. While many pathogens persist only for hours, common nosocomial pathogens can survive for days to weeks under laboratory conditions and thereby potentially form a continuous source of transmission if no adequate inactivation procedures are performed (Kirchhoff et al., 2021).

Many pathogens require a living host to survive, while others may be able to persist in a dormant state outside of a living host. Nonetheless, all pathogens require mechanisms to transfer from one host to another. Understanding how infectious pathogens spread is critical to prevent the transmission of infectious diseases. In addition to respiratory, fecal-oral and sexual transmission,

the transmission of pathogens between hosts via fomites plays an important role in the spread of infectious diseases eg. desk, trolley, bed rail, bed cover, medical chart, weight machine etc (Wißmann et al., 2021).

#### IV) Hospital-acquired Infections

Almost any pathogen can, on occasion, cause hospital infection but those that are able to survive in the hospital environment for long periods and develop resistance to antibiotics and disinfectants are particularly important in this respect. *S aureus* strains, resistant to multiple antibiotics, spread globally in the 1950s and 1960s, colonising hospitals and causing nosocomial infection. The original strains have since been replaced by other strains but staphylococci continue to be one of the commonest causative agents of hospital infection (MRSA). In recent decades, the Gram-negative bacilli of the family Enterobacteriaceae – *E coli*, *Klebsiella*, *Enterobacter*, *Proteus* and *Serratia* – have become the most important group of hospital pathogens with multiple drug resistance (Haque et al., 2018).

Healthcare-associated infections in nearly half of ICU patients eventually lead to death, especially when caused by *Acinetobacter* spp., *Klebsiella* spp., or *Pseudomonas* spp. The use of endotracheal tubes and urinary catheters may further expose patients to the risk of healthcare-associated infection. Therefore, to reduce these infections, effective strategies to combat antibiotic-resistant bacteria must be implemented, along with stricter adherence to infection control programmes and enhancement of infection control using feasible and affordable tools and resources. Our findings could be used by policy-makers to develop more practical protocols for hand hygiene, reducing contact with patients, and using invasive devices. Staff training programmes, along with continuous supervision and monitoring, are also essential to prevent the spread of infection (Rimaz et al., 2023).

#### V) Identification of bacterial isolates

Bacterial identification methods used in routine identification of pathogens in medical microbiology include a combination approach of biochemical tests, mass spectrometry or molecular biology techniques. Extensive publicly-available databases of DNA sequence data from pathogenic bacteria have been amassed in recent years; this provides an opportunity for



using bacterial genome sequencing for identification purposes. Whole genome sequencing is increasing in popularity, although at present it remains a relatively expensive approach to bacterial identification and typing. Complexity-reduced bacterial genome sequencing provides an alternative. We evaluate genomic complexity-reduction using restriction enzymes and sequencing to identify bacterial isolates (Talamantes-Becerra et al., 2019)

Methods for accurate bacterial identification are critical for medical diagnosis and treatment of infectious diseases. Traditional identification based on the study of phenotypic characteristics or biochemical testing provide an inexpensive option. However, phenotypic similarities between bacterial strains may lead to incorrect diagnosis. In the last decade, sequencing techniques such as 16S rRNA gene sequencing and other PCR methods have provided novel approaches for bacterial identification. DNA sequence-based results for bacterial identification and strain typing at a lower cost than whole genome sequencing. A bioinformatics pipeline, Curr3.1 DNA Fragment Analysis Software, was developed to automate the data analysis. Automation enables time-savings, facilitates reproducibility and reduces the probability of human mistakes. The final report produced by the analytical pipeline indicates the closest identified bacterial reference sequence including genus, species and in some cases, strain typing information (Buszewski et al., 2017).

## VI) Resistant bacteria

Antibiotic resistance in bacteria has emerged as a medical catastrophe. This results from the speed at which bacteria multiply and are spread, and the ease with which they can change their genetic material or acquire new genes. They exert biochemical resistance by preventing entry of the drug, by rapidly extruding the drug, or by enzymatically inactivating the drug or altering its molecular target. The presence of antibiotics in the internal environments of human beings and animals provides a selective pressure for any resistant organisms to become predominant. Examples of antibiotic resistance in several important human pathogens are *Streptococcus pneumoniae*, enterococci, staphylococci, enteric bacilli, *Haemophilus influenzae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, and *Mycobacterium tuberculosis*. the widespread availability of antibiotics, accompanied by their overprescription, acts as a double-edged sword, since the overuse and/or misuse of antibiotics leads to a growing number of multidrug-resistant microbes.



This, in turn, has recently emerged as a global public health challenge facing both clinicians and their patients (Kwiatkowska et al., 2023). Causes of antibiotic resistance are different in aspects eg. antibiotic-resistant genes, inappropriate use and prescription of antibiotics, the extensive use of antibiotics in agriculture and so on. In addition to intrinsic resistance, bacteria can acquire resistance to particular antimicrobial agents through the transfer of genetic material conferring resistance. Amongst the most common bacterial resistance strategies are: drug target site changes, increased cell wall permeability to antibiotics, antibiotic inactivation, and efflux pumps. A better understanding of the interplay between the mechanisms of antibiotic actions and bacterial defense strategies against particular antimicrobial agents is crucial for developing new drugs or drug combinations (Baran et al., 2023).

## VII) Antibiogram Profiling

An antibiogram profiling is an overall profile of antimicrobial susceptibility testing results of a specific microorganism to a battery of antimicrobial drugs. This profile is generated by the laboratory using aggregate data from a hospital or healthcare system; data are summarized periodically and presented showing percentages of organisms tested that are susceptible to a particular antimicrobial drug. Only results for antimicrobial drugs that are routinely tested and clinically useful should be presented to clinicians. The Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS) published guidelines entitled "Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data" for use when creating an antibiogram. CLSI guidelines recommend compiling the antibiogram at least annually, including only the first isolate per patient in the period analyzed, and including only organisms for which  $\geq 30$  isolates were tested in the period analyzed. Antibiograms are compiled mainly by microbiology laboratory technologists, but may be a collaborative effort involving the lab, pharmacy, infection preventionists, and clinicians (Ferreira et al., 2021)

Results revealed that Antibiograms of *S. aureus* and CoNS showed 100% and 78% resistance, respectively, against penicillin. *Acinetobacter* spp. showed 100% resistance to ceftriaxone and ampicillin. Overall prevalence of multidrug-resistant bacteria was 57.7% (Birru et al., 2021)

### 2.1.1 The rise of antimicrobial resistant bacteria

In addition to evolution and random mutations driven by selection due to antibiotic pressure or other environmental stress factors, AMR can arise through horizontal gene transfer between species, including the exchange of pathogenicity islands, via plasmids, transposons, chromosomal cassettes or prophages. Resistance genes can then be transmitted to other areas, including where antibiotic usage is significantly lower, via the movement of humans or through the food chain. (Munita et al., 2016)

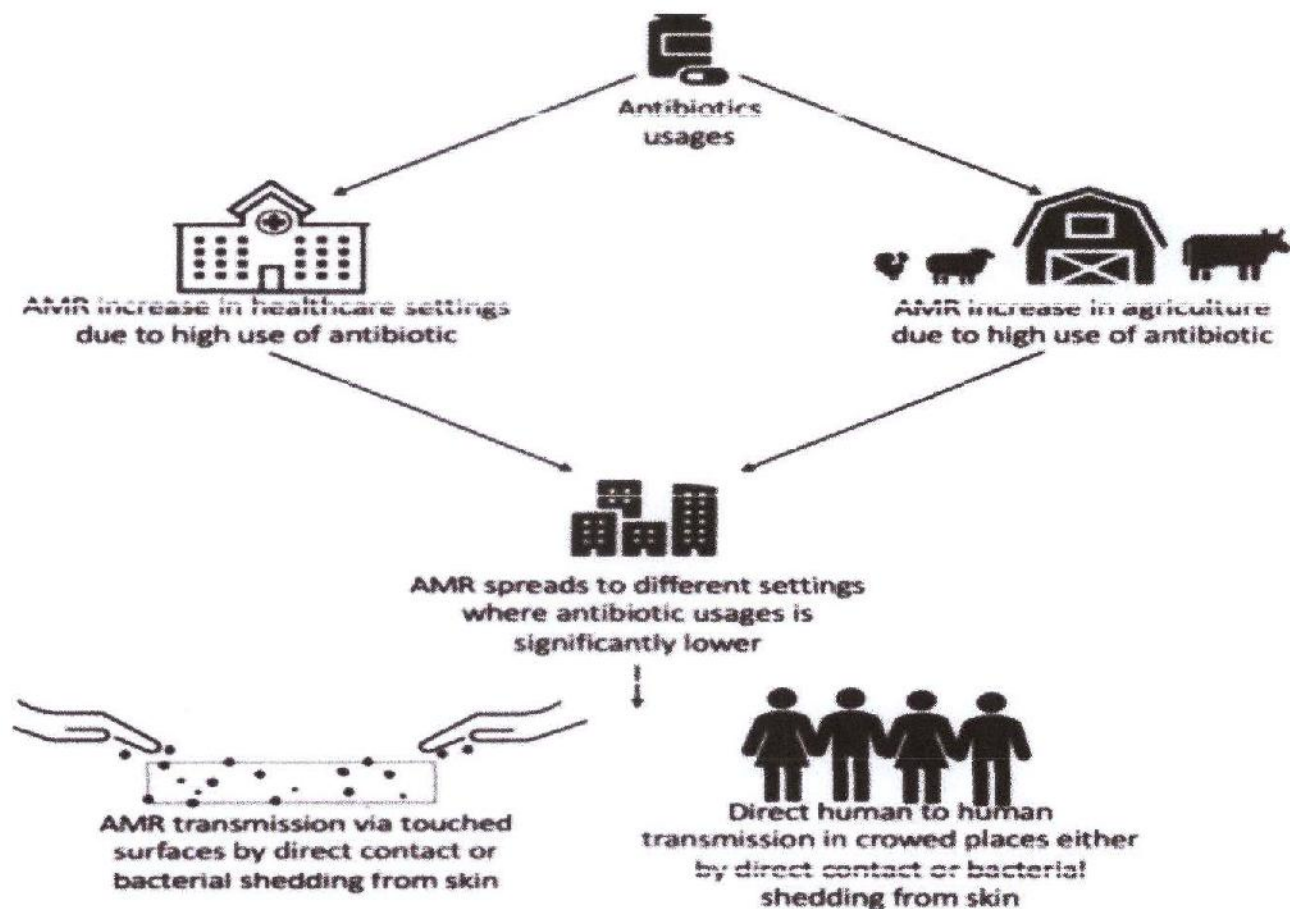


Fig. 2.1.1: The transmission of AMR bacteria from high antibiotic usage areas to humans within public settings (Munita et al., 2016).



### 2.1.2 Antibiotic resistance in human medicine

In the community of affluent countries, the excessive prescription by general practitioners, even in the absence of appropriate indications, plays an important role in the inappropriate use of antibiotics. Diagnostic uncertainty often fosters over-prescription especially when the clinical picture of viral or bacterial aetiology is similar. Self-medication also plays an important part. In many developing countries, excessive use is due to the easy availability of antimicrobial drugs that can be purchased without prescription of a physician or other qualified health professional ( Ugwu et al., 2020).

In both situations, there is the perception that antibiotics are the “wonder drugs” that can rapidly cure any kind of ailments. In the hospital setting, the intensive and prolonged use of antimicrobial drugs is probably the main contributor to the emergence and spread of highly antibiotic-resistant nosocomial infections; but other factors can play an important role: presence of highly susceptible immunosuppressed patients ( Murray et al., 2022).

According to a recent point prevalence survey on healthcare associated infections in Europe, 35.0% of the hospitalised patients in 2011 were receiving antibiotics ( Hauwermeiren et al., 2019). The inappropriate use of antibiotics is also associated with other common behaviour patterns, such as failure to complete the recommended treatment or self-medication. Self-medication with antimicrobials almost always involves unnecessary, inadequate, and ill-timed dosing, creating an ideal environment for microbes to adapt rather than be eliminated. Self-medication with antimicrobials is common in many areas of world, particularly in developing countries with loose regulatory systems where antibiotics are sold over the counter drugs, but also in some affluent countries. Antibiotics are medications that are used to prevent and treat bacterial infections. (Strathdee et al., 2020). Antibiotic resistance, on the contrary, occurs when bacteria change as a result of antibiotic use. A scenario in which bacteria evolve and cease to respond to drugs, making infections more difficult to cure and raising the risk of disease spread, serious sickness, and death. Bacterial resistance refers to a bacterium's ability to withstand or tolerate the effects of antibiotics. The ability of a microorganism to withstand an antimicrobial's growth inhibitory or killing activity at clinically achievable concentrations ( Murray et al., 2022).



Antibiotic resistance is on the rise in all parts of the world as bacterial infections are one of the leading causes of illness and mortality. New resistance mechanisms emerge regularly and spread internationally, putting the capacity to treat prevalent infectious diseases in jeopardy. Because of its potential to spread internationally and the resulting restricted treatment options, antibiotic resistance is a major concern in healthcare ( Ugwu et al., 2020).

Recently, the WHO named antimicrobial resistance among the top 10 global public health threats facing humanity. Antibiotic-resistant infections are estimated to claim up to 10 million lives per year by 2050, costing the global economy about \$100 trillion (Strathdee et al.,2020)

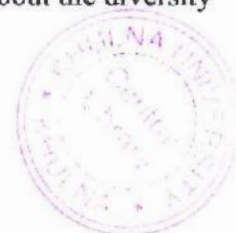
As antimicrobial resistance increases in many parts of the world, it becomes increasingly important to not just search for new antimicrobial agents but to come up with strategies to further reduce the emergence and rate of resistance or possibly counter the speedy development of antibiotic resistance by pathogenic microorganisms. It is mentioned that unsuccessful treatment of bacterial infections associated with antibiotic resistance claims at least 700,000 every year globally, and is projected to be associated with 10 million deaths per year by 2050 ( Strathdee et al.,2020).

### 2.1.3 The threat from antimicrobial resistance

The rise of antimicrobial resistance (AMR) is a global crisis which threatens the lifesaving role of antibiotics, the use of which is one of the cornerstones of modern medicine (Hutchings et al., 2019).

A report by Lord O'Neil commissioned by the UK government in 2014, estimated that by 2050 10 million people worldwide could die annually from infections that no longer respond to antibiotics, with the economic cost of this could reach USD 100 trillion annually if no concrete global action is undertaken (Katherine et al., 2019). The rapid increase and spread of AMR is driven by many factors, including the overreliance on and misuse of antibiotics in healthcare settings and in agriculture, including in livestock feed as a growth promotor and metaphylaxis or as an "infrastructural" response to poor hygiene, overcrowding and climate stress (Chang et al., 2019).

Only a few studies have focussed on general public settings in built-up environments but the studies that have been conducted generally shown that public settings have a high abundance of AMR resistance across different species. Some of these species pose a public health risk as they have genetic linkage to isolates that have caused infections in humans and livestock animals (Cave et al., 2021). The main issues with many of these studies is that they tend to focus on relevant clinical bacteria (including those belonging to the genus *Staphylococcaceae* and *Enterobacteriaceae*), recovered from surfaces and from the air: little is known about the diversity of antimicrobial resistant bacterial species (Kahsay et al., 2019).



#### 2.1.4 Disinfection

Disinfection removes micro-organisms without complete sterilization. Disinfection is used to destroy organisms present on delicate or heat-sensitive instruments which cannot be sterilized or when single use items are not available. Disinfection is not a sterilizing process and must not be used as a convenient substitute for sterilization (Bakar et al., 2018). Thermal disinfection is not appropriate for instruments that will be used in critical sites as these instruments must be sterile. Certain products and processes will provide different levels of disinfection. These levels are classified as:

- I. High-level disinfection : Destroys all micro-organisms except some bacterial spores (especially if there is heavy contamination).
- II. Intermediate disinfection : Inactivates *Mycobacterium tuberculosis*, vegetative bacteria, most viruses and most fungi, but does not always kill bacterial spores.
- III. Low-level disinfection : Can kill most bacteria, some viruses and some fungi, but cannot be relied on to kill more resistant bacteria such as *M. tuberculosis* or bacterial spores. The two methods of achieving disinfection are thermal and chemical disinfection (Haque et al., 2018).

##### a) Thermal disinfection (pasteurization)

If an instrument is able to withstand the process of heat and moisture and is not required to be sterile, then thermal disinfection is appropriate. By using heat and water at temperatures that



destroy pathogenic, vegetative agents, this is a very efficient method of disinfection. The level of disinfection depends on the water temperature and the duration the instrument is exposed to that temperature (Sartelli et al., 2018).

#### b) Chemical disinfection

The performance of chemical disinfectants is dependent on a number of factors including : temperature, contact time, concentration, pH, presence of organic or inorganic matter and the numbers and resistance of the initial bioburden on a surface. Instrument grade disinfectants are classified as high, intermediate or low level. When used according to the manufacturers' guidelines, disinfectants will fall into one of these levels (Bakar et al., 2018).

#### c) Selection of disinfectant

There is no single ideal disinfectant. Different grades of disinfectants are used for different and various purposes. Only instrument grade disinfectants are suitable to use on medical instruments and equipment. Hospital grade or household grade disinfectants must not be used on instruments, they are only suitable for environmental purposes. Monitoring of the disinfectant is important if it is a multi-use solution. It is important that it is stored correctly and according to the manufacturer instructions. Be sure not to contaminate the solution when pouring out for use. Glutaraldehyde is generally the most appropriate chemical disinfectant that will provide high- level disinfection. This chemical must be used under very strict controlled conditions and in a safe working in those environment. Glutaraldehyde 2% is an appropriate high level disinfectant for endoscopes, respiratory therapy equipment and for material that is destroyed by heat. An immersion time of > 20 min is required. Flexible endoscopes are very easy to damage and particularly difficult to disinfect. It is extremely important that meticulous mechanical cleaning must always precede sterilization or disinfection procedures. For the selection of disinfectants see APIC Guidelines for selection and use of disinfectants (Bakar et al., 2018).

### 2.1.5 Factors contributing to the emergence of antibiotic resistance

Antibiotic resistance is a natural phenomenon that occurs when microorganisms are exposed to antibiotic drugs. Under the selective pressure of antibiotics, susceptible bacteria are killed or inhibited, while bacteria that are naturally (or intrinsically) resistant or that have acquired

antibiotic-resistant traits have a greater chance to survive and multiply. Not only the overuse of antibiotics but also the inappropriate use contribute to the increase of antibiotic resistance (Francesca et al., 2015).

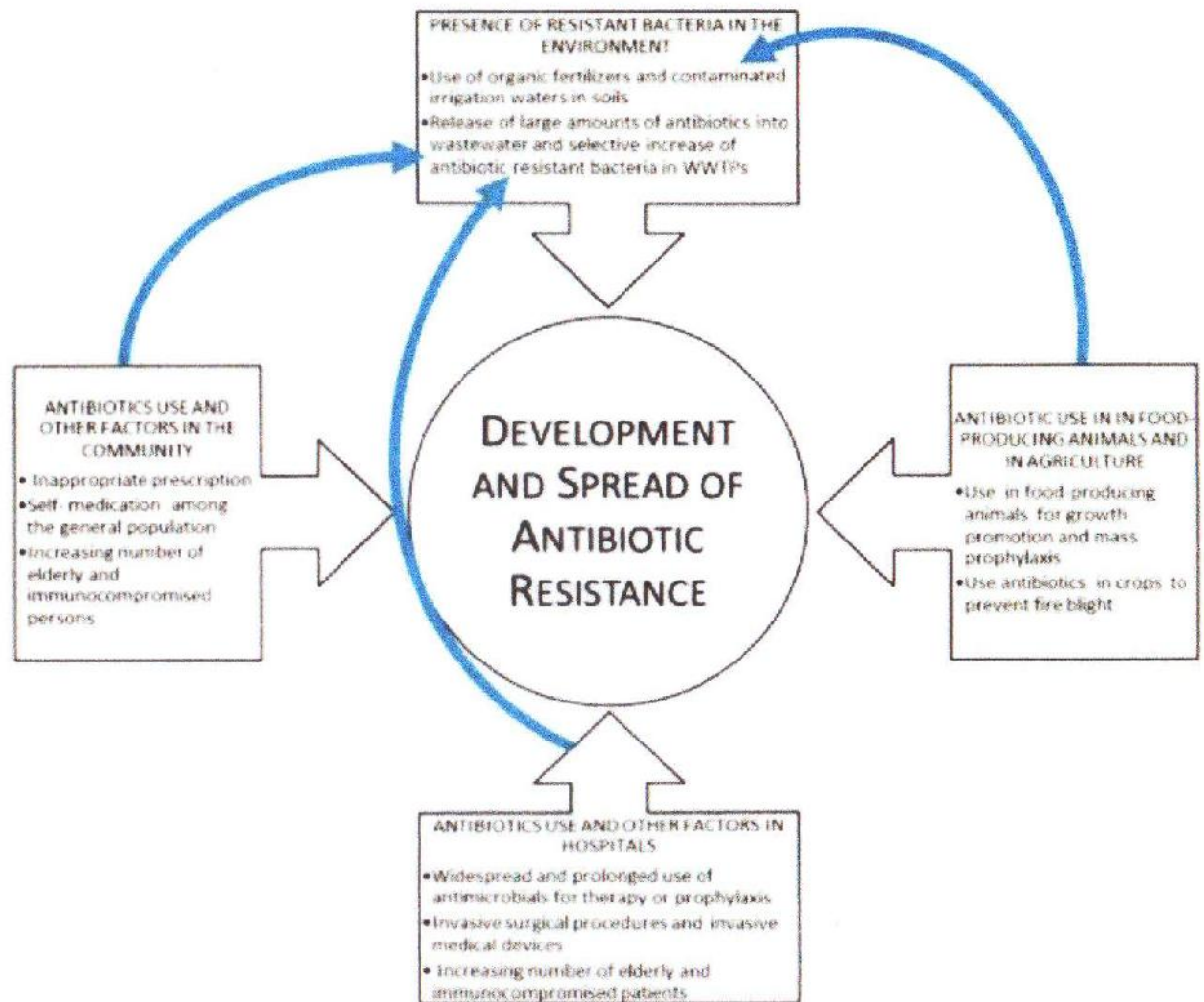


Fig 2.1.2 : Factors involved in the spread of antibiotic resistance (Francesca et al., 2015).



### 2.1.6 Bacterial infection with their transmission mode

| Name of Bacteria                   | Infection in Human body                                 | Mode of transmission              |
|------------------------------------|---------------------------------------------------------|-----------------------------------|
| 1. <i>Staphylococcus aureus</i>    | Pneumonia, Vomiting, Fever                              | Nosocomial                        |
| 2. <i>Klebsiella pneumoniae</i>    | Pneumonia, Fever, Urinary tract infection, Vomiting     | Nosocomial                        |
| 3. <i>Pseudomonas aeruginosa</i>   | Urinary tract infection, Pneumonia, Fever               | Water, Contaminated surface       |
| 4. <i>E. coli</i>                  | Urinary tract infection, Vomiting, Fever                | Nosocomial, Water, Food           |
| 5. <i>Salmonella typhimurium</i>   | Diarrhoea, Fever, Stomach pain                          | Airborne, Droplets                |
| 6. <i>Corynebacterium spp</i>      | Diphtheria, Pneumonia, Urinary tract infection, Fever   | Direct contact, Droplets          |
| 7. <i>Streptococcus pneumoniae</i> | Tonsillitis, Throat infection, Pneumonia                | Contaminated surface              |
| 8. <i>Acinetobacter spp</i>        | Meningitis, Urinary tract infection, Pneumonia, Fatigue | Contaminated surface, Droplets    |
| 9. <i>Shigella spp</i>             | Diarrhoea, Vomiting, Stomach pain                       | Food, Water, Contaminated surface |

Table 2.1.3: Different bacterial species with their infection in human body and mode of transmission

(Zoppi *et al.*, 2020 & Karin *et al.*, 2019)

# **Chapter Three**

## **Materials and Method**



### 3 Materials and Method

## Flowchart of my overall work

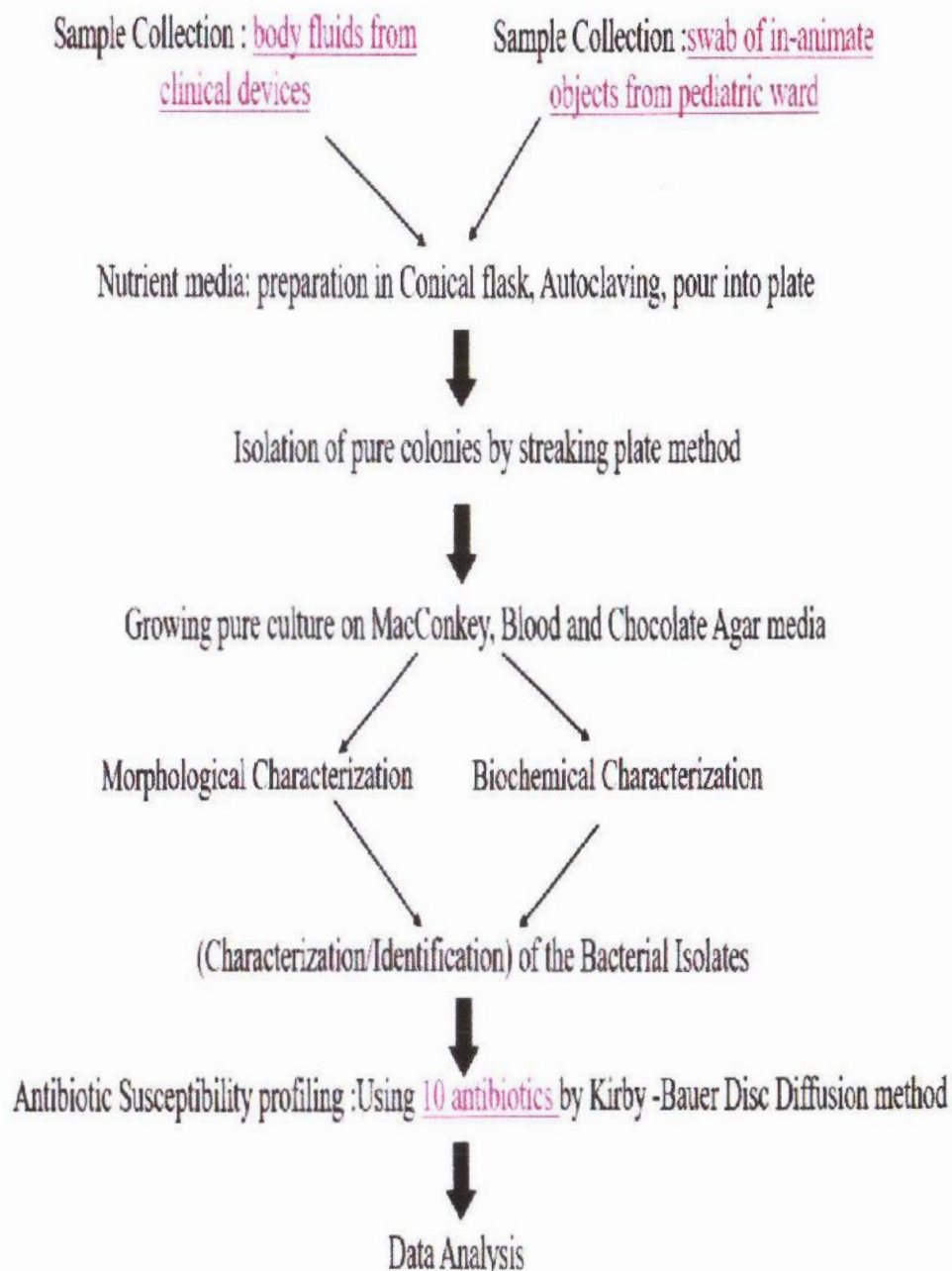


Fig 3: Overall flowchart of my research work

### 3.1 Study Area and Sample Collection

This study was conducted in Pediatric Ward of Ad-din Akij Medical College, Khulna Shishu Hospital and Khulna Medical College Hospital of Khulna city from 7 February 2023 to 20 January 2024. The research took place in the Animal Biotechnology Laboratory under the Biotechnology and Genetic Engineering Discipline at Khulna University, Bangladesh. Sample collection occurred at Ad-Din Akij Medical College Hospital (AAMCH) between February 7, 2023 to March 12, 2023, Khulna Shishu Hospital(KSH) between September 15, 2023 to October 25, 2023 and the final study was carried out at Khulna Medical College Hospital (KMCH) in Khulna City, Bangladesh, between December 10, 2023 to January 20, 2024.

A total of 40 samples were collected from various inanimate objects including Bed Rail, Bed Cover, Door Handle, Medical Chart, Weight Machine, Scissor, Desk, Calculator, Telephone, Trolley, Stethoscope and Sink at Pediatric Ward in Ad-Din Akij Medical College Hospital (AAMCH) further 40 Samples were collected from inanimate objects at Pediatric Ward in Khulna Shishu Hospital (KSH) and 25 Samples were obtained from inanimate objects at Pediatric Ward in Khulna Medical College Hospital (KMCH).

A total of 25 discarded patient care equipment items, including Canula, Catheter, Oxygen Nebulizer and Suction Tube from pediatric patient admitted to Pediatric ward in Ad-Din Akij Medical College Hospital (AAMCH), 20 discarded patient care equipment from Pediatric ward in Khulna Shishu Hospital (KSH), and 20 discarded patient care equipment from Pediatric ward in Khulna Medical College Hospital (KMCH) were collected for the study.

|                  | AAMCH                                 |                     | KSH                                       |                     | KMCH                                     |                     | Overall                          |                     |
|------------------|---------------------------------------|---------------------|-------------------------------------------|---------------------|------------------------------------------|---------------------|----------------------------------|---------------------|
|                  | In-<br>animate<br>objects             | Clinical<br>devices | In-<br>animate<br>objects                 | Clinical<br>devices | In-<br>animate<br>objects                | Clinical<br>devices | In-<br>animate<br>objects        | Clinical<br>devices |
| Total<br>Samples | 40                                    | 50                  | 40                                        | 40                  | 25                                       | 40                  | 105                              | 130                 |
| Study period     | February 7, 2023<br>to March 12, 2023 |                     | September 15, 2023<br>to October 25, 2023 |                     | December 10, 2023<br>to January 20, 2024 |                     | February, 2023-<br>January, 2024 |                     |

Table 3.1.1 : Total isolated sample (n=235) at 3 different Hospitals in Khulna City

| Serial No. | Inanimate Objects       | Clinical devices | Body Fluids<br>Collected from<br>Clinical devices |
|------------|-------------------------|------------------|---------------------------------------------------|
| I.         | Bed Rail                | Canula           | Blood                                             |
| II.        | Bed Cover               | Suction Tube     | Intestinal fluid                                  |
| III.       | Door Handle             | OG tube          | Nasal swab                                        |
| IV.        | Medical Chart           | Oxygen Nebulizer | Nasal swab                                        |
| V.         | Cardiopulmonary Monitor |                  |                                                   |
| VI.        | Blood pressure Monitor  |                  |                                                   |
| VII.       | Weight Machine          |                  |                                                   |
| VIII.      | Scissor                 |                  |                                                   |
| IX.        | Stethoscope             |                  |                                                   |
| X.         | Desk                    |                  |                                                   |
| XI.        | Calculator              |                  |                                                   |
| XII.       | Telephone               |                  |                                                   |
| XIII.      | Trolley                 |                  |                                                   |
| XIV.       | Sink                    |                  |                                                   |

Table 3.1.2 : List of body fluids from Clinical devices and swabs of inanimate objects which has been used as sample source



### 3.2 Research tools and procedures:

Literature review for compiling related secondary information from books, journal, reports etc. available at Khulna university central library and internet resources will be explored for reviewing most recent relevant information.

### 3.3 Experimental Materials

The experiments required various equipment like i.Electronic Balance, ii.pH Meter, iii.Magnetic Stirrer, iv.Autoclave, v.Freezer, vi.Electric microscope, vii.Laminar air flow, viii.Incubator, ix.Refrigerator, and x. Water bath xi.Zip lock bag xii.Spirit lamp xiii.Inoculating loop xiv.Ice box

| Serial No. | Equipment           | Function                                                                                                                                                                                                                         |
|------------|---------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| I.         | Electronic Balance  | Electronic balance is an instrument used in the accurate measurement of weight of materials                                                                                                                                      |
| II.        | pH Meter            | A pH meter is a scientific instrument that measures the hydrogen-ion activity in water-based solutions, indicating its acidity or alkalinity expressed as pH.                                                                    |
| III.       | Hot plate           | A hot plate is a laboratory device used to heat samples, solutions, and materials uniformly without the danger associated with the open flame at precise temperatures.                                                           |
| IV.        | Autoclave           | An autoclave is a machine that uses steam under pressure to kill harmful bacteria, viruses, fungi, and spores on items that are placed inside a pressure vessel.                                                                 |
| V.         | Refrigerator,       | The primary function of a laboratory refrigerator is to maintain a defined, internal storage temperature <sup>1</sup> . It is used to store and preserve various samples and chemicals.                                          |
| VI.        | Electric microscope | An electron microscope is a device that uses a beam of electrons to magnify an object's image. It is a technique for obtaining high resolution images of biological (such as bacteria, fungi etc.) and non-biological specimens. |
| VII.       | Laminar air flow    | It is extensively used in laboratories to maintain the aseptic or contaminant-free working atmosphere.                                                                                                                           |

|       |            |                                                                                                                                                                                                                                                   |
|-------|------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| VIII. | Incubator  | An incubator is a device used to grow and maintain microbiological cultures or cell cultures. The incubator maintains optimal temperature, humidity and other conditions such as the CO <sub>2</sub> and oxygen content of the atmosphere inside. |
| IX.   | Freezer    | The function of laboratory freezers are storing temperature-sensitive materials like volatile chemicals, biological specimens, and cellular components.                                                                                           |
| X.    | Water bath | A water bath is a laboratory equipment used to incubate samples in water at a constant temperature over a long period of time.                                                                                                                    |

Table 3.3.1: Function of Lab Equipment

### 3.4 Media and chemicals

The chemicals and reagents used in the experiments were Nutrient broth, Agar powder, MacConkey agar, Blood agar base, sheep blood, Gram Iodine, Safranin, Crystal Violet, Alcohol, Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) Mueller Hinton Agar Media, TSI media, Urease agar media, Simon's Citrate Agar Media, Kovacs Oxidase Reagent(1 percent tetra-methyl-pphenylenediamine dihydrochloride), Whatman's filter paper, methyl red-Voges-Proskauer(MRVP) broth, Methyl red solution, 0.02%, plasma, Mueller Hinton Agar, sterile 0.8% saline and Sodium Chloride. Media are manufactured by Himedia manufactures company.

### 3.5 Sample Processing

#### 3.5.1. Inanimate Surface Samples

Samples were transported to the laboratory in cool conditions and processed within two hours of collection. Sterilized cotton swab dipped in 0.85% saline water is used to wipe inner surface of clinical devices. Tip of the cotton swab is rubbed on a small area of agar plate (Blood agar, MacConkey agar) and from that area streaking is done by inoculation loop and incubated at 37°C for 24 hours. Clinicals devices (Central venus catheter, Used syringe, Nasal canula, Nasogastric



tube, Suction tube) were collected from Pediatric Ward in Hospitals by using zip-lock bag and brought into Animal Biotechnology Lab in Khulna university.

### 3.5.2 Patient's Body Fluids Samples from Clinical devices

Discarded patient care equipment such as canulas, OG(Orogastric) tubes, and NG(Nasogastric Tube), suction tubes were collected from Pediatric patient upto 5 years old. Each sample was labeled with the patient's age and brief clinical history. Patients who had been admitted for at least 1 or 2 days were included in the study. All specimen collections were performed with the assistance of nurses. Samples were transported to the laboratory immediately using a cool box for further processing. Body fluid was collected from the discarded patient care equipment by sterilized cotton swab and inoculating loop.

### 3.5.3 Isolates from Inanimate objects and Body Fluids from PCE

Nutrient agar, blood agar, and MacConkey agar media were used for isolating bacteria from samples. These media were prepared following the manufacturer's instructions. Swab samples and samples like body fluid (Blood, Oral-Gastric content, Nasal-Gastric Content, Nasal-Oral Contents, Respiratory secretions) from patient care equipment were inoculated on all three media types. The agar plates were then incubated at 37°C for 24 hours. After incubation, colonies with specific characteristics in terms of form, surface texture, and edge were selected and streaked onto new plates.

## 3.6 Media Preparation

### 3.6.1 Nutrient agar

It contains nutrients that suitable to subculture a wide range of microorganisms and makes it an excellent agar media to check on the purity before any biochemical or serological test. Besides, the addition of agar solidifies nutrient agar, which makes it suitable for the cultivation of microorganisms.



### Preparation of Nutrient agar

Firstly, I suspended 28g of nutrient agar powder in 1L of distilled water which was mixed and dissolved completely. Then sterilized properly by autoclaving at 121°C for 15 minutes. Next the liquid poured into the petri dish and wait for the medium to solidify. I was sure that the media was prepared in the clean environment to prevent any contamination. When the agar was solidified which was ready to use (Adrian et al., 2021).

### 3.6.2 Nutrient Broth

Basically, the nutrient broth is the nutrient agar that lacks the solidifying agent, agar powder. It remains in liquid form at room temperature and are usually used to maintain the stocks of microorganisms. In general, it was used to grow fastidious organisms. Also, we can enrich our nutrient broth with blood, serum, sugars etc for special purposes.

### Preparation of Nutrient broth

I added 13g of nutrient agar powder in 1L of distilled water. Then mixed and dissolved it completely and poured them into the final containers (eg. conical flask). Finally, sterilized by autoclaving at 121°C for 15 minutes to prevent any contamination (Adrian et al., 2021).

### 3.6.3 MacConkey agar (MAC)

**MacConkey agar** was the first solid differential media to be formulated which was developed in the 20th century by Alfred Theodore MacConkey. **MacConkey agar** is a selective and differential media used for the isolation and differentiation of non-fastidious gram-negative rods, particularly members of the family Enterobacteriaceae and the genus *Pseudomonas*.

### Preparation of MacConkey Agar

I suspended 49.53 grams of dehydrated medium in 1000 ml purified/distilled water and provided heat in boiling to dissolve the medium completely. Then sterilized properly by autoclaving at 15

lbs pressure (121°C) for 15 minutes and cooled to 45-50°C. Finally, Mixed well before pouring into sterile Petri plates (Jung et al., 2022)

#### 3.6.4 Blood Agar (BA)

Blood Agar are **enriched medium** used to culture those bacteria or microbes that do not grow easily. Such bacteria are called "fastidious" as they demand a special, enriched nutritional environment as compared to the routine bacteria. Blood Agar is used to grow a wide range of pathogens particularly those that are more difficult to grow such as *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Neisseria* species. It is also required to detect and differentiate haemolytic bacteria, especially *Streptococcus* species. It is also a differential media in allowing the detection of hemolysis (destroying the RBC) by cytolytic toxins secreted by some bacteria, such as certain strains of *Bacillus*, *Streptococcus*, *Enterococcus*, *Staphylococcus*, and *Aerococcus*. Blood agar can be made selective for certain pathogens by the addition of antibiotics, chemicals or dyes.

#### Preparation of Blood Agar

I Suspended 28 g of nutrient agar powder in 1 litre of distilled water and provided heat this mixture while stirring to fully dissolve all components. Then autoclaved the dissolved mixture at 121 degrees Celsius for 15 minutes. Once the nutrient agar had been autoclaved, allowed it to cool but not solidify. When the agar cooled to 45-50 °C, Added 5% (vol/vol) sterile defibrinated blood that had been warmed to room temperature and mixed gently but well. Finally, I avoided air bubbles and dispensed into sterile plates while liquid (Sagar et al.,2022).

#### 3.6.5 Chocolate agar

It is essentially the same as blood agar except that during preparation the red blood cells are lysed when added to molten agar base. As a result, the cell lysis releases intracellular nutrients such as hemoglobin, hemin , and the coenzyme nicotinamide adenine dinucleotide into the agar which is utilized by fastidious bacteria. Red blood cell lysis gives the medium a chocolate-brown coloration when prepared from which the agar gets its name.



### Preparation of Chocolate Agar

To prepare chocolate agar, blood agar was firstly heated until it become molten. This process causes the red blood cells within the agar to rupture, releasing their intracellular nutrients. The resulting medium provided the fastidious bacteria with the essential components they need for growth. This enriched medium was then poured into Petri dishes or other containers and allowed to solidify. In the laboratory, chocolate agar was frequently used to isolate and cultivate fastidious pathogens from clinical samples. The medium's composition made it highly supportive of the growth of bacteria that might not thrive on other types of media. By providing the necessary nutrients, chocolate agar enables the identification and further study of these bacteria, aiding in the diagnosis and treatment of associated infections. In summary, chocolate agar is a specialized agar medium used for the cultivation and isolation of fastidious bacteria. It is similar to blood agar but contains lysed red blood cells, releasing important nutrients for the growth of specific bacterial pathogens (Sagar et al.,2022).

### 3.6.6 Bacterial Glycerol Stock

Bacterial glycerol stocks are important for long-term storage of bacteria. Although we can store bacteria at  $-20^{\circ}\text{C}$ , many labs also create bacterial glycerol stocks. This way, when we want to make more plasmid DNA, the plasmid will already be in your desired bacterial strain and we will not need to obtain more competent cells and retransform. Bacteria on an LB agar plate can be stored at  $4^{\circ}\text{C}$  for a few weeks. However, if we want to store bacteria for a longer time, we will need to establish glycerol stocks. The addition of glycerol stabilizes the frozen bacteria, preventing damage to the cell membranes and keeping the cells alive. A glycerol stock of bacteria can be stored stably at  $-80^{\circ}\text{C}$  for many years.

### Preparation of Bacterial Glycerol Stocks

At first it should be followed the steps for Inoculating an Overnight Liquid Culture. After i had been successfully done the bacterial growth and added 500  $\mu\text{L}$  of the overnight culture to 500  $\mu\text{L}$



of 50% glycerol in a 2 mL screw top tube or cryovial and gently mixed. Freezing the glycerol stock tube at  $-80^{\circ}\text{C}$ . The stock was then stable for years, as long as it is kept at  $-80^{\circ}\text{C}$ . Subsequent freeze and thawed cycles to reduce shelf life. To recover bacteria from your glycerol stock, I had to open the tube and use a sterile loop, toothpick or pipette tip to scrape some of the frozen bacteria off of the top. It had been done to grow the bacteria overnight at the appropriate temperature. Finally, Growth conditions, including copy number and growth temperature, could be found on your plasmid's information page and the next day I started for further processing an overnight culture for plasmid DNA prep the following day (Adrian et al., 2021).

### 3.6.7 Mueller Hinton agar

It is a culture media suitable for the isolation of pathogenic *Neisseria* species. With the addition of different supplements, it can be used for different Antimicrobial Susceptibility Testing (AST).

#### Preparation of Mueller Hinton agar

I Suspended 38g of your Muller Hinton Powder in 1L of distilled water. Then mixed and dissolved them completely. Sterilized by autoclaving at  $121^{\circ}\text{C}$  for 15 minutes. Finally, poured the liquid into the petri dish and wait for the medium to solidify. I was sure that you was preparing the agar in the clean environment to prevent any contamination. Once the agar was solidify, the agar was ready to use. (Adrian 2021)

| Sl.No. | Media              | Purposes                                                                                             |
|--------|--------------------|------------------------------------------------------------------------------------------------------|
| 01     | Nutrient agar      | To grow a wide variety of nonfastidious microorganism                                                |
| 02     | Macconkey agar     | To differentiate the gram negative bacteria based on their lactose metabolism eg. Klebsiella, E.coli |
| 03     | Blood agar         | To grow fastidious organisms and determine hemolysis eg. Streptococcus                               |
| 04     | Chocolate agar     | To isolate nonselective pathogenic bacteria eg. Haemophilus influenzae                               |
| 05     | Muller-Hinton agar | To determine antibiotics susceptibility eg. Disk-diffusion method                                    |
| 06     | Nutrient broth     | To maintain the stocks of microorganisms                                                             |

Table 3.3.2: Different media used in the study

### 3.7 Bacteria isolation and growth of colony:

- Media (Blood agar, MacConkey agar, Mueller Hinton agar media) is prepared according to the media labeling instruction and other necessary equipment( petri dish, inoculation loop) are sterilized by autoclaving at 121 degree Celsius for 15 minute.
- Sterilized cotton swab dipped in 0.85% saline water is used to wipe inner surface of clinical devices)
- Tip of the cotton swab is rubbed on a small area of agar plate (Blood agar, MacConkey agar) and from that area streaking is done by inoculation loop and incubated at 37°C for 24 hours.

#### 3.7.1 Gram Staining

Gram staining was performed to identify bacterial strains based on Gram's reaction. A fresh culture was spread and heat-fixed on a glass slide. It was then allowed to cool, followed by air-drying. The heat-fixed (passing it over a heat source, such as a flame, several times) smear was stained with crystal violet and subsequently rinsed in tap water. Gram's iodine was applied, rinsed, and acetone-alcohol (decolorizer) was used to remove excess color. Afterward, the smear was rinsed and safranin was applied. Finally, the slide was examined under an electric microscope (Zeiss-Primo Star).

### 3.8 Biochemical Test:

- Catalase Test
- Oxidase Test
- Methyl Red (MR)
- Citrate Utilization Test
- Triple Sugar Iron Test (TSI)
- Urease Test
- Coagulase Test





| Sl.No. | Bacteria                          | Oxidase | Catalase | MR | Citrate | TSI | Urease | Coagulase |
|--------|-----------------------------------|---------|----------|----|---------|-----|--------|-----------|
| 01     | <u>Streptococcus</u>              | -       | -        | +  | -       | -   | -      | -         |
| 02     | <u>Staphylococcus</u>             | -       | +        | +  | -       | -   | +      | +         |
| 03     | <u>Pseudomonas</u>                | +       | +        | -  | +       | -   | -      | -         |
| 04     | <u>Shigella</u>                   | -       | +        | -  | -       | +   | -      | -         |
| 05     | <u>Klebsiella</u>                 | -       | -        | -  | +       | -   | +      | -         |
| 06     | <u>E.coli</u>                     | -       | -        | +  | -       | +   | -      | -         |
| 07     | <u>Vibrio cholerae</u>            | +       | -        | -  | +       | -   | +      | -         |
| 08     | <u>Mycobacterium tuberculosis</u> | -       | +        | -  | -       | -   | +      | -         |
| 09     | <u>Salmonella</u>                 | -       | -        | -  | +       | -   | -      | -         |
| 10     | <u>Acinetobacter spp</u>          | -       | +        | -  | +       | -   | -      | -         |
| 11     | <u>Corynebacterium nigricans</u>  | -       | +        | +  | -       | -   | -      | -         |

Table 3.3.3: Bacterial identification based on biochemical test

(Sagar *et al.*,2022 & Ulfat *et al.*,2022)

### 3.8.1 Catalase Test

- Requirement: 3% H<sub>2</sub>O<sub>2</sub>, Glass slide, Inoculation loop, Micropipette, Dropper
- Procedure: This test demonstrate the presence of catalase, an enzyme that catalyses the release of oxygen from hydrogen peroxide. It is used to differentiate those bacteria that produces an enzyme catalase, eg. staphylococci, from non catalase producing bacteria eg. streptococci.

To begin with a glass slide was taken. A little amount of the organism was picked up with a sterile inoculation loop and gently smeared on a glass slide from an 18-24 hour fresh culture. Then, using a dropper or pipette, a drop of 3 percent H<sub>2</sub>O<sub>2</sub> was dropped on the smeared region of the glass slide. Against the dark chamber,

bubbles were observed to form. Catalase positive bacteria cause bubbles to appear on the glass slide, which was recorded carefully at the time.

### 3.8.2 Oxidase Test

- Requirement: Kovacs Oxidase reagent, Whatman's filter paper, Inoculation loop, Dropper
- Procedure: The oxidase test is a biological reaction that determines the presence of cytochrome oxidase, an enzyme that catalyzes the transport of electrons between electron sources in bacteria. This experiment can be performed with a variety of reagents. Kovacs Oxidase Reagent outperforms the others. The test reagent N, N, N', N'- tetramethyl-p-phenylenediamine dihydrochloride serves as an artificial electron acceptor for the enzyme oxidase in this experiment.

A strip of Whatman's No 1 filter paper was soaked in a 1 percent solution of tetramethyl-p-phenylenediamine dihydrochloride that had been newly synthesized. After that, a sterile inoculation loop was used to transmit the colony. Within a few seconds, the color changed. Oxidase-positive bacteria have a rich purple hue that appears within 5-10 seconds, whereas negative bacteria have no color that appears after 60 seconds. This test is used to assist in the identification of Pseudomonas, Neisseria, Alcaligens, Aeromonas, Campylobacter, Vibrio, Brucella and Pasteurella all of which produce the enzyme cytochrome oxidase.

### 3.8.3 Methyl Red (MR)

- Requirement: MR-VP broth, Methyl red solution, Test tube, Inoculation loop, Dropper
- Procedure: The methyl red test is used to measure an organism's capacity to form stable acid end products during glucose fermentation. The test microorganisms were cultured in a broth medium containing glucose. If the



bacteria can use glucose, stable acid is produced, and the color of the methyl redshifts from yellow to red as a result.

To carry out this test, make MR-VP broth, which was then evenly poured into each test tube. Organisms were inoculated into the medium after an 18-24 hour pure culture. For 24 hours, the test tube was incubated in an incubator at 37°C, 5 or 6 drops of methyl red reagent were added after 24 hours of incubation. Finally, the color difference was noticed.

#### 3.8.4 Citrate Utilization Test

- Requirement: Simmon's Citrate Agar, Test tube, Inoculation loop
- Procedure: The citrate utilization test is used to distinguish organisms in the Enterobacteriaceae family based on their capacity to utilize citrate as only sources of energy. The Simmon's formula is the most often used citrate medium in the citrate utilization test.

The medium was poured into a slanted tube. With an inoculation loop, a single colony was selected and the slant was streaked back and forth. For up to 4-7 days, the slant was incubated aerobically at 35 to 37°C. The angle revealed the color change from green to blue.

#### 3.8.5 Triple Sugar Iron Test (TSI)

- Requirement: Triple sugar iron, Test tube, Inoculation loop
- Procedure: The test determines the organism's ability to create hydrogen sulfide which aids in the identification of members of the Enterobacteriaceae family. This experiment was conducted with a triple sugar iron medium (TSI).

To begin, the TSI medium was prepared and placed into the test tube before being autoclaved. Inoculated organisms were collected from an 18-24 hour pure culture.

The culture was then incubated for 24 hours at 37°C. The blackening of the media was seen.

#### 3.8.6 Urease Test

- Requirement: Christensen's Urea Agar, Test tube, Inoculation loop
- Procedure: When amino acids are broken down, they make urea. When water is added to urea, CO<sub>2</sub> and ammonia are made. When ammonia is made, the medium becomes more alkaline. The pH changes when the color of phenol red changes from light orange at pH 6.8 to pink pH 8.1. Rapid organisms that produce urease turn the whole medium pink in 24 hours. Negative organisms don't change color or turn yellow when they make acid and weakly positive organisms may take a few days.

Firstly, the media was prepared with all the ingredients and sterilized at 121°C for 15 minutes. Then the test tubes were filled with 4-5 ml of medium. The test tubes were allowed to cool before use. Inoculated organisms were collected from 28-24 hour pure culture. The culture was then incubated for 24 hours at 37°C. Rapid organisms that produce urease turn the whole medium pink in 24 hours. Negative organisms don't change color or turn yellow when they make acid and weakly positive organisms may take a few days.

#### 3.8.7 Coagulase Test

- Requirement: Human plasma, Glass slide, Inoculation loop, Dropper
- Procedure: A drop of distilled water was placed at both ends of the of the grease-free slides. A colony of each test organism was emulsified at both ends on each slide. A drop of human plasma was added to one of the slides while the other served as a control. Clumping of the organisms looked for within 10 seconds. In the case of positive tests, the fibrinogen was converted directly to fibrin. Clumping was shown for the positive test, while a negative test showed no clumping.



|    |               |      |                             |     |       |     |
|----|---------------|------|-----------------------------|-----|-------|-----|
| 2. | Azithromycin  | 15µg | Beta-Hemolytic Streptococci | ≥18 | 14-17 | ≤13 |
|    |               |      | Streptococcus pneumonia     | ≥18 | 14-17 | ≤13 |
|    |               |      | Staphylococcus aureus       | ≥18 | 14-17 | ≤13 |
| 3. | Ciprofloxacin | 5µg  | Acinetobacter spp.          | ≥21 | 16-20 | ≤15 |
|    |               |      | Enterobacteriaceae          | ≥21 | 16-20 | ≤15 |
|    |               |      | Enterococcus                | ≥21 | 16-20 | ≤15 |
|    |               |      | Staphylococcus aureus       | ≥21 | 16-20 | ≤15 |
|    |               |      | Pseudomonas Aeruginosa      | ≥21 | 16-20 | ≤15 |
| 4. | Imipenem      | 10µg | Acinetobacter spp.          | ≥16 | 14-15 | ≤13 |
|    |               |      |                             | ≥16 | 20-22 | ≤19 |
|    |               |      | Enterobacteriaceae          |     |       |     |
|    |               |      | Staphylococcus aureus       | ≥16 | 14-15 | ≤13 |
|    |               |      | Pseudomonas Aeruginosa      | ≥16 | 14-15 | ≤13 |
| 5. | Gentamicin    | 10µg | Acinetobacter spp.          | ≥15 | 13-14 | ≤12 |
|    |               |      |                             | ≥15 | 13-14 | ≤12 |
|    |               |      | Enterobacteriaceae          |     |       |     |
|    |               |      | Staphylococcus aureus       | ≥15 | 13-14 | ≤12 |
|    |               |      | Pseudomonas Aeruginosa      | ≥15 | 13-14 | ≤12 |
| 6. | Cefixime      | 5µg  | Enterobacteriaceae          | ≥19 | 16-18 | ≤15 |

### 3.9 Antimicrobial susceptibility testing:

Once the bacteria is isolated and identified from each sample collected, the standard Kirby-Bauer disk diffusion method was used to determine the antimicrobial susceptibility profiles of the isolates. An inoculum for each isolate was prepared by emulsifying colonies from an overnight pure culture in sterile normal saline (0.85%) in test tubes with the turbidity adjusted to 0.5 McFarland standards. The bacterial suspension was uniformly streaked on MHA plates using sterile swabs and left for 3 minutes before the introduction of the antibiotics. The antimicrobial susceptibility testing was performed using Mueller-Hinton medium against ampicillin, chloramphenicol, ciprofloxacin, clindamycin, erythromycin, gentamicin, streptomycin, tetracycline, cephalothin, cefotaxime etc. The choice of those antibiotic agents is based on the commonly available drugs and drugs which are frequently prescribed by physicians following the suggestion by Magiorakos .

The zones of inhibition were measured and compared with National Committee for Clinical Laboratory Standards (NCCLS) guidelines. Antibiotics used in the disc diffusion method and their diameter of the zone of inhibition (mm) according to CLSI guideline 2022.

| Serial No. | Antimicrobial Agent | Disc Content | When Testing           | Susceptible | Intermediate | Resistant |
|------------|---------------------|--------------|------------------------|-------------|--------------|-----------|
| 1.         | Amikacin            | 30µg         | Acinetobacter spp.     | ≥ 17        | 15-16        | ≤ 14      |
|            |                     |              | Enterobacteriaceae     | ≥ 17        | 15-16        | ≤ 14      |
|            |                     |              | Pseudomonas Aeruginosa | ≥ 17        | 15-16        | ≤ 14      |
|            |                     |              | Staphylococcus aureus  | ≥ 17        | 15-16        | ≤ 14      |



|     |              |      |                             |     |       |     |
|-----|--------------|------|-----------------------------|-----|-------|-----|
| 7.  | Erythromycin | 15µg | Beta-Hemolytic Streptococci | ≥21 | 16-20 | ≤15 |
|     |              |      | Enterococcus                | ≥23 | 14-22 | ≤13 |
|     |              |      | Streptococcus pneumonia     | ≥21 | 16-20 | ≤15 |
|     |              |      | Staphylococcus aureus       | ≥23 | 14-22 | ≤13 |
| 8.  | Colistin     | 10µg | Pseudomonas Aeruginosa      | ≥11 | -     | ≤10 |
| 9.  | Penicillin   | 10µg | Beta-Hemolytic Streptococci | ≥24 | -     |     |
|     |              |      | Enterococcus                | ≥15 | -     | ≤14 |
|     |              |      | Staphylococcus aureus       | ≥29 | -     | ≤28 |
| 10. | Cefotaxime   | 30µg | Acinetobacter spp.          | ≥23 | 15-22 | ≤14 |
|     |              |      | Enterobacteriaceae          | ≥26 | 23-25 | ≤22 |
|     |              |      | Staphylococcus aureus       | ≥23 | 15-22 | ≤14 |

Table 3.9.1 : Antibiotics and Zone of inhibition used in the disc diffusion method (CLSI .,2023)

### 3.9.1 Antibigram profiling:

Antibiotic-sensitivity profiles of all isolates will be inspected using 10 antibiotics. generally prescribed by the Physician . Selected antibiotics are i.Ciprofloxacin, ii.Amikacin, iii.Imipenem, iv.Gentamicin, v.Cefixime, vi.Erythromycin, vii.Azithromycin, viii.Cefotaxime, ix.Colistin, and x.Penicillin. Antibigrams of Gram-positive and Gram-negative bacteria should be isolated, respectively. It will be observed the bacteria which are highly sensitive to most of the antibiotics

tested and susceptibility patterns exhibited by isolates of different multidrug resistant bacteria by Kirby disk diffusion method (Chaoui et al., 2019).

### 3.10 Statistical Analysis

The data was entered, validated and analyzed using Excel 2016 (Microsoft Corporation) and GraphPad Prism 8.0.2

### 3.11 Ethical Considerations

The research received ethical approval and clearance from the Ethical Committee of Ad-Din Akij Medical College Hospital in Khulna, Bangladesh. All the participants were briefed about the study and informed consent obtained from each of the participant . Details of each participant were extracted from the hospital records using serialized questionnaire, the same serial number on each questionnaire was maintained during coding therefore ensuring the anonymity of the participant details. Written informed consent was taken from each respondent and from caregivers for children before they were requested to give data and sample. All information obtained from this study was kept confidential and utilized only for the study. Positive findings were reported to the attending physicians for the proper management of patients. Patient records were kept safe as data safety record .

# **Chapter Four**

## **Results**





## 4. Results

### 4.1 Isolation of bacteria

This research work was conducted to isolate, identify and determine the antibiogram of Health care associated bacteria. For my research prospect, total 235 samples were taken from Ad-Din Akij Medical College Hospital (AAMCH) including 90, Khulna Shishu Hospital (KSH) including 80 and Khuina Medical College (KMC) including 65 in where total 134 (57%) samples were found different types of bacteria. The study revealed the highest percentage of bacterial growth in Khulna Medical College Hospital that is 69% whereas Ad-Din Akij Medical College showed the lowest rate of bacterial growth that is 48% from In-animate objects and body fluid from Clinical devices. It also reveals that bacteria found comparatively more in Clinical devices on average 72% than In-animate objects i.e 50% as well. Overall prevalence of multidrug-resistant bacteria was 57%.

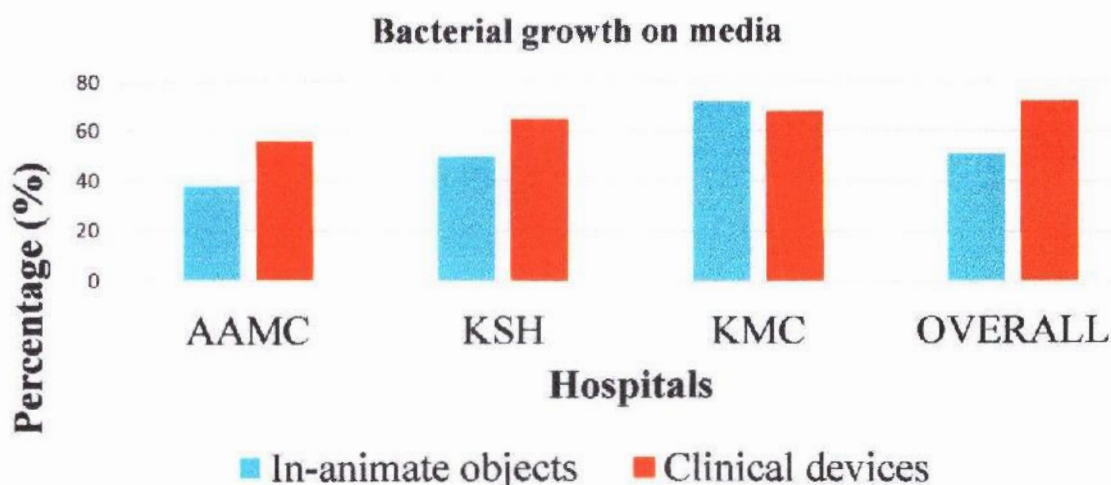


Fig 4 : Growth of total bacterial isolates comparison between In-animate objects and Patient care equipments at 3 different hospitals in Khulna City

## 4.2 Identification of bacteria

### A. Colony Morphology of the Isolates

#### 4.2.1 Macroscopic observation of all bacterial isolates from inanimate objects

| No. of isolates | Nutrient Agar | Blood Agar           | Macconkey   | Elevation | Shape     |
|-----------------|---------------|----------------------|-------------|-----------|-----------|
| 9               | White Creamy  | White Creamy         | Pink        | Convex    | Round     |
| 8               | White Creamy  | White Creamy         | Pink Opaque | Convex    | Irregular |
| 11              | Grayish White | Grayish White        | Transparent | Convex    | Irregular |
| 7               | White         | White Beta Hemolysis | No Growth   | Convex    | Round     |
| 14              | Transparent   | Beta Hemolysis       | No Growth   | Convex    | Irregular |
| 2               | Transparent   | Gamma Hemolysis      | No Growth   | Flat      | Round     |
| 2               | White         | Grayish White        | Pink        | Convex    | Round     |

Table 4.2.1: Overall Macroscopic Observation of isolates from inanimate objects in 3 Hospitals

#### 4.2.2 Macroscopic observation of all bacterial isolates from Clinical devices

| No. of isolates | Nutrient Agar | Blood Agar           | Macconkey   | Elevation | Shape     |
|-----------------|---------------|----------------------|-------------|-----------|-----------|
| 15              | White Creamy  | White Creamy         | Pink        | Convex    | Round     |
| 12              | White Creamy  | White Creamy         | Pink Opaque | Convex    | Irregular |
| 17              | Grayish White | Grayish White        | Transparent | Convex    | Irregular |
| 19              | White         | White Beta Hemolysis | No Growth   | Convex    | Round     |
| 10              | Transparent   | Beta Hemolysis       | No Growth   | Convex    | Irregular |
| 4               | Transparent   | Gama Hemolysis       | No Growth   | Flat      | Round     |
| 4               | White         | Grayish White        | Pink        | Convex    | Round     |

Table 4.2.2: Overall Macroscopic Observation of isolates from Clinical devices in 3 Hospitals



## B. Gram Staining

### 4.2.3 Gram Staining of all bacterial isolates

|         | Gram Staining  |                |                  |                |
|---------|----------------|----------------|------------------|----------------|
|         | In-animate     |                | Clinical Devices |                |
|         | Positive (+ve) | Negative (-ve) | Positive (+ve)   | Negative (-ve) |
| AAMCH   | 9              | 6              | 18               | 10             |
| KSH     | 6              | 14             | 12               | 14             |
| KMCH    | 6              | 12             | 9                | 18             |
| Overall | 21             | 32             | 39               | 42             |

Table 4.2.3: Overall Gram staining results of isolated bacteria from inanimate objects and Clinical devices in 3 hospitals

The isolates collected from inanimate objects at KSH and KMCH shows a greater part of gram negative bacteria. Whereas study on AAMCH reveals mostly gram positive bacteria. Rate of gram positive bacteria in each hospital are 63%(AAMCH), 39%(KSH) and 33%%(KMCH) and the rate of Gram Negative bacteria are 37%%(AAMCH), 61%(KSH) and 67%(KMCH). Combined result is 45% gram positive and 55% gram positive bacteria.

### C. Biochemical Test Results

#### 4.2.4 Biochemical test results of isolated bacteria from inanimate objects

| Sample Code                                                                                  | Gram Staining | Oxidase | Catalase | MR | Citrate | Urease | Coagulase |
|----------------------------------------------------------------------------------------------|---------------|---------|----------|----|---------|--------|-----------|
| PB-10, PB-12, PB-15                                                                          | +             | -       | -        | +  | -       | -      | -         |
| PB-1, PB-6, PB-7, PB-9, PB-13, PB-14, PB-41, PB-37, PB-36, PB-35, PB-50, PB-84, PB-87, PB-88 | +             | -       | +        | +  | -       | +      | +         |
| PB-2, PB-8, PB-44, PB-46, PB-86, PB-96                                                       | -             | +       | +        | -  | +       | -      | -         |
| PB-4, PB-45                                                                                  | -             | -       | +        | -  | -       | -      | -         |
| PB-11, PB-34, PB-43, PB-33, PB-83, PB-85, PB-89, PB-95, PB-97, PB-98, PB-99                  | -             | -       | -        | -  | +       | +      | -         |
| PB-3, PB-51, PB-39, PB-38, PB-47, PB-100                                                     | -             | -       | -        | +  | -       | -      | -         |
| PB-5, PB-48, PB-94                                                                           | -             | -       | -        | -  | +       | -      | -         |
| PB-49, PB-52, PB-40, PB-90                                                                   | -             | -       | +        | -  | +       | -      | -         |

|                            |   |   |   |   |   |   |   |
|----------------------------|---|---|---|---|---|---|---|
| PB-42, PB-91, PB-92, PB-93 | + | - | + | + | - | - | - |
|----------------------------|---|---|---|---|---|---|---|

Table 4.2.4 Biochemical Test Result of In-animate objects (n=53)

#### 4.2.5 Biochemical test results of isolated bacteria from Clinical devices

| Sample Code                                                                                                                                                                                                                                                                                                 | Gram Staining | Oxidase | Catalase | MR | Citrate | Urease | Coagulase |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------|---------|----------|----|---------|--------|-----------|
| PB-73, PB-73(a)                                                                                                                                                                                                                                                                                             | +             | -       | -        | +  | -       | -      | -         |
| PB-16, PB-16(a),<br>PB-17, PB-17(a),<br>PB-18(a), PB-20(a), PB-21, PB-21(a), PB-22, PB-22(a), PB-24(a),<br>PB-25(a), PB-26, PB-26(a), PB-28, PB-28(a), PB-31, PB-31(a), PB-54, PB-56, PB-56(a), PB-58, PB-58(a), PB-59, PB-59(a), PB-66, PB-66(a), PB-69, PB-72, PB-72(a), PB-79, PB-79(a), PB-80, PB-80(a) | +             | -       | +        | +  | -       | +      | +         |



|                                                                                                                                                                                                  |   |   |   |   |   |   |   |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---|---|---|---|---|---|---|
| PB-19, PB-19(a),<br>PB-27(a), PB-29,<br>PB-29(a), PB-<br>30(a), PB-76, PB-<br>77, PB-77(a)                                                                                                       | - | + | + | - | + | - | - |
| PB-23, PB-23(a),<br>PB-32, PB-32(a),<br>PB-55, PB-57, PB-<br>57(a), PB-62, PB-<br>62(a), PB-70, PB-<br>70(a), PB-71, PB-<br>71(a), PB-78, PB-<br>78(a), PB-81, PB-<br>81(a), PB-82, PB-<br>82(a) | - | - | - | - | + | + | - |
| PB-61, PB-61(a),<br>PB-63, PB-63(a),<br>PB-65, PB-67,<br>PB-67(a), PB-68,<br>PB-68(a)                                                                                                            | - | - | - | + | - | - | - |
| PB-64, PB-64(a),<br>PB-74, PB-75,<br>PB-75(a)                                                                                                                                                    | - | - | - | - | + | - | - |
| PB-53, PB-53(a),<br>PB-60,                                                                                                                                                                       | + | - | + | + | - | - | - |

Table 4.2.5: Biochemical Test Results of isolated bacteria from Clinical devices (n=81)

#### 4.2.6 Overall Biochemical test results of all bacterial isolates

| Hospital | No. of Bacterial isolates | Oxidase |     | Catalase |     | Citrate |     | MR  |     | Urease |     | Coagulase |     |
|----------|---------------------------|---------|-----|----------|-----|---------|-----|-----|-----|--------|-----|-----------|-----|
|          |                           | +ve     | -ve | +ve      | -ve | +ve     | -ve | +ve | -ve | +ve    | -ve | +ve       | -ve |
| AAMCH    | Inanimate objects         | 2       | 13  | 12       | 3   | 4       | 11  | 10  | 5   | 7      | 8   | 6         | 9   |
|          | Clinical devices          | 6       | 22  | 28       | -   | 10      | 18  | 18  | 10  | 22     | 6   | 18        | 10  |
| KSH      | Inanimate objects         | 2       | 18  | 20       | -   | 9       | 11  | 10  | 10  | 8      | 12  | 5         | 15  |
|          | Clinical devices          | -       | 26  | 26       | -   | 7       | 19  | 19  | 7   | 14     | 12  | 9         | 17  |
| KMCH     | Inanimate objects         | 2       | 16  | 18       | -   | 11      | 7   | 7   | 11  | 10     | 8   | 3         | 15  |
|          | Clinical devices          | 3       | 24  | 25       | 2   | 16      | 11  | 11  | 16  | 17     | 10  | 7         | 20  |

Table 4.2.6: Overall Biochemical test result of bacterial isolates from inanimate objects and body fluids from Clinical devices at 3 different Hospitals

#### 4.2.7 Triple Sugar Iron test results

|       | Gas production+<br>Red/Yellow | Red/Yellow+<br>No gas | Red/Red+<br>No gas | Gas production+<br>Yellow/Yellow | Red/Black+<br>gas | Black+<br>No gas |
|-------|-------------------------------|-----------------------|--------------------|----------------------------------|-------------------|------------------|
| AAMCH | 11                            | 14                    | 6                  | 7                                | 5                 | -                |
| KSH   | 15                            | 9                     | 9                  | 5                                | 6                 | 2                |
| KMCH  | 10                            | 7                     | 9                  | 12                               | 7                 | -                |

Table 4.2.7: Overall TSI test results of isolated bacteria from inanimate objects and Clinical devices in 3 hospitals

### 4.3 Estimated Organisms

#### A. Ad-din Akij Medical College Hospital (AAMCH)

| Equipment        | Number of<br>Equipment | Bacteria found on<br>Equipment | Sample<br>Code | Number of<br>Equipment on<br>which bacteria<br>found |
|------------------|------------------------|--------------------------------|----------------|------------------------------------------------------|
| Desk             | 3                      | Staphylococcus                 | PB-1           | 2                                                    |
|                  |                        | Pseudomonas                    | PB-2           |                                                      |
| Bed Rail         | 3                      | E.coli                         | PB-3           | 1                                                    |
| Bed Cover        | 5                      | Shigella                       | PB-4           | 2                                                    |
|                  |                        | Salmonella                     | PB-5           |                                                      |
| Oxygen Nebulizer | 4                      | Staphylococcus                 | PB-6           | 1                                                    |
| Medical Chart    | 3                      | Staphylococcus                 | PB-7           | 1                                                    |
| Telephone        | 2                      | Pseudomonas                    | PB-8           | 1                                                    |



|                       |           |                |       |          |
|-----------------------|-----------|----------------|-------|----------|
| Stethoscope           | 5         | Staphylococcus | PB-9  | 1        |
| Scissor               | 2         | Streptococcus  | PB-10 | 1        |
| Sink                  | 3         | Klebsiella     | PB-11 | 2        |
|                       |           | Streptococcus  | PB-12 |          |
| Door Handle           | 3         | Staphylococcus | PB-13 | 1        |
| Fan Switch            | 2         | Staphylococcus | PB-14 | 1        |
| Weight Machine        | 2         | Streptococcus  | PB-15 | 1        |
| Non used suction tube | 1         | No growth      |       | 0        |
| Non used Canula       | 1         | No growth      |       | 0        |
| Non used syringe      | 1         | No growth      |       | 0        |
|                       | Total -40 |                |       | Total-15 |

Table 4.3.1: Bacteria of inanimate objects from Pediatric Ward at Ad-din Akij Medical College Hospital

In Ad-din Akij Medical College Hospital (AAMCH) results showed on table 4.3.1 that the predominant bacteria were found on desk, sink and bed cover mostly. Besides, door handle, weight machine, medical chart, stethoscope, fan switch etc were containing different bacterial species eg. *Pseudomonas* spp., *Klebsiella* spp., *Staphylococcus* spp., *E.coli*, *Shigella* spp., *streptococcus* spp. in where *staphylococcus* spp. are mostly found.

| Patient's Name | Age | First Sample (Equipment) & Date of Culture | Bacteria from first culture and Bacterial Code | Second Sample (Equipment) & Date of Culture | Bacteria from second culture and Bacteria Code | Infected disease & duration at stay in hospital |
|----------------|-----|--------------------------------------------|------------------------------------------------|---------------------------------------------|------------------------------------------------|-------------------------------------------------|
| Sami           | 5m  | Blood (Canula)<br>15.02.23                 | <i>Staphylococcus</i> (PB-16)                  | Blood (Canula)<br>26.02.23                  | <i>Staphylococcus</i> (PB-16a)                 | Fever (12 days)                                 |

|         |    |                                                      |                           |                                                      |                            |                                         |
|---------|----|------------------------------------------------------|---------------------------|------------------------------------------------------|----------------------------|-----------------------------------------|
| Adib    | 3Y | Blood<br>(Canula)<br>15.02.23                        | Staphylococcus<br>(PB-17) | Intestinal<br>fluid<br>(Suction<br>tube)<br>25.02.23 | Staphylococcus<br>(PB-17a) | Fever<br>(11 days)                      |
| Aroshi  | 9m | Intestinal<br>fluid<br>(Suction<br>tube)<br>15.02.23 | No growth                 | Blood<br>(Canula)<br>28.02.23                        | Klebsiella<br>(PB-18a)     | Pneumonia<br>(14 days)                  |
| Fatema  | 3m | Blood<br>(Canula)<br>16.02.23                        | Pseudomonas<br>(PB-19)    | Intestinal<br>fluid<br>(Suction<br>tube)<br>25.02.23 | Pseudomonas<br>(PB-19a)    | Urinary tract<br>infection<br>(10 days) |
| Jubayer | 5m | Blood<br>(Canula)<br>16.02.23                        | No growth                 |                                                      |                            | (5 days)                                |
| Anas    | 5Y | Blood<br>(Canula)<br>16.02.23                        | No growth                 |                                                      |                            | (5 days)                                |
| Rihani  | 7m | Nasal swab<br>18.02.23                               | No growth                 | Intestinal<br>fluid<br>(Suction<br>tube)<br>08.03.23 | Staphylococcus<br>(PB-20a) | Respiratory<br>distress<br>(18 Days)    |
| Abir    | 2m | Intestinal<br>fluid<br>(Suction<br>tube)<br>18.02.23 | Staphylococcus<br>(PB-21) | Blood<br>(Canula)<br>28.02.23                        | Staphylococcus<br>(PB-21a) | Respiratory<br>distress<br>(11 days)    |

|          |    |                                                      |                           |                                                      |                            |                                         |
|----------|----|------------------------------------------------------|---------------------------|------------------------------------------------------|----------------------------|-----------------------------------------|
| Arafat   | 7y | Blood<br>(Canula)<br>20.02.23                        | Staphylococcus<br>(PB-22) | Blood<br>(Canula)<br>06.03.23                        | Staphylococcus<br>(PB-22a) | Fever<br>(15 Days)                      |
| Arian    | 6m | Blood<br>(Canula)<br>20.02.23                        | Klebsiella<br>(PB-23)     | Blood<br>(Canula)<br>02.03.23                        | Klebsiella<br>(PB-23a)     | Pneumonia<br>(11 Days)                  |
| Aradhya  | 3Y | Intestinal<br>fluid<br>(Suction<br>tube)<br>22.02.23 | No Growth                 | Intestinal<br>fluid<br>(Suction<br>tube)<br>02.03.23 | Staphylococcus<br>(PB-24a) | Respiratory<br>distress<br>(9 days)     |
| Khadija  | 4m | Nasal swab<br>22.02.23                               | No Growth                 | Blood<br>(Canula)<br>05.03.23                        | Staphylococcus<br>(PB-25a) | Vomiting<br>(12 days)                   |
| Ayesha-1 | 2Y | Nasal swab<br>25.02.23                               | Staphylococcus<br>(PB-26) | Intestinal<br>fluid<br>(Suction<br>tube)<br>05.03.23 | Staphylococcus(<br>PB-26a) | Fever<br>(9 days)                       |
| Mosiur   | 2m | Intestinal<br>fluid<br>(Suction<br>tube)<br>25.02.23 | No Growth                 | Urine<br>(Catheter)<br>12.03.23                      | Pseudomonas<br>(PB-27a)    | Urinary tract<br>infection<br>(16 days) |
| Jannat   | 4Y | Blood<br>(Canula)<br>28.02.23)                       | No growth                 |                                                      |                            | (4 Days)                                |
| Ayesha-2 | 5Y | Intestinal<br>fluid                                  | Staphylococcus<br>(PB-28) | Blood<br>(Canula)<br>10.03.23                        | Staphylococcus<br>(PB-28a) | Pneumonia<br>(11 days)                  |



|        |    |                                             |                        |                                             |                         |                                   |
|--------|----|---------------------------------------------|------------------------|---------------------------------------------|-------------------------|-----------------------------------|
|        |    | (Suction tube)<br>28.02.23                  |                        |                                             |                         |                                   |
| Asura  | 2Y | Urine (Catheter)<br>02.03.23                | No growth              | Blood (Canula)<br>05.03.23                  | No growth               | (3 days)                          |
| Fahim  | 5m | Intestinal fluid (Suction tube)<br>02.03.23 | No growth              |                                             |                         | (2 days)                          |
| Raihan | 4m | Blood (Canula)<br>05.03.23                  | No growth              |                                             |                         | (3 days)                          |
| A.Saad | 5Y | Blood (Canula)<br>05.03.23                  | Pseudomonas (PB-29)    | Blood (Canula)<br>12.03.23                  | Pseudomonas (PB-29a)    | Urinary tract infection (8 days)  |
| Toha   | 3Y | Blood (Canula)<br>05.03.23                  | No growth              | Nasal swab<br>14.03.23                      | Pseudomonas (PB-30a)    | Urinary tract infection (10 days) |
| Ayman  | 2m | Nasal swab<br>08.03.23                      | Staphylococcus (PB-31) | Intestinal fluid (Suction tube)<br>14.03.23 | Staphylococcus (PB-31a) | Pneumonia (9 days)                |
| Talha  | 3m | Blood (Canula)<br>08.03.23                  | Klebsiella (PB-32)     | Blood (Canula)<br>14.03.23                  | Klebsiella (PB-32a)     | Pneumonia (9 days)                |
| Rahim  | 4Y | Nasal swab (12.03.23)                       | No growth              |                                             |                         | (4 days)                          |

|        |    |                                                      |           |  |      |          |
|--------|----|------------------------------------------------------|-----------|--|------|----------|
| Morium | 6m | Intestinal<br>fluid<br>(Suction<br>tube)<br>14.03.23 | No growth |  |      | (3 days) |
| n=25   |    |                                                      | n=11      |  | n=17 |          |

Table 4.3.2 : Bacteria of body fluids from Clinical devices at Pediatric Ward in Ad-din Akij Medical College Hospital

Result showed on table 4.3.2 that most of the pediatric patients were infected by staphylococcus spp. , Klebsiella spp. and infected by fever as well as pneumonia. Patient care equipments were mostly contaminated (68%) and found the antimicrobial bacteria were Staphylococcus spp the highest rate and the other prevalence bacteria were Klebsiella spp.,Pseudomonas spp.,E.coli, Salmonella spp.,Streptococcus perspectivevely.

| In-animate Objects                   | Bacterial Genus          | Body fluids from Patient Care<br>Equipment                                                                                                                                 |
|--------------------------------------|--------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| PB-1, PB-6, PB-7, PB-9, PB-13, PB-14 | Staphylococcus<br>(n=24) | PB-16, PB-16(a), PB-17, PB-17(a),<br>PB-18(a), PB-20(a), PB-21, PB-21(a),<br>PB-22, PB-22(a), PB-24(a), PB- 25(a),<br>PB-26, PB-26(a), PB-28, PB-28(a),<br>PB-31, PB-31(a) |
| PB-11                                | Klebsiella<br>(n=5)      | PB-23, PB-23(a), PB-32, PB-32(a)                                                                                                                                           |
| PB-2, PB-8                           | Pseudomonas<br>(n=8)     | PB-19, PB-19(a), PB-27(a), PB-29,<br>PB-29(a), PB-30(a)                                                                                                                    |
| PB-3                                 | E.coli<br>(n=1)          |                                                                                                                                                                            |
| PB-5                                 | Salmonella<br>(n=1)      |                                                                                                                                                                            |
| PB-10, PB-12, PB-15                  | Streptococcus<br>(n=3)   |                                                                                                                                                                            |

|      |                   |  |
|------|-------------------|--|
| PB-4 | Shigella<br>(n=1) |  |
|------|-------------------|--|

Table 4.3.4: Summary of Inanimate objects and Clinical devices at Ad-Din Akij Medical College Hospital

It reveals (table 4.3.4) that the *Staphylococcus* spp. are mostly present in in-animate objects and fluids from patient care equipments which is 24 isolates out of 32 and next highst number is *Pseudomonas* i.e 8.

| Isolated bacteria             | Patient Name | Age | Infected disease        | Bacteria found on inanimate objects |
|-------------------------------|--------------|-----|-------------------------|-------------------------------------|
| <i>Staphylococcus aureus</i>  | Sam1         | 5m  | Fever                   | Desk                                |
|                               | Adib         | 3Y  | Fever                   | Oxygen Nebulizer                    |
|                               | Aroshi       | 9m  | Pneumonia               | Medical Chart                       |
|                               | Rihan        | 7m  | Respiratory distress    | Stethoscope                         |
|                               | Abir         | 2m  | Respiratory distress    | Door Handle                         |
|                               | Arafat       | 7y  | Fever                   | Fan Switch                          |
|                               | Aradhya      | 3Y  | Respiratory distress    |                                     |
|                               | Khadija      | 4m  | Vomiting                |                                     |
|                               | Ayesha-1     | 2Y  | Fever                   |                                     |
|                               | Ayesha-2     | 5Y  | Pneumonia               |                                     |
|                               | Ayman        | 2m  | Pneumonia               |                                     |
| <i>Pseudomonas aeruginosa</i> | Fatema       | 3m  | Urinary tract infection | Desk                                |
|                               | Mosiur       | 2m  | Urinary tract infection | Telephone                           |
|                               | A.Saad       | 5Y  | Urinary tract infection |                                     |
|                               | Toha         | 3Y  | Urinary tract infection |                                     |
| <i>Klebsiella pneumoniae</i>  | Arian        | 6m  | Pneumonia               | Sink                                |
|                               | Talha        | 3m  | Pneumonia               |                                     |
|                               |              |     |                         | Scissor                             |



|                                 |  |  |  |                |
|---------------------------------|--|--|--|----------------|
| <i>Streptococcus pneumoniae</i> |  |  |  | Sink           |
|                                 |  |  |  | Weight Machine |
| <i>E. coli</i>                  |  |  |  | Bed Rail       |
| <i>Salmonella typhimurium</i>   |  |  |  | Bed Cover      |
| <i>Shigella spp</i>             |  |  |  | Bed Cover      |

Table 4.3.5: Summary of Inanimate objects and Clinical devices at Ad-Din Akij Medical College Hospital

#### B. Khulna Shishu Hospital

| Equipment        | Number of Equipment | Bacteria found on Equipment | Sample Code | Number of Equipment on which bacteria found |
|------------------|---------------------|-----------------------------|-------------|---------------------------------------------|
| Desk             | 4                   | Klebsiella                  | PB-34       | 3                                           |
|                  |                     | Staphylococcus              | PB-41       |                                             |
|                  |                     | Klebsiella                  | PB-43       |                                             |
| Bed Rail         | 3                   | Staphylococcus              | PB-37       | 2                                           |
|                  |                     | E.coli                      | PB-51       |                                             |
| Bed Cover        | 2                   | Pseudomonas spp             | PB-44       | 1                                           |
| Oxygen Nebulizer | 3                   | Acinetobacter spp           | PB-49       | 1                                           |
| Medical Chart    | 4                   | E.coli                      | PB-39       | 2                                           |
|                  |                     | Salmonella spp              | PB-48       |                                             |
| Telephone        | 4                   | Klebsiella                  | PB-33       | 2                                           |
|                  |                     | Corynebacterium spp         | PB-42       |                                             |
| Stethoscope      | 3                   | Acinetobacter spp           | PB-52       | 1                                           |
| Scissor          | 2                   | Acinetobacter spp           | PB-40       | 1                                           |
| Sink             | 2                   | E.coli                      | PB-38       | 2                                           |
|                  |                     | E.coli                      | PB-47       |                                             |
| Door Handle      | 1                   | Staphylococcus              | PB-36       | 1                                           |
| Fan Switch       | 2                   | Shigella                    | PB-45       | 1                                           |
| Weight Machine   | 4                   | Staphylococcus              | PB-35       | 2                                           |

|                       |          |                |       |          |
|-----------------------|----------|----------------|-------|----------|
|                       |          | Pseudomonas    | PB-46 |          |
| Sucking Machine       | 3        | Staphylococcus | PB-50 | 1        |
| Non used suction tube | 1        |                |       | 0        |
| Non used canula       | 1        |                |       | 0        |
| Non used syringe      | 1        |                |       | 0        |
|                       | Total-40 |                |       | Total-20 |

Table 4.3.6: Bacteria isolated from inanimate objects at Khulna Shishu Hospital

In Khulna Shishu Hospital, Result showed on table 4.3.6 that the predominant bacteria were found on desk, bed rail, medical chart, telephone, sink and bed weight machine mostly. Besides, door handle, weight machine, medical chart, stethoscope, fan switch etc were containing different bacterial species eg. Pseudomonas spp., Klebsiella spp., Staphylococcus spp., E.coli, Shigella spp., streptococcus spp. in where staphylococcus spp. were mostly found.

| Patient's Name | Age | First Sample (Equipment) & Date of Culture  | Bacteria from first culture and Sample Code | Second Sample (Equipment) & Date of Culture | Bacteria from second culture and Sample Code | Infected disease & duration of stay at hospital |
|----------------|-----|---------------------------------------------|---------------------------------------------|---------------------------------------------|----------------------------------------------|-------------------------------------------------|
| Abdullah       | 2m  | Blood (Canula)<br>21.09.23                  | Corynebacterium (PB-53)                     | Blood (Canula)<br>28.09.23                  | Corynebacterium (PB-53a)                     | Diphtheria (7 days)                             |
| Hima           | 4m  | Nasal swab<br>21.09.23                      | Staphylococcus (PB-54)                      | Blood (Canula)<br>27.09.23                  | No growth                                    | Fever (6 days)                                  |
| Humaira        | 3Y  | Intestinal fluid (Suction tube)<br>21.09.23 | No growth                                   | Blood (Canula)<br>12.10.23                  | Klebsiella (PB-55)                           | Pneumonia (21 days)                             |

|        |    |                                                      |                           |                                 |                                |                                        |
|--------|----|------------------------------------------------------|---------------------------|---------------------------------|--------------------------------|----------------------------------------|
| Dipto  | 7m | Intestinal<br>fluid<br>(Suction<br>tube)<br>23.09.23 | Staphylococcus<br>(PB-56) | Blood<br>(Canula)<br>29.09.23   | Staphylococcus<br>(PB-56a)     | Fever<br>(6 days)                      |
| Ali    | 3Y | Nasal swab<br>(23.09.23)                             | Klebsiella<br>(PB-57)     | Blood<br>(Canula)<br>30.09.23   | Klebsiella<br>(PB-57a)         | Pneumonia<br>(7 days)                  |
| Arko   | 2Y | Intestinal<br>fluid<br>(Suction<br>tube)<br>23.09.23 | Staphylococcus<br>(PB-58) | Blood<br>(Canula)<br>28.09.23   | Staphylococcus<br>(PB-58a)     | Vomiting<br>(5 days)                   |
| Abrar  | 3Y | Nasal swab<br>25.09.23                               | Staphylococcus<br>(PB-59) | Blood<br>(Canula)<br>04.10.23   | Staphylococcus<br>(PB-59a)     | Fever<br>(9 Days)                      |
| Asad   | 4Y | Intestinal<br>fluid<br>(Suction<br>tube)<br>25.09.23 | No growth                 | Urine<br>(Catheter)<br>03.10.23 | Corynebacteriu<br>m<br>(PB-60) | Urinary tract<br>infection<br>(8 days) |
| Eti    | 2Y | Blood<br>(Canula)<br>30.09.23                        | E.coli<br>(PB-61)         | Blood<br>(Canula)<br>12.10.23   | E.coli<br>(PB-61a)             | Diarrhoea<br>(12 Days)                 |
| Miran  | 5m | Nasal swab<br>30.09.23                               | Klebsiella<br>(PB-62)     | Urine<br>(Catheter)<br>07.10.23 | Klebsiella<br>(PB-62a)         | Pneumonia<br>(7 Days)                  |
| Kawsar | 7m | Nasal swab<br>04.10.23                               | E.coli<br>(PB-63)         | Nasal swab<br>10.10.23          | E.coli<br>(PB-63a)             | Diarrhoea<br>(6 days)                  |



|        |    |                                                |                           |                                                |                            |                        |
|--------|----|------------------------------------------------|---------------------------|------------------------------------------------|----------------------------|------------------------|
| Tasnim | 6m | Intestinal fluid<br>(Suction tube)<br>04.10.23 | Salmonella spp<br>(PB-64) | Blood<br>(Canula)<br>10.10.23                  | Salmonella spp<br>(PB-64a) | Typhoid<br>(6 days)    |
| Arian  | 9m | Intestinal fluid<br>(Suction tube)<br>06.10.23 | E.coli<br>(PB-65)         | Blood<br>(Canula)<br>14.10.23                  | No growth                  | Diarrhoea<br>(8 days)  |
| Ayman  | 4Y | Nasal swab<br>06.10.23                         | Staphylococcus<br>(PB-66) | Blood<br>(Canula)<br>18.10.23                  | Staphylococcus<br>(PB-66a) | Pneumonia<br>(12 days) |
| Sima   | 3Y | Blood<br>(Canula)<br>06.10.23                  | E.coli<br>(PB-67)         | Blood<br>(Canula)<br>20.10.23                  | E.coli<br>(PB-67a)         | Diarrhoea<br>(14 days) |
| Prema  | 2Y | Urine<br>(Catheter)<br>10.10.23                | No growth                 | Nasal swab<br>20.10.23                         | No growth                  | 10 days                |
| Arpon  | 3m | Nasal swab<br>14.10.23                         | No growth                 | Intestinal fluid<br>(Suction tube)<br>23.10.23 | No growth                  | 9 days                 |
| Zerin  | 2m | Blood<br>(Canula)<br>14.10.23                  | No growth                 | Blood<br>(Canula)<br>27.10.23                  | No growth                  | 13 days                |
| Suma   | 4m | Nasal swab<br>(20.10.23)                       | No growth                 | Blood<br>(Canula)<br>25.10.23                  | No growth                  | 5 days                 |
| Puja   | 2m | Intestinal fluid                               | No growth                 | Blood<br>(Canula)<br>27.10.23                  | No growth                  | 7 days                 |

|      |  |                               |      |  |      |  |
|------|--|-------------------------------|------|--|------|--|
|      |  | (Suction<br>tube)<br>20.10.23 |      |  |      |  |
| n=20 |  |                               | n=13 |  | n=13 |  |

Table 4.3.7: Bacteria isolated from body fluids of 20 pediatric patient from Clinical devices at Khulna Shishu Hospital

Result showed on table 4.3.7 that most of the pediatric patients were infected by staphylococcus spp. , Klebsiella spp. and infected by fever as well as pneumonia. Patient care equipments were mostly contaminated (75%) and In-animate objects (50%) found the antimicrobial bacteria in where Staphylococcus spp the highst rate and the other prevalence bacteria were Klebsiella spp.,Pseudomonas spp.,E.coli, Salmonella spp.,Streptococcus perspectivevely.

| Sample Code of In-animate Object  | Bacterial Genus          | Sample Code of Body fluid isolated from Patient Care Equipment            |
|-----------------------------------|--------------------------|---------------------------------------------------------------------------|
| PB-41, PB-37, PB-36, PB-35, PB-50 | Staphylococcus<br>(n=14) | PB-54, PB-56, PB-56(a), PB-58, PB-58(a), PB-59, PB-59(a), PB-66, PB-66(a) |
| PB-34, PB-43, PB-33               | Klebsiella<br>(n=8)      | PB-55, PB-57, PB-57(a), PB-62, PB-62(a)                                   |
| PB-44, PB-46                      | Pseudomonas<br>(n=2)     |                                                                           |
| PB-51, PB-39, PB-38, PB-47        | E.coli<br>(n=11)         | PB-61, PB-61(a), PB-63, PB-63(a), PB-65, PB-67, PB-67(a)                  |
| PB-48                             | Salmonella<br>(n=3)      | PB-64, PB-64(a)                                                           |
| PR-42                             | Corynebacterium<br>(n=4) | PR-53, PR-53(a), PR-60                                                    |
| PB-49, PB-52, PB-40               | Acinetobacter<br>(n=3)   |                                                                           |

|       |                   |  |
|-------|-------------------|--|
| PB-45 | Shigella<br>(n=1) |  |
|-------|-------------------|--|

4.3.8 Table: Summary of Inanimate objects and Patient care equipments at Khulna Shishu Medical College Hospital

It reveals (table 4.3.8) that the *Staphylococcus* spp. are mostly present in in-animate objects and fluids from patient care equipments which is 14 isolates out of 35 and next highst number is *E.coli* i.e 11.

| Isolated bacteria             | Patient Name | Age | Infected disease        | Bacteria found on inanimate objects |
|-------------------------------|--------------|-----|-------------------------|-------------------------------------|
| <i>Staphylococcus aureus</i>  | Hima         | 4m  | Fever                   | Weight Machine                      |
|                               | Dipto        | 7m  | Fever                   | Door Handle                         |
|                               | Arko         | 2Y  | Vomiting                | Bed Rail                            |
|                               | Abrar        | 3Y  | Fever                   | Desk                                |
|                               | Ayman        | 4Y  | Pneumonia               | Sucking Machine                     |
| <i>Pseudomonas aeruginosa</i> |              |     |                         | Bed Cover                           |
|                               |              |     |                         | Weight Machine                      |
| <i>Klebsiella pneumoniae</i>  | Humaira      | 3Y  | Pneumonia               | Telephone                           |
|                               | Ali          | 3Y  | Pneumonia               | Desk                                |
|                               | Miran        | 5m  | Pneumonia               |                                     |
| <i>E.coli</i>                 | Eti          | 2Y  | Diarrhoea               | Sink                                |
|                               | Kawsar       | 7m  | Diarrhoea               | Medical Chart                       |
|                               | Arian        | 9m  | Diarrhoea               | Bed Rail                            |
|                               | Sima         | 3Y  | Diarrhoea               |                                     |
| <i>Salmonella typhimurium</i> | Tasnim       | 6m  | Typhoid                 | Medical Chart                       |
| <i>Corynebacterium spp.</i>   | Abdullah     | 2m  | Diphtheria              | Telephone                           |
|                               | Asad         | 4Y  | Urinary tract infection |                                     |
|                               |              |     |                         | Scissor                             |



|                           |  |  |  |                  |
|---------------------------|--|--|--|------------------|
| <i>Acinetobacter spp.</i> |  |  |  | Oxygen Nebulizer |
|                           |  |  |  | Stethoscope      |
| <i>Shigella spp.</i>      |  |  |  | Fan Switch       |

Table 4.3.9 : Summary of Inanimate objects and Clinical devices at Khulna Shishu Hospital

### C. Khulna Medical College Hospital

| Equipment        | Number of Equipment | Bacteria found on Equipment | Sample Code | Number of Equipment on which bacteria found |
|------------------|---------------------|-----------------------------|-------------|---------------------------------------------|
| Desk             | 3                   | Klebsiella                  | PB-89       | 3                                           |
|                  |                     | Acinetobacter spp.          | PB-90       |                                             |
|                  |                     | Klebsiella                  | PB-98       |                                             |
| Bed Rail         | 2                   | Corynebacterium             | PB-93       | 2                                           |
|                  |                     | Klebsiella                  | PB-99       |                                             |
| Bed Cover        | 1                   | Corynebacterium             | PB-92       | 1                                           |
| Oxygen Nebulizer | 2                   | Staphylococcus              | PB-87       | 2                                           |
|                  |                     | Klebsiella                  | PB-95       |                                             |
| Medical Chart    | 1                   | Salmonella spp.             | PB-94       | 1                                           |
| Telephone        | 2                   | Staphylococcus              | PB-84       | 1                                           |
| Stethoscope      | 2                   | Klebsiella                  | PB-83       | 1                                           |
| Scissor          | 2                   | Klebsiella                  | PB-85       | 1                                           |
| Sink             | 1                   | E.coli                      | PB-100      | 1                                           |
| Door Handle      | 1                   | Pseudomonas                 | PB-86       | 1                                           |
| Fan Switch       | 1                   | Pseudomonas                 | PB-96       | 1                                           |
| Weight Machine   | 1                   | Klebsiella                  | PB-97       | 1                                           |
| Sucking Machine  | 1                   | Staphylococcus              | PB-88       | 1                                           |
| Trolley          | 2                   | Corynebacterium             | PB-91       | 1                                           |

|                       |          |  |  |          |
|-----------------------|----------|--|--|----------|
| Non used suction tube | 1        |  |  | 0        |
| Non used canula       | 1        |  |  | 0        |
| Non used syringe      | 1        |  |  | 0        |
|                       | Total-25 |  |  | Total-18 |

Table 4.3.1.1: Bacteria isolated from inanimate objects at Khulna Medical College Hospital

In Khulna Medical College Hospital, Result showed on table 4.3.1.1 that the predominant bacteria were found on desk, bed rail, Oxygen nebulizer, sink and bed cover mostly. Besides, door handle, weight machine, medical chart, stethoscope, fan switch etc were containing different bacterial species eg. *Pseudomonas* spp., *Klebsiella* spp., *Staphylococcus* spp., *E.coli*, *Shigella* spp., *streptococcus* spp. in where *staphylococcus* spp. are mostly found.

| Patient's Name | Age | First Sample (Equipment) & Date of Culture  | Bacteria from first culture and Sample Code | Second Sample (Equipment) & Date of Culture | Bacteria from second culture and Sample Code | Infected disease & duration of stay at hospital |
|----------------|-----|---------------------------------------------|---------------------------------------------|---------------------------------------------|----------------------------------------------|-------------------------------------------------|
| Mita           | 4m  | Blood (Canula)<br>02.12.23                  | <i>E.coli</i> (PB-68)                       | Blood (Canula)<br>12.12.23                  | <i>E.coli</i> (PB-68a)                       | Urinary tract infection (10 days)               |
| Oishi          | 5m  | Nasal swab<br>02.12.23                      | <i>Staphylococcus</i> (PB-69)               | Blood (Canula)<br>10.12.23                  | No growth                                    | Fever (8 days)                                  |
| Hira           | 4Y  | Intestinal fluid (Suction tube)<br>02.12.23 | No growth                                   | Blood (Canula)<br>05.12.23                  | No Growth                                    | 3days                                           |
| Mustain        | 8m  | Intestinal fluid (Suction tube)<br>05.12.23 | <i>Klebsiella</i> (PB-70)                   | Blood (Canula)<br>15.12.23                  | <i>Klebsiella</i> (PB-70a)                   | Pneumonia (10 days)                             |

|          |    |                                                |                           |                                 |                            |                                        |
|----------|----|------------------------------------------------|---------------------------|---------------------------------|----------------------------|----------------------------------------|
| Probir   | 3Y | Nasal swab<br>05.12.23                         | Klebsiella<br>(PB-71)     | Blood<br>(Canula)<br>12.12.23   | Klebsiella<br>(PB-71a)     | Pneumonia<br>(7 days)                  |
| Ayesha-3 | 2Y | Intestinal fluid<br>(Suction tube)<br>05.12.23 | Staphylococcus<br>(PB-72) | Blood<br>(Canula)<br>15.12.23   | Staphylococcus<br>(PB-72a) | Fever<br>(10 days)                     |
| Shohagi  | 4Y | Nasal swab<br>07.12.23                         | Streptococcus<br>(PB-73)  | Blood<br>(Canula)<br>(12.12.23) | Streptococcus<br>(PB-73a)  | Tonsilitis<br>(5 Days)                 |
| Sohid    | 2Y | Intestinal fluid<br>(Suction tube)<br>07.12.23 | No growth                 | Blood<br>(Canula)<br>13.12.23   | Salmonella<br>(PB-74)      | Typhoid<br>(6 days)                    |
| Zisan    | 2Y | Blood<br>(Canula)<br>07.12.23                  | Salmonella (PB-75)        | Blood<br>(Canula)<br>19.12.23   | Salmonella<br>(PB-75a)     | Typhoid<br>(12 Days)                   |
| Arohi    | 8m | Nasal swab<br>10.12.23                         | No Growth                 | Blood<br>(Canula)<br>20.12.23   | Pseudomonas<br>(PB-76)     | Pneumonia<br>(10 Days)                 |
| Billah   | 6m | Nasal swab<br>10.12.23                         | Pseudomonas<br>(PB-77)    | Nasal swab<br>17.12.23          | Pseudomonas<br>(PB-77a)    | Urinary tract<br>infection<br>(7 days) |
| Bilash   | 2Y | Intestinal fluid<br>(Suction tube)<br>10.12.23 | Klebsiella<br>(PB-78)     | Blood<br>(Canula)<br>17.12.23   | Klebsiella<br>(PB-78a)     | Pneumonia<br>(7 days)                  |
| Rudro    | 6m | Intestinal fluid<br>(Suction tube)<br>10.12.23 | Staphylococcus<br>(PB-79) | Blood<br>(Canula)<br>14.12.23   | Staphylococcus<br>(PB-79a) | Pneumonia<br>(4 days)                  |
| Antu     | 3Y | Nasal swab<br>10.12.23                         | Staphylococcus<br>(PB-80) | Blood<br>(Canula)<br>30.12.23   | Staphylococcus<br>(PB-80a) | Fever<br>(20 days)                     |
| Salma    | 2Y | Blood                                          | Klebsiella                | Blood                           | Klebsiella                 | Vomiting                               |



|         |    |                                                       |                                      |                                                      |                                        |                                        |
|---------|----|-------------------------------------------------------|--------------------------------------|------------------------------------------------------|----------------------------------------|----------------------------------------|
| Rima    | 2Y | (Canula)<br>15.12.23<br>Blood<br>(Canula)<br>15.12.23 | (PB-81)<br><br>Klebsiella<br>(PB-82) | (Canula)<br>22.12.23<br>Nasal swab<br>25.12.23       | (PB-81a)<br><br>Klebsiella<br>(PB-82a) | (7 days)<br><br>Pneumonia<br>(10 days) |
| Robin   | 3m | Nasal swab<br>15.12.23                                | No growth                            | Intestinal<br>fluid<br>(Suction<br>tube)<br>24.12.23 | No growth                              | 9 days                                 |
| Zara    | 7m | Blood<br>(Canula)<br>20.12.23                         | No growth                            | Blood<br>(Canula)<br>27.12.23                        | No growth                              | 7 days                                 |
| Jahid   | 3m | Urine<br>(Catheter)<br>24.12.23                       | No growth                            | Blood<br>(Canula)<br>30.12.23                        | No growth                              | 6 days                                 |
| Sabrina | 5Y | Intestinal fluid<br>(Suction tube)<br>24.12.23        | No growth                            | Urine<br>(Catheter)<br>30.12.23                      | No growth                              | 6 days                                 |
| n=20    |    |                                                       | n=13                                 |                                                      | n=14                                   |                                        |

Table 4.3.1.2 : Bacteria isolated from body fluids of 20 pediatric patient from Clinical devices at Khulna Medical College Hospital

Result showed on table 4.3.1.2 that most of the pediatric patients were infected by staphylococcus spp. , Klebsiella spp. and infected by fever as well as pneumonia. Patient care equipments were mostly contaminated (68%) and found the antimicrobial bacteria were Staphylococcus spp the highest rate and the other prevalence bacteria were Klebsiella spp.,Pseudomonas spp.,E.coli, Salmonella spp.,Streptococcus perspectivevely.

| In-animate Objects                              | Bacterial Genus          | Body fluids from Patient Care Equipment                                             |
|-------------------------------------------------|--------------------------|-------------------------------------------------------------------------------------|
| PB-84, PB-87, PB-88                             | Staphylococcus<br>(n=10) | PB-69, PB-72, PB-72(a), PB-79, PB-79(a), PB-80, PB-80(a)                            |
| PB-83, PB-85, PB-89, PB-95, PB-97, PB-98, PB-99 | Klebsiella<br>(n=17)     | PB-70, PB-70(a), PB-71, PB-71(a), PB-78, PB-78(a), PB-81, PB-81(a), PB-82, PB-82(a) |
| PB-86, PB-96                                    | Pseudomonas<br>(n=5)     | PB-76, PB-77, PB-77(a)                                                              |
| PB-100                                          | E.coli<br>(n=3)          | PB-68, PB-68(a)                                                                     |
| PB-94                                           | Salmonella<br>(n=4)      | PB-74, PB-75, PB-75(a)                                                              |
| PB-91, PB-92, PB-93                             | Corynebacterium<br>(n=3) |                                                                                     |
|                                                 | Streptococcus<br>(n=2)   | PB-73, PB-73(a)                                                                     |
| PB-90                                           | Acinetobacter<br>(n=1)   |                                                                                     |

Table 4.3.1.3: Summary of Inanimate objects and Patient care equipments at Khulna Medical College Hospital

It reveals (table 4.3.1.3) that Klebsiella spp. are mostly present in in-animate objects and fluids from patient care equipments which is 17 isolates out of 3 and next highest number is Staphylococcus spp. is 10, Pseudomonas i.e 5 as well.

| Sources          | Bacteria found on Equipment | Sample Code | Number of Equipment on which bacteria found |
|------------------|-----------------------------|-------------|---------------------------------------------|
| Desk             | Staphylococcus              | PB-1        | 8                                           |
|                  | Pseudomonas                 | PB-2        |                                             |
|                  | Klebsiella                  | PB-34       |                                             |
|                  | Staphylococcus              | PB-41       |                                             |
|                  | Klebsiella                  | PB-43       |                                             |
|                  | Klebsiella                  | PB-89       |                                             |
|                  | Acinetobacter spp.          | PB-90       |                                             |
|                  | Klebsiella                  | PB-98       |                                             |
| Bed Rail         | E.coli                      | PB-3        | 5                                           |
|                  | Staphylococcus              | PB-37       |                                             |
|                  | E.coli                      | PB-51       |                                             |
|                  | Corynebacterium             | PB-93       |                                             |
|                  | Klebsiella                  | PB-99       |                                             |
| Bed Cover        | Shigella                    | PB-4        | 4                                           |
|                  | Salmonella                  | PB-5        |                                             |
|                  | Pseudomonas spp             | PB-44       |                                             |
|                  | Corynebacterium             | PB-92       |                                             |
| Oxygen Nebulizer | Staphylococcus              | PB-6        | 4                                           |
|                  | Acinetobacter spp           | PB-49       |                                             |
|                  | Staphylococcus              | PB-87       |                                             |
|                  | Klebsiella                  | PB-95       |                                             |
| Medical Chart    | Staphylococcus              | PB-7        | 4                                           |
|                  | E.coli                      | PB-39       |                                             |
|                  | Salmonella spp              | PB-48       |                                             |
|                  | Salmonella spp.             | PB-94       |                                             |
| Telephone        | Pseudomonas                 | PB-8        | 4                                           |
|                  | Klebsiella                  | PB-33       |                                             |
|                  | Corynebacterium             | PB-42       |                                             |



|                 |                   |        |             |
|-----------------|-------------------|--------|-------------|
|                 | Staphylococcus    | PB-84  |             |
| Stethoscope     | Staphylococcus    | PB-9   | 3           |
|                 | Acinetobacter spp | PB-52  |             |
|                 | Klebsiella        | PB-83  |             |
| Scissor         | Streptococcus     | PB-10  | 3           |
|                 | Acinetobacter spp | PB-40  |             |
|                 | Klebsiella        | PB-85  |             |
| Sink            | Klebsiella        | PB-11  | 5           |
|                 | Streptococcus     | PB-12  |             |
|                 | E.coli            | PB-38  |             |
|                 | E.coli            | PB-47  |             |
|                 | E.coli            | PB-100 |             |
| Door Handle     | Staphylococcus    | PB-13  | 3           |
|                 | Staphylococcus    | PB-36  |             |
|                 | Pseudomonas       | PB-86  |             |
| Fan Switch      | Staphylococcus    | PB-14  | 3           |
|                 | Shigella          | PB-45  |             |
|                 | Pseudomonas       | PB-96  |             |
| Weight Machine  | Streptococcus     | PB-15  | 4           |
|                 | Staphylococcus    | PB-35  |             |
|                 | Pseudomonas       | PB-46  |             |
|                 | Klebsiella        | PB-97  |             |
| Trolley         | Corynebacterium   | PB-91  | 1           |
| Sucking machine | Staphylococcus    | PB-50  | 2           |
|                 | Staphylococcus    | PB-88  |             |
|                 |                   |        | Total(n)=53 |

Table 4.3.1.5: Isolated Bacteria on inanimate objects at 3 different hospitals in Khulna City

| Sources                            | Bacteria found on Sample | Sample Code                                                                                                                                           | Number of Equipment on which bacteria found |
|------------------------------------|--------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------|
| Blood<br>(Canula)                  | Staphylococcus           | PB-16, PB-16(a), PB-17, PB-18(a), PB-21(a), PB-22, PB-22(a), PB-25(a), PB-28(a), PB-56(a), PB-58(a), PB-59(a), PB-66(a), PB-72(a), PB-79(a), PB-80(a) | 45                                          |
|                                    | Klebsiella               | PB-23, PB-23(a), PB-32, PB-32(a), PB-55, PB-57(a), PB-70(a), PB-71(a), PB-78(a), PB-81, PB-81(a), PB-82                                               |                                             |
|                                    | Pseudomonas              | PB-19, PB-29, PB-29(a), PB-76                                                                                                                         |                                             |
|                                    | E.coli                   | PB-61, PB-61(a), PB-67, PB-67(a), PB-68, PB-68(a)                                                                                                     |                                             |
|                                    | Salmonella               | PB-64(a), PB-74, PB-75, PB-75(a)                                                                                                                      |                                             |
|                                    | Corynebacterium          | PB-53, PB-53(a)                                                                                                                                       |                                             |
|                                    | Streptococcus            | PB-73(a)                                                                                                                                              |                                             |
| Intestinal fluid<br>(Suction tube) | Staphylococcus           | PB-17(a), PB-20(a), PB-21, PB-24(a), PB-26(a), PB-28, PB-31(a), PB-56, PB-58, PB-72, PB-79                                                            | 15                                          |
|                                    | Klebsiella               | PB-70                                                                                                                                                 |                                             |
|                                    | Pseudomonas              | PB-19(a)                                                                                                                                              |                                             |
|                                    | E.coli                   | PB-65                                                                                                                                                 |                                             |
|                                    | Salmonella               | PB-64                                                                                                                                                 |                                             |

|                     |                 |                                                 |             |
|---------------------|-----------------|-------------------------------------------------|-------------|
| Urine<br>(Catheter) | Klebsiella      | PB-62(a)                                        | 3           |
|                     | Pseudomonas     | PB-27(a)                                        |             |
|                     | Corynebacterium | PB-60                                           |             |
| Nasal<br>Swab       | Staphylococcus  | PB-26, PB-31, PB-54, PB-59, PB-66, PB-69, PB-80 | 18          |
|                     | Klebsiella      | PB-57, PB-62, PB-71, PB-78, PB-82(a)            |             |
|                     | Pseudomonas     | PB-30(a), PB-77, PB-77(a)                       |             |
|                     | E.coli          | PB-63, PB-63(a)                                 |             |
|                     | Streptococcus   | PB-73                                           |             |
|                     |                 |                                                 | Total(n)=81 |

Table 4.3.1.6 : Isolated Bacteria of Body fluids from Clinical devices at 3 different hospitals in Khulna City

#### D. Overall 3 Hospitals at Khulna City

| In-animate Object                                                                            | Bacterial Genus          | Body fluid isolated from Patient Care Equipment                                                                                                                                                                                                                                                    |
|----------------------------------------------------------------------------------------------|--------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| PB-1, PB-6, PB-7, PB-9, PB-13, PB-14, PB-41, PB-37, PB-36, PB-35, PB-50, PB-84, PB-87, PB-88 | Staphylococcus<br>(n=48) | PB-16, PB-16(a), PB-17, PB-17(a), PB-18(a), PB-20(a), PB-21, PB-21(a), PB-22, PB-22(a), PB-24(a), PB-25(a), PB-26, PB-26(a), PB-28, PB-28(a), PB-31, PB-31(a), PB-54, PB-56, PB-56(a), PB-58, PB-58(a), PB-59, PB-59(a), PB-66, PB-66(a), PB-69, PB-72, PB-72(a), PB-79, PB-79(a), PB-80, PB-80(a) |



|                                                                                   |                          |                                                                                                                                                                               |
|-----------------------------------------------------------------------------------|--------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| PB-11, PB-34, PB-43, PB-33, PB-83,<br>PB-85, PB-89, PB-95, PB-97, PB-98,<br>PB-99 | Klebsiella<br>(n=30)     | PB-23, PB-23(a), PB-32, PB-32(a),<br>PB-55, PB-57, PB-57(a), PB-62, PB-<br>62(a), PB-70, PB-70(a), PB-71, PB-<br>71(a), PB-78, PB-78(a), PB-81, PB-<br>81(a), PB-82, PB-82(a) |
| PB-2, PB-8, PB-44, PB-46, PB-86,<br>PB-96                                         | Pseudomonas<br>(n=15)    | PB-19, PB-19(a), PB-27(a), PB-29,<br>PB-29(a), PB-30(a), PB-76, PB-77,<br>PB-77(a)                                                                                            |
| PB-3, PB-51, PB-39, PB-38, PB-47,<br>PB-100                                       | E.coli<br>(n=15)         | PB-61, PB-61(a), PB-63, PB-63(a),<br>PB-65, PB-67, PB-67(a), PB-68, PB-<br>68(a)                                                                                              |
| PB-5, PB-48, PB-94                                                                | Salmonella<br>(n=8)      | PB-64, PB-64(a), PB-74, PB-75, PB-<br>75(a)                                                                                                                                   |
| PB-10, PB-12, PB-15,                                                              | Streptococcus<br>(n=5)   | PB-73, PB-73(a)                                                                                                                                                               |
| PB-4, PB-45                                                                       | Shigella<br>(n=2)        |                                                                                                                                                                               |
| PB-42, PB-91, PB-92, PB-93                                                        | Corynebacterium<br>(n=7) | PB-53, PB-53(a), PB-60,                                                                                                                                                       |
| PB-49, PB-52, PB-40, PB-90                                                        | Acinetobacter<br>(n=4)   |                                                                                                                                                                               |

Table 4.3.1.7 : Summary of total isolated bacteria (n=134) from 3 different hospitals at Khuina City

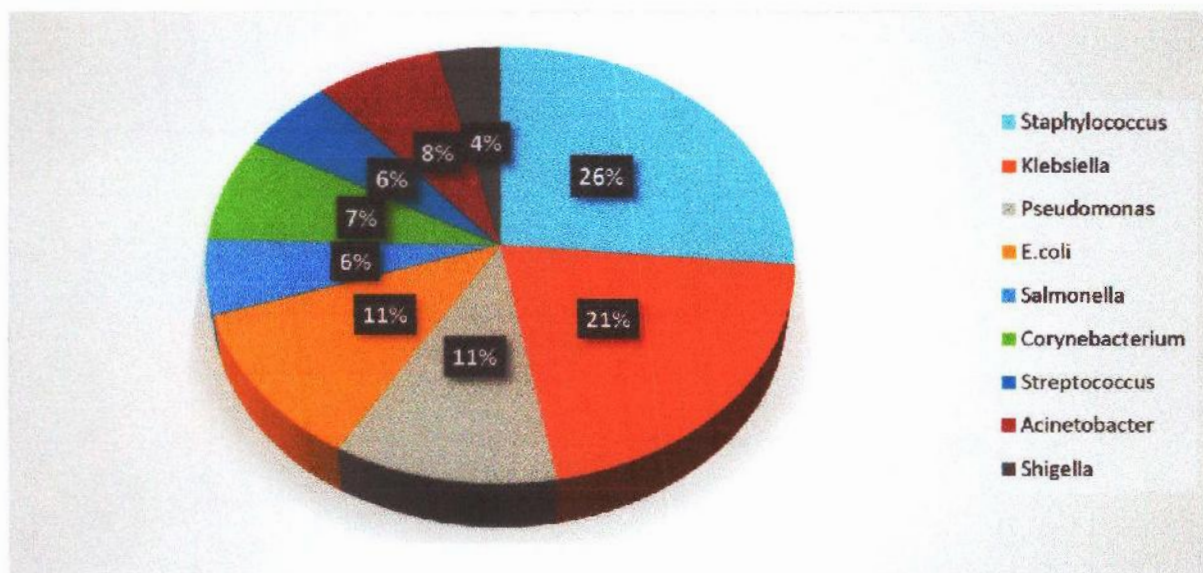


Fig 4.3.1.8: Bacteria found on inanimate objects at 3 different hospitals in Khulna City

Result showed (Fig. 4.3.1.8) from all bacterial isolates (n=53) of Inanimate objects were mostly contaminated as antibiotic resistant bacteria by *Staphylococcus* spp. (26%) and perspective other species were *Klebsiella* spp. (21%), *Pseudomonas* spp. (11%), *E.coli* (11%), *Salmonella* spp.(6%), *Corynebacterium* spp. (7%), *Streptococcus* spp.(6%), *Acinetobacter* spp. (8%) and *Shigella* (4%) the lowest rate of contamination .

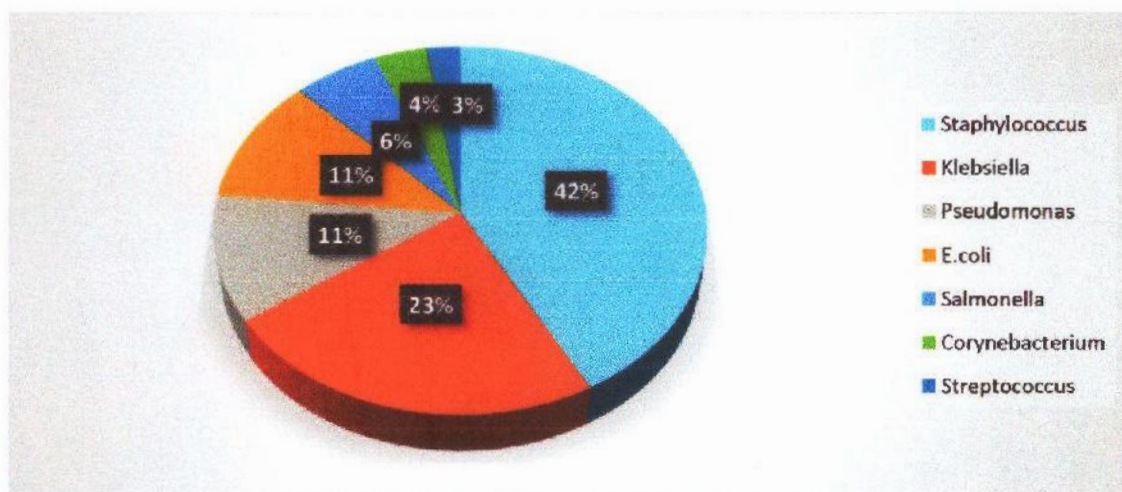


Fig 4.3.1.9: Bacteria found on Clinical devices at 3 different hospitals in Khulna City



Result showed (Fig 4.3.1.9) from all bacterial isolates (n=81) of Clinical devices were mostly contaminated as antibiotic resistant bacteria by *Staphylococcus* spp. (42%) and perspective other species were *Klebsiella* spp. (23%), *Pseudomonas* spp. (11%), *E.coli* (11%), *Salmonella* spp.(6%), *Corynebacterium* spp. (4%), *Streptococcus* spp.(3%) the lowest rate of contamination

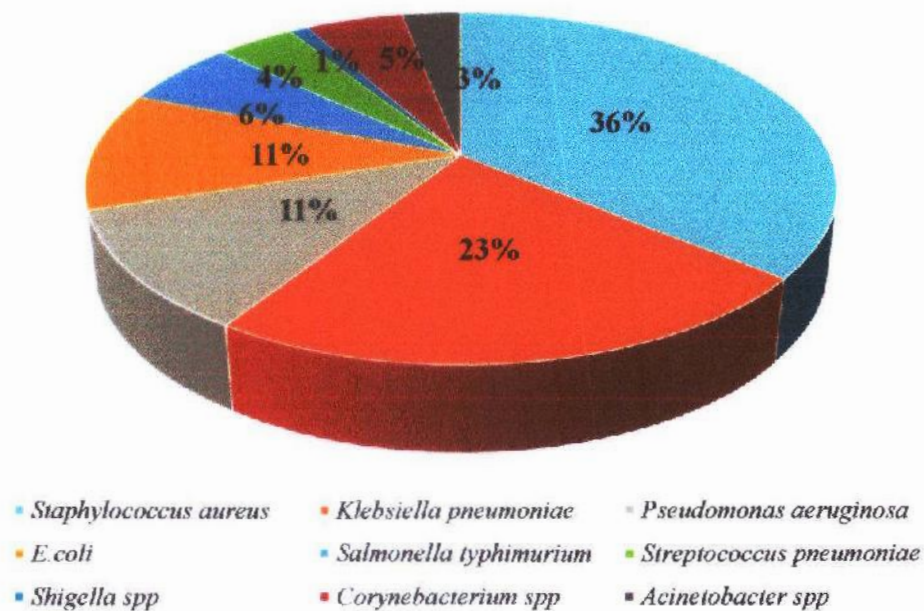


Fig 4.3.2.1: Overall bacterial prevalence on inanimate objects and clinical devices at 3 different Hospitals

Result showed (Fig 4.3.2.1) from all isolates (n=134) that Inanimate objects and Patient care equipments were mostly contaminated as antibiotic resistant bacteria by *Staphylococcus* spp. (36%) and perspective other species were *Klebsiella* spp. (23%), *Pseudomonas* spp. (11%), *E.coli* (11%), *Salmonella* spp.(6%), *Corynebacterium* spp. (5%), *Streptococcus* spp.(4%), *Acinetobacter* spp. (3%) and *Shigella* (1%) the lowest rate of contamination .



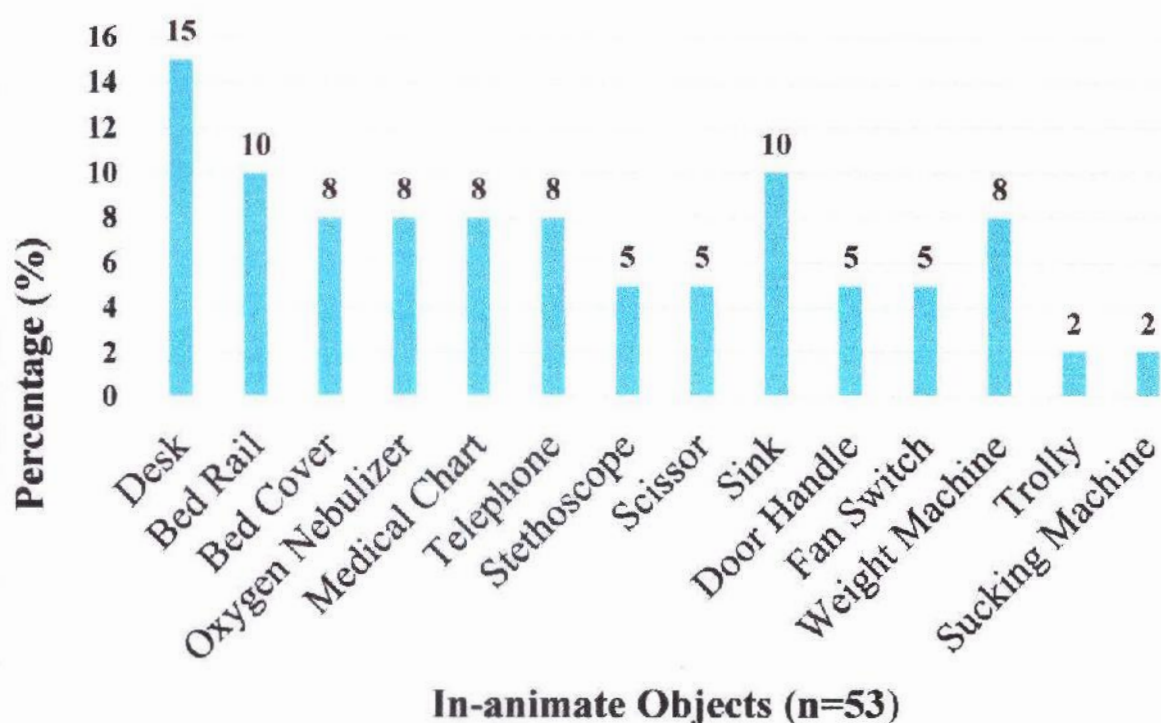


Fig 4.3.2.2: Bacteria found on different inanimate objects at Pediatric ward from 3 different hospitals in Khulna City

Result showed on In-animate objects (Fig 4.3.2.2) that the predominant bacteria were found on desk (15%), bed rail (10%), bed cover (8%), medical chart (8%), sink (10%) Oxygen Nebulizer (8%), Telephone (8%) and weight machine (8%) mostly. Besides, door handle, stethoscope, fan switch, trolley etc were containing different bacterial species eg. *Pseudomonas* spp., *Klebsiella* spp., *Staphylococcus* spp., *Corynebacterium* spp., *Acinetobacter* spp., *E.coli*, *Shigella* spp., *Streptococcus* spp. in where *Staphylococcus* spp. were mostly found.

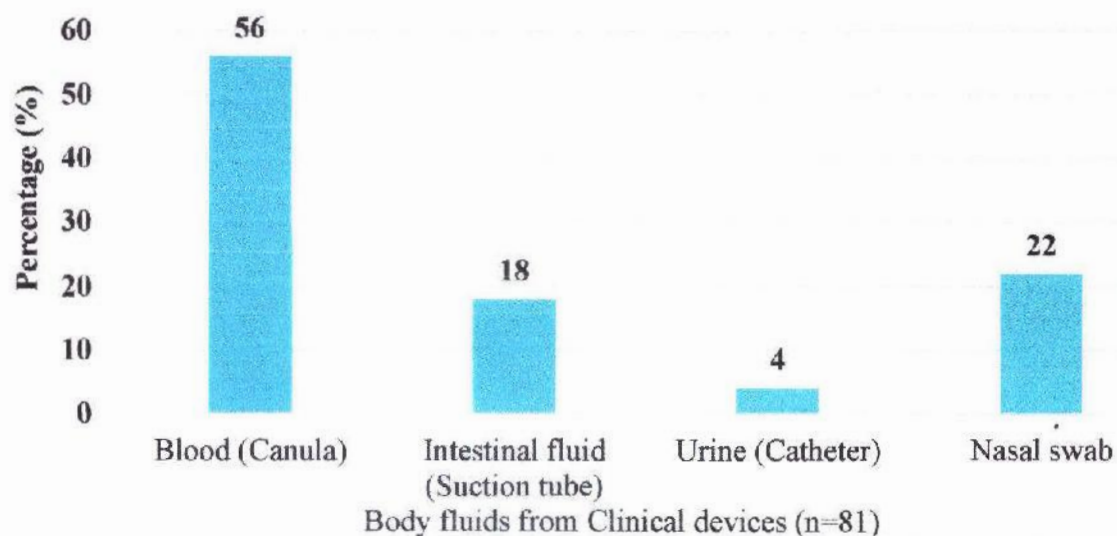


Fig 4.3.2.3: Bacteria found on body fluids from Clinical devices at Pediatric ward from 3 different hospitals in Khulna City

Result showed on body fluids from Clinical devices (Fig 4.3.2.3) that the predominant bacteria were found on blood (Canula) 56%. *Staphylococcus* spp. were mostly found and respectively other species are *Pseudomonas* spp., *Klebsiella* spp., *Corynebacterium* spp., *Acinetobacter* spp., *E.coli*, *Shigella* spp., *streptococcus* spp.

#### 4.3.1 Male-Female Infection

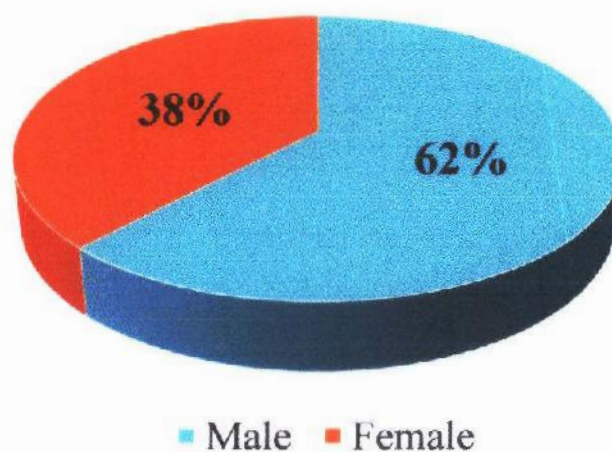


Fig 4.3.2.4 :Male and Female infection at 3 different Hospitals in Khulna City

Overall all bacterial isolates of body fluids from Clinical devices showed 62% infection on Male pediatric patient whereas 38% infection on Female pediatric patient at 3 different hospitals in Khulna City.

#### 4.4 Antibiotic Resistance Activity Analysis

##### A. Ad-Din Akij Medical College Hospital

| Sample Code | Bacteria       | Antibiotic disc |     |     |     |    |     |   |     |    |   |
|-------------|----------------|-----------------|-----|-----|-----|----|-----|---|-----|----|---|
|             |                | AK              | AZM | CFM | IMP | GM | CTX | E | CIP | CT | P |
| PB-1        | Staphylococcus | S               | R   | R   | R   | S  | R   | R | R   | R  | R |
| PB-2        | Pseudomonas    | S               | R   | R   | I   | I  | R   | R | R   | R  | R |
| PB-3        | E.coli         | S               | R   | R   | S   | S  | R   | S | S   | R  | S |
| PB-4        | Shigella       | S               | S   | S   | R   | S  | S   | I | S   | R  | I |
| PB-5        | Salmonella     | I               | S   | R   | R   | S  | R   | R | S   | I  | R |
| PB-6        | Staphylococcus | S               | R   | S   | R   | S  | R   | R | R   | R  | R |
| PB-7        | Staphylococcus | S               | I   | I   | R   | S  | R   | S | S   | R  | R |
| PB-8        | Pseudomonas    | R               | S   | R   | S   | I  | R   | R | R   | R  | R |
| PB-9        | Staphylococcus | S               | S   | I   | R   | S  | R   | R | R   | R  | R |
| PB-10       | Streptococcus  | S               | I   | R   | R   | S  | R   | R | S   | S  | R |
| PB-11       | Klebsiella     | R               | S   | R   | R   | I  | R   | I | S   | I  | R |
| PB-12       | Streptococcus  | S               | S   | R   | S   | S  | I   | R | S   | S  | R |
| PB-13       | Staphylococcus | S               | I   | R   | R   | I  | I   | S | R   | R  | R |
| PB-14       | Staphylococcus | S               | R   | R   | R   | S  | R   | R | R   | R  | R |
| PB-15       | Streptococcus  | S               | I   | I   | S   | S  | R   | R | S   | R  | R |

Table 4.4.1.1. : Antibigram profile of different bacterial isolates from inanimate objects at Ad-Din Akij Medical College Hospital

| Bacteria & Sample Code | Antibiotic disc |     |     |     |    |     |   |     |    |   |
|------------------------|-----------------|-----|-----|-----|----|-----|---|-----|----|---|
|                        | AK              | AZM | CFM | IMP | GM | CTX | E | CIP | CT | P |
| Staphylococcus (PB-16) | R               | S   | R   | S   | S  | R   | S | R   | S  | R |



|                            |   |   |   |   |   |   |   |   |   |   |
|----------------------------|---|---|---|---|---|---|---|---|---|---|
| Staphylococcus<br>(PB-16a) | R | R | R | S | S | R | S | R | S | R |
| Staphylococcus<br>(PB-17)  | S | S | R | R | S | R | S | R | R | R |
| Staphylococcus<br>(PB-17a) | S | S | R | R | R | R | S | R | S | R |
| Staphylococcus<br>(PB-18a) | S | S | R | R | I | R | S | S | S | R |
| Pseudomonas<br>(PB-19)     | R | S | R | S | S | R | S | R | R | S |
| Pseudomonas<br>(PB-19a)    | R | S | R | S | R | R | S | R | R | S |
| Staphylococcus<br>(PB-20a) | S | S | R | R | S | R | S | S | R | R |
| Staphylococcus<br>(PB-21)  | S | I | R | R | S | S | R | R | R | R |
| Staphylococcus<br>(PB-21a) | R | R | R | R | R | R | R | R | R | R |
| Staphylococcus<br>(PB-22)  | S | S | R | R | S | S | R | S | R | R |
| Staphylococcus<br>(PB-22a) | S | S | R | R | S | S | R | R | S | R |
| Klebsiella<br>(PB-23)      | S | I | R | R | S | R | R | R | R | I |
| Klebsiella<br>(PB-23a)     | S | R | R | R | S | R | R | R | R | R |
| Staphylococcus<br>(PB-24a) | S | S | R | S | S | S | S | S | S | R |
| Staphylococcus<br>(PB-25a) | S | S | R | R | S | S | R | R | R | R |
| Staphylococcus<br>(PB-26)  | S | S | R | R | I | R | S | S | S | R |

|                            |   |   |   |   |   |   |   |   |   |   |
|----------------------------|---|---|---|---|---|---|---|---|---|---|
| Staphylococcus<br>(PB-26a) | S | S | R | S | R | R | S | S | S | R |
| Pseudomonas<br>(PB-27a)    | S | R | I | R | R | R | R | R | R | R |
| Staphylococcus<br>(PB-28)  | S | I | R | I | S | R | R | R | S | R |
| Staphylococcus<br>(PB-28a) | S | R | R | R | S | R | R | R | R | R |
| Pseudomonas<br>(PB-29)     | S | S | R | R | S | S | S | R | R | R |
| Pseudomonas<br>(PB-29a)    | S | S | R | R | S | S | S | R | R | R |
| Pseudomonas<br>(PB-30a)    | S | I | R | S | S | R | R | S | R | R |
| Staphylococcus<br>(PB-31)  | S | S | R | S | S | S | S | S | R | R |
| Staphylococcus<br>(PB-31a) | S | S | R | S | S | I | S | S | R | R |
| Klebsiella<br>(PB-32)      | I | S | R | S | S | S | S | I | R | R |
| Klebsiella<br>(PB-32a)     | I | S | R | R | S | R | R | R | R | R |

Table 4.4.1.2: Bacteria isolated from body fluids of 25 pediatric patient from Clinical devices at Ad-Din Akij Medical College Hospital

#### B. Khulna Shishu Hospital

| Sample<br>Code | Name of the<br>isolated Bacteria | Antibiotic disc |     |     |     |    |     |   |     |    |   |
|----------------|----------------------------------|-----------------|-----|-----|-----|----|-----|---|-----|----|---|
|                |                                  | AK              | AZM | CFM | IMP | GM | CTX | E | CIP | CT | P |
| PB-33          | Klebsiella                       | I               | R   | S   | R   | R  | S   | R | R   | R  | R |
| PB-34          | Klebsiella                       | R               | R   | S   | R   | R  | S   | R | R   | S  | R |
| PB-35          | Staphylococcus                   | R               | I   | R   | R   | R  | S   | R | R   | S  | R |

|       |                 |   |   |   |   |   |   |   |   |   |   |
|-------|-----------------|---|---|---|---|---|---|---|---|---|---|
| PB-36 | Staphylococcus  | R | R | R | R | R | S | R | R | R | R |
| PB-37 | Staphylococcus  | R | R | R | R | R | S | R | R | R | R |
| PB-38 | E.coli          | S | R | S | S | R | S | S | R | S | R |
| PB-39 | E.coli          | S | S | S | S | R | I | R | R | S | R |
| PB-40 | Acinetobacter   | S | R | S | R | R | R | R | R | S | R |
| PB-41 | Staphylococcus  | R | R | R | R | R | R | R | R | S | R |
| PB-42 | Corynebacterium | S | S | R | S | S | R | S | R | R | R |
| PB-43 | Klebsiella      | S | R | R | R | R | R | R | R | R | R |
| PB-44 | Pseudomonas     | R | S | R | R | R | R | S | R | R | R |
| PB-45 | Shigella        | S | S | R | R | S | R | S | R | R | R |
| PB-46 | Pseudomonas     | S | R | R | R | S | R | S | R | R | R |
| PB-47 | E.coli          | S | S | R | I | S | R | I | S | R | I |
| PB-48 | Salmonella      | S | R | R | R | R | R | R | S | R | R |
| PB-49 | Acinetobacter   | S | S | R | R | S | R | S | S | R | R |
| PB-50 | Staphylococcus  | R | S | R | R | S | R | S | R | R | R |
| PB-51 | E.coli          | S | S | S | S | I | R | R | I | I | I |
| PB-52 | Acinetobacter   | S | S | R | R | R | R | R | R | S | R |

Table 4.4.1.3 : Antibigram profile of bacteria isolated from inanimate objects at Khulna Shishu Hospital





| Sample Code | Name of the Bacteria | Antibiotic disc |     |     |     |    |     |   |     |    |   |
|-------------|----------------------|-----------------|-----|-----|-----|----|-----|---|-----|----|---|
|             |                      | AK              | AZM | CFM | IMP | GM | CTX | E | CIP | CT | P |
| PB-53       | Corynebacterium      | S               | R   | R   | R   | R  | R   | R | R   | S  | R |
| PB-53a      | Corynebacterium      | S               | R   | R   | R   | R  | R   | R | R   | S  | R |
| PB-54       | Staphylococcus       | S               | R   | R   | R   | S  | R   | R | R   | R  | R |
| PB-55       | Klebsiella           | S               | S   | S   | R   | R  | S   | S | S   | R  | R |
| PB-56       | Staphylococcus       | R               | R   | R   | R   | I  | R   | S | R   | R  | R |
| PB-56a      | Staphylococcus       | R               | R   | R   | R   | I  | R   | S | R   | R  | R |
| PB-57       | Klebsiella           | R               | S   | R   | R   | R  | R   | R | S   | R  | R |
| PB-57a      | Klebsiella           | R               | S   | R   | R   | R  | R   | R | S   | R  | R |
| PB-58       | Staphylococcus       | I               | R   | S   | R   | S  | R   | R | R   | R  | R |
| PB-58a      | Staphylococcus       | R               | R   | S   | R   | S  | R   | R | R   | R  | R |
| PB-59       | Staphylococcus       | R               | R   | R   | R   | R  | R   | R | R   | S  | R |
| PB-59a      | Staphylococcus       | R               | R   | R   | R   | R  | R   | R | R   | S  | R |
| PB-60       | Corynebacterium      | S               | S   | R   | R   | S  | R   | R | R   | R  | R |

| Bacteria Code | Bacteria       | Antibiotic disc |     |     |     |    |     |   |     |    |   |
|---------------|----------------|-----------------|-----|-----|-----|----|-----|---|-----|----|---|
|               |                | AK              | AZM | CFM | IMP | GM | CTX | E | CIP | CT | P |
| PB-61         | E.coli         | S               | I   | S   | R   | S  | R   | I | S   | I  | S |
| PB-61a        | E.coli         | S               | I   | S   | R   | S  | R   | R | S   | I  | S |
| PB-62         | Klebsiella     | R               | S   | R   | R   | R  | R   | S | R   | R  | R |
| PB-62a        | Klebsiella     | R               | S   | R   | R   | R  | R   | S | R   | R  | R |
| PB-63         | E.coli         | S               | S   | I   | R   | S  | I   | S | R   | R  | R |
| PB-63a        | E.coli         | S               | S   | I   | R   | S  | I   | S | R   | R  | R |
| PB-64         | Salmonella spp | S               | R   | R   | S   | R  | S   | S | R   | R  | S |
| PB-64a        | Salmonella spp | S               | R   | R   | S   | R  | S   | S | R   | R  | S |
| PB-65         | E.coli         | S               | R   | S   | R   | S  | S   | S | R   | S  | R |
| PB-66         | Staphylococcus | R               | S   | R   | R   | R  | S   | R | R   | R  | R |
| PB-66a        | Staphylococcus | R               | S   | R   | R   | R  | S   | R | R   | R  | R |
| PB-67         | E.coli         | S               | S   | I   | I   | S  | R   | S | R   | I  | S |
| PB-67a        | E.coli         | S               | S   | I   | R   | S  | R   | S | R   | S  | S |

Table 4.4.1.4 : Antibigram profile of bacteria isolated from body fluids of 20 pediatric patient from Clinical devices at Khulna Shishu Hospital

### C. Khulna Medical College Hospital

| Bacteria &<br>Sample Code  | Antibiotic disc |     |     |     |    |     |   |     |    |   |
|----------------------------|-----------------|-----|-----|-----|----|-----|---|-----|----|---|
|                            | AK              | AZM | CFM | IMP | GM | CTX | E | CIP | CT | P |
| E.coli<br>(PB-68)          | S               | R   | I   | S   | S  | R   | I | S   | R  | I |
| E.coli<br>(PB-68a)         | S               | R   | S   | S   | S  | R   | R | S   | S  | R |
| Staphylococcus<br>(PB-69)  | S               | R   | R   | S   | S  | S   | R | R   | S  | R |
| Klebsiella<br>(PB-70)      | S               | I   | R   | S   | S  | R   | I | S   | R  | R |
| Klebsiella<br>(PB-70a)     | S               | S   | R   | S   | S  | R   | S | S   | R  | R |
| Klebsiella<br>(PB-71)      | R               | R   | R   | R   | R  | R   | R | R   | R  | R |
| Klebsiella<br>(PB-71a)     | R               | R   | R   | R   | R  | R   | R | R   | R  | R |
| Staphylococcus<br>(PB-72)  | R               | R   | R   | R   | R  | R   | R | R   | R  | R |
| Staphylococcus<br>(PB-72a) | R               | R   | R   | R   | R  | R   | R | R   | R  | R |
| Streptococcus<br>(PB-73)   | R               | R   | R   | S   | S  | S   | R | R   | R  | R |
| Streptococcus<br>(PB-73a)  | R               | R   | R   | S   | S  | S   | R | R   | R  | R |
| Salmonella<br>(PB-74)      | S               | R   | R   | S   | S  | S   | R | R   | R  | R |
| Salmonella<br>(PB-75)      | S               | R   | R   | R   | S  | S   | R | R   | R  | R |
| Salmonella<br>(PB-75a)     | S               | R   | R   | R   | S  | S   | R | R   | R  | R |



|                            |   |   |   |   |   |   |   |   |   |   |
|----------------------------|---|---|---|---|---|---|---|---|---|---|
| Pseudomonas<br>(PB-76)     | R | S | R | S | S | I | R | S | R | R |
| Pseudomonas<br>(PB-77)     | R | R | R | R | R | R | R | R | R | R |
| Pseudomonas<br>(PB-77a)    | R | R | R | R | R | R | R | R | R | R |
| Klebsiella<br>(PB-78)      | S | R | R | S | S | I | R | R | R | R |
| Klebsiella<br>(PB-78a)     | S | R | R | S | S | R | R | R | R | R |
| Staphylococcus<br>(PB-79)  | R | R | R | S | S | S | R | R | R | R |
| Staphylococcus<br>(PB-79a) | R | R | R | S | S | S | R | R | R | R |
| Staphylococcus<br>(PB-80)  | S | R | S | R | R | S | S | S | R | R |
| Staphylococcus<br>(PB-80a) | S | R | S | R | R | S | S | S | R | R |
| Klebsiella<br>(PB-81)      | S | I | I | S | R | I | R | R | R | S |
| Klebsiella<br>(PB-81a)     | S | I | I | S | R | S | R | R | R | I |
| Klebsiella<br>(PB-82)      | R | R | R | R | R | S | S | R | R | R |
| Klebsiella<br>(PB-82a)     | R | R | R | R | R | S | S | R | R | R |

Table 4.4.1.5: Antibigram profile of bacteria isolated from body fluids of 20 pediatric patient from Clinical devices at Khulna Medical College Hospital

| Sample Code | Bacteria        | Antibiotic disc |     |     |     |    |     |   |     |    |   |
|-------------|-----------------|-----------------|-----|-----|-----|----|-----|---|-----|----|---|
|             |                 | AK              | AZM | CFM | IMP | GM | CTX | E | CIP | CT | P |
| PB-83       | Klebsiella      | S               | R   | R   | S   | S  | R   | R | R   | S  | R |
| PB-84       | Staphylococcus  | R               | R   | R   | I   | S  | R   | R | S   | R  | R |
| PB-85       | Klebsiella      | I               | S   | R   | R   | R  | R   | R | R   | R  | R |
| PB-86       | Pseudomonas     | R               | R   | S   | S   | S  | I   | R | S   | S  | R |
| PB-87       | Staphylococcus  | I               | S   | R   | R   | I  | S   | I | R   | R  | R |
| PB-88       | Staphylococcus  | R               | R   | I   | S   | S  | S   | S | I   | R  | R |
| PB-89       | Klebsiella      | R               | I   | S   | I   | R  | S   | R | R   | I  | R |
| PB-90       | Acinetobacter   | S               | R   | R   | R   | R  | S   | R | R   | R  | R |
| PB-91       | Corynebacterium | R               | R   | R   | R   | R  | R   | R | R   | R  | R |
| PB-92       | Corynebacterium | R               | R   | R   | S   | R  | R   | I | R   | I  | R |
| PB-93       | Corynebacterium | I               | R   | R   | R   | S  | I   | R | R   | R  | R |
| PB-94       | Salmonella      | R               | R   | R   | S   | I  | I   | I | R   | R  | R |
| PB-95       | Klebsiella      | S               | R   | S   | S   | S  | S   | S | R   | S  | R |
| PB-96       | Pseudomonas     | R               | R   | R   | R   | R  | R   | R | R   | R  | R |
| PB-97       | Klebsiella      | S               | R   | R   | S   | S  | S   | I | R   | R  | R |
| PB-98       | Klebsiella      | R               | R   | R   | S   | S  | R   | R | R   | I  | R |
| PB-99       | Klebsiella      | R               | S   | I   | R   | S  | S   | R | R   | S  | R |
| PB-100      | E.coli          | R               | R   | R   | S   | R  | R   | R | S   | R  | R |

Table 4.4.1.6 : Antibigram profile of bacteria isolated from inanimate objects at Khulna Medical College Hospital

### A.Ad-Din Akij Medical College Hospital

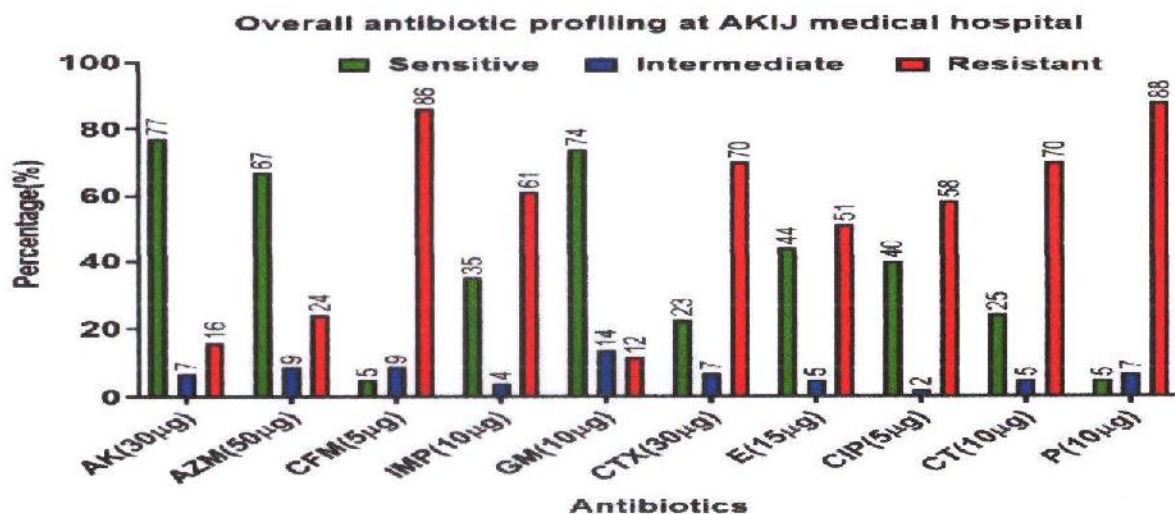


Fig 4.4.1.7 : Antibiotic Profiling of all bacterial isolates at Ad-Din Akij Medical College Hospital

The Overall bacteria shows (Fig 4.4.1.7) that bacteria perspective resistant against Penicilin (88%), Cefixime (86%), Cefotaxime (70%), Colistin (70%) whereas Amikacin is 77% and Gentamicin is 74% sensitive so far.

### B.Khulna Shishu Hospital

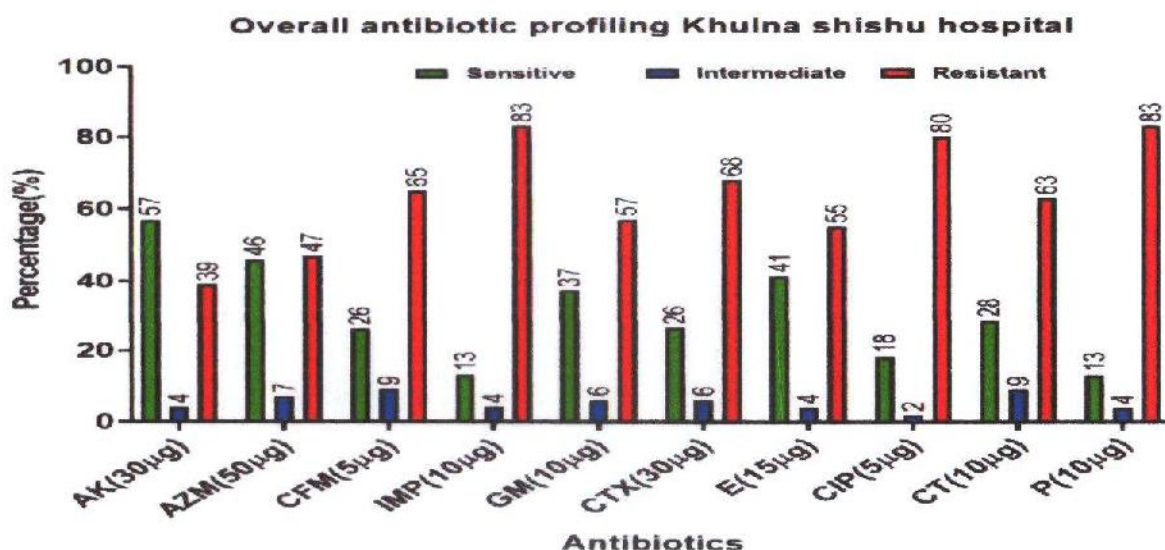


Fig 4.4.1.8 : Antibiotic profiling of all bacterial isolates at Khulna shishu hospital



The Overall bacteria shows (Fig 4.4.1.8) bacteria perspective resistant against Penicilin (83%), Ciprofloxacin 80%, Cefotaxime 68% and Emipenem (83%).

#### C.Khulna Medical College Hospital

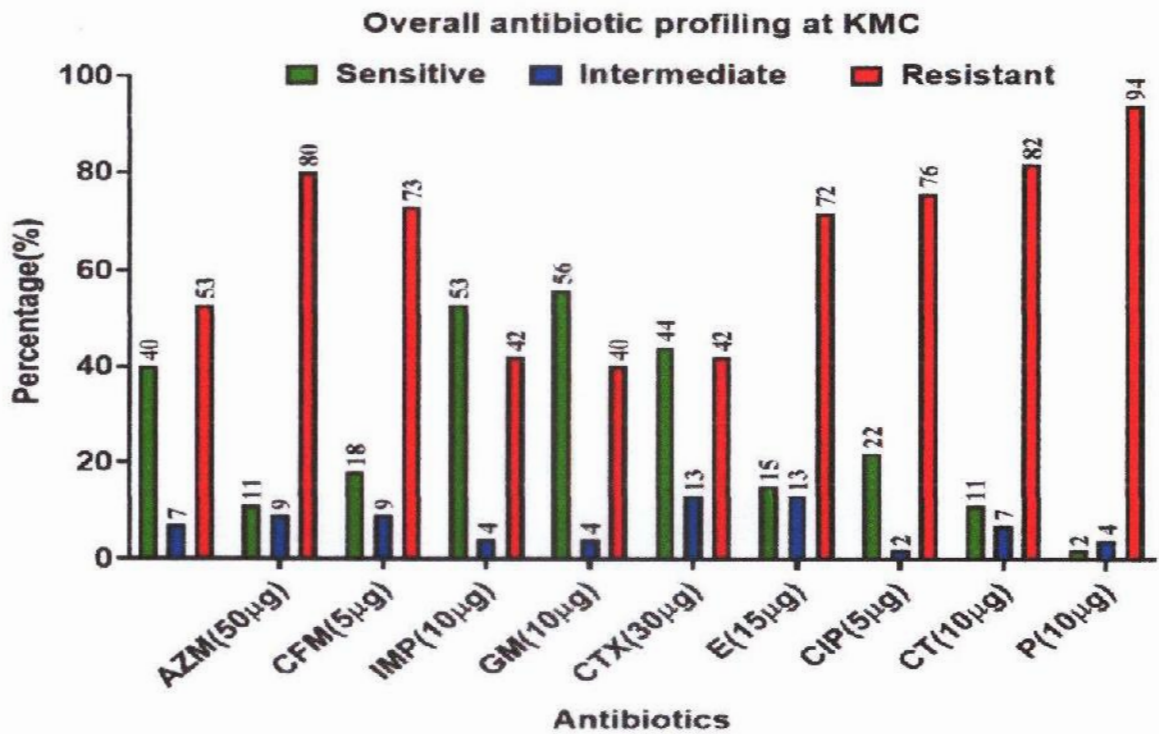


Fig 4.4.1.9 : Antibiotic profiling of all bacterial isolates at Khulna Medical College Hospital

The Overall bacteria shows (Fig 4.4.1.9) bacteria perspective resistant against Penicilin (94%), Colistin (82%), Azithromicin 80%, Ciprofloxacin 76%, Cefotaxime 73% and Emipenem (72%).

#### D.Overall 3 Hospitals in Khulna City

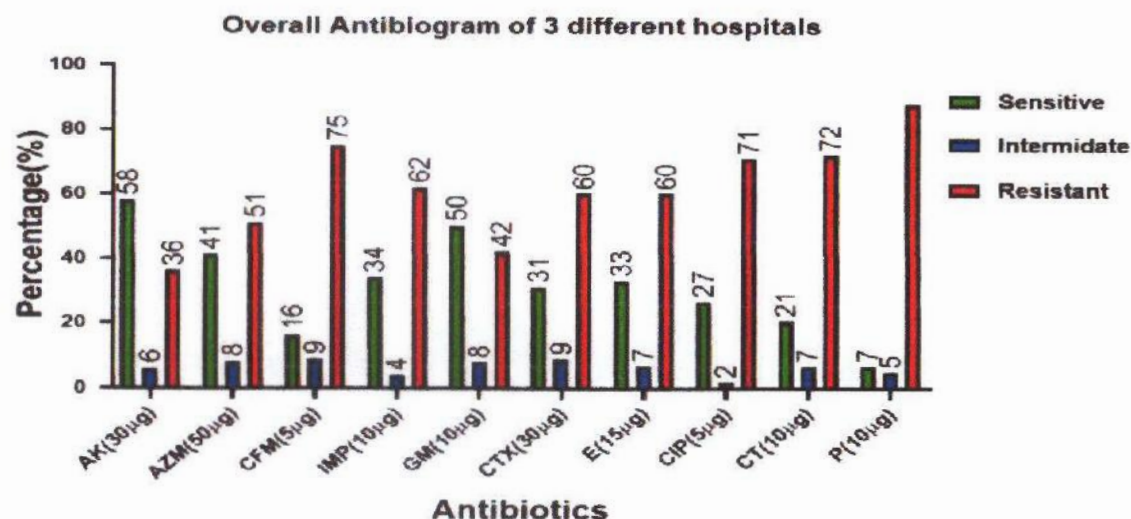


Fig 4.4.2.1 : Overall Antibigram of 3 different Hospitals at Khulna City

The study explained that mostly resistant Antibiotic against all bacteria is Penicillin (88%), Cefixime (75%), Colistin (72%), Ciprofloxacin (71%) and Imipenem (62%) whereas mostly sensitive Amikacin (58%) and Gentamicin (50%) and other antibiotic is different level of resistant.



## Nutrient Media Culture

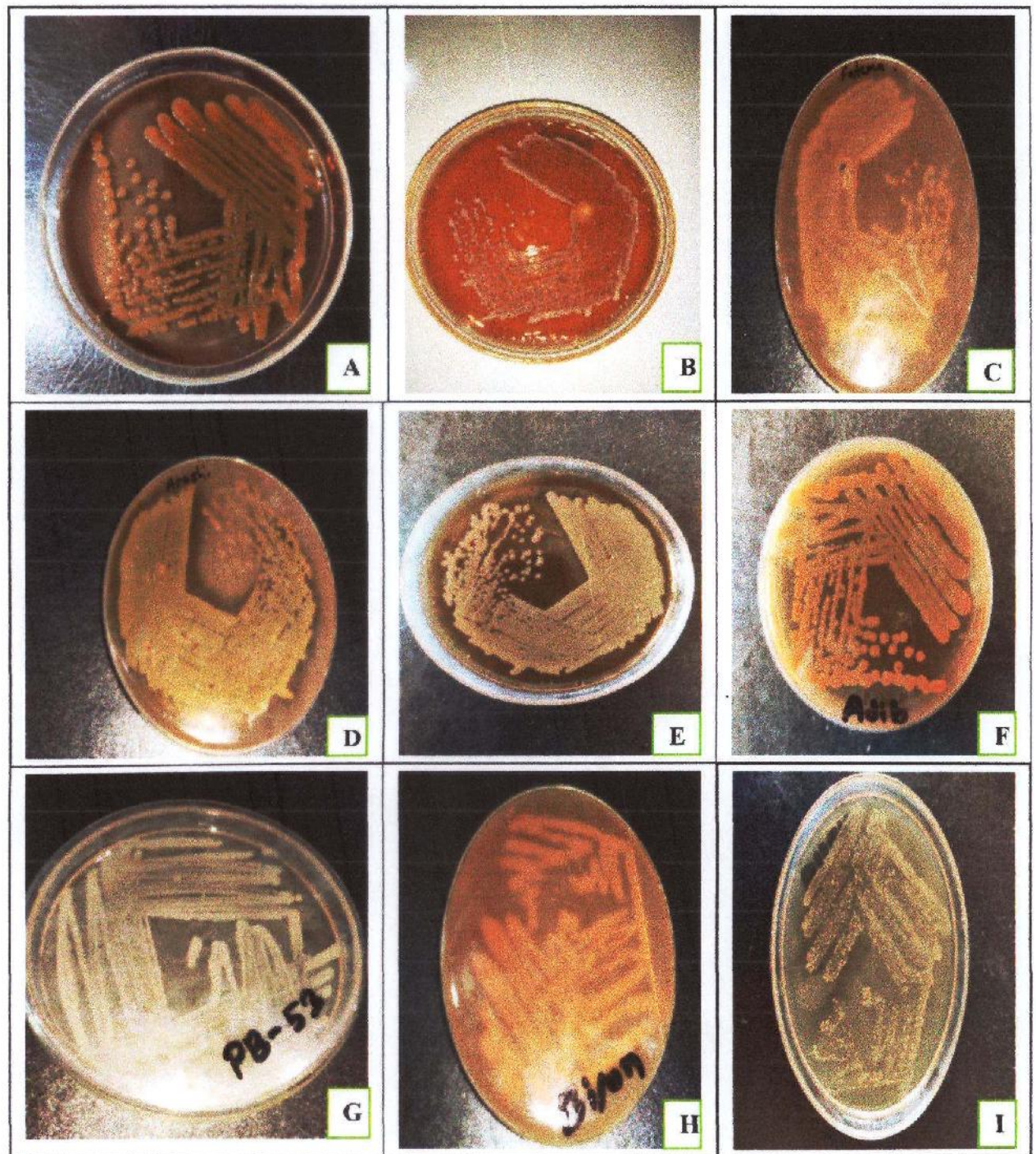


Fig 4.1.1: Growth of bacterial isolates on Nutrient agar media



## Macconkey agar

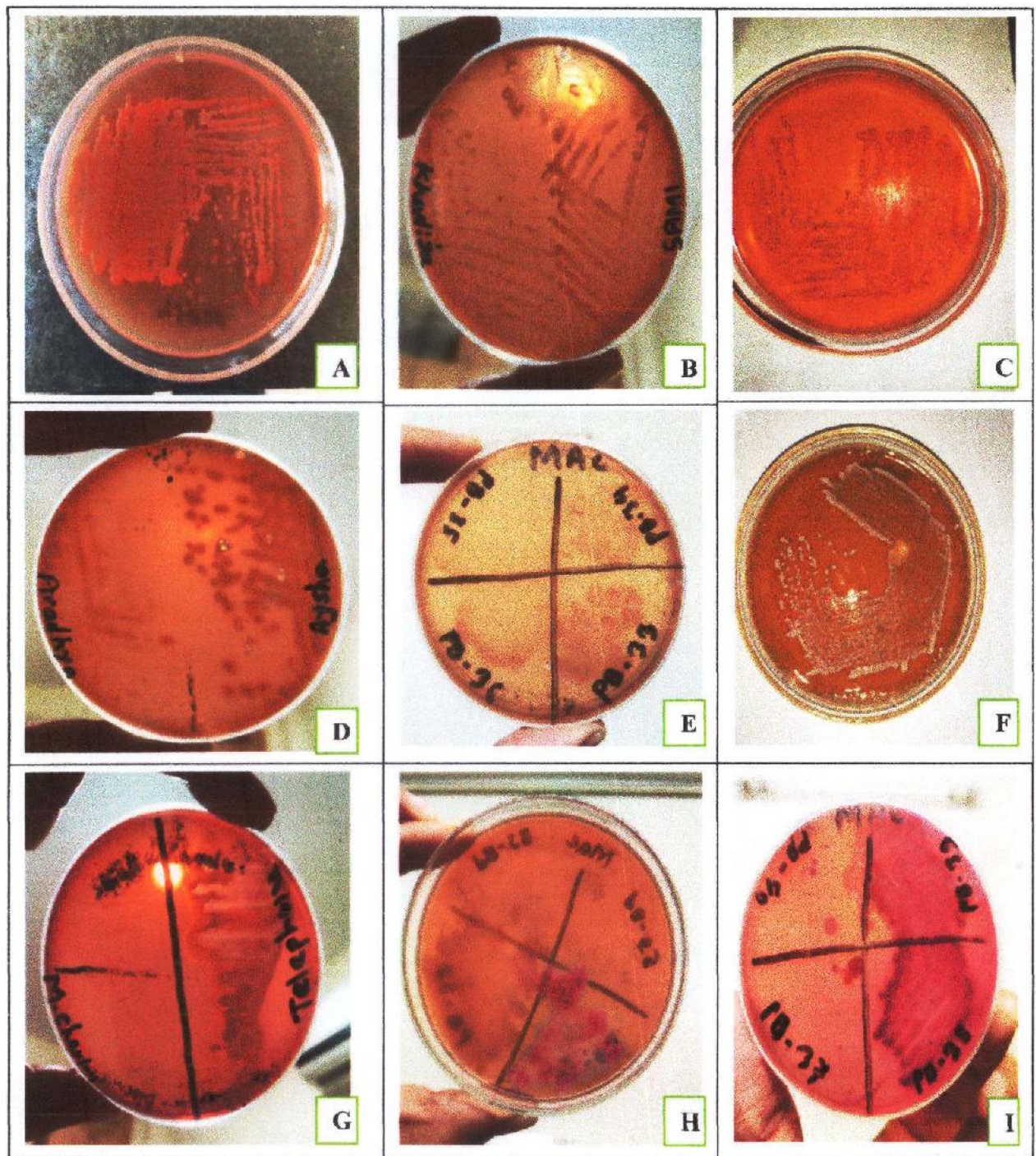


Fig 4.1.2: Growth of bacterial isolates on Macconkey agar media



## Blood Agar

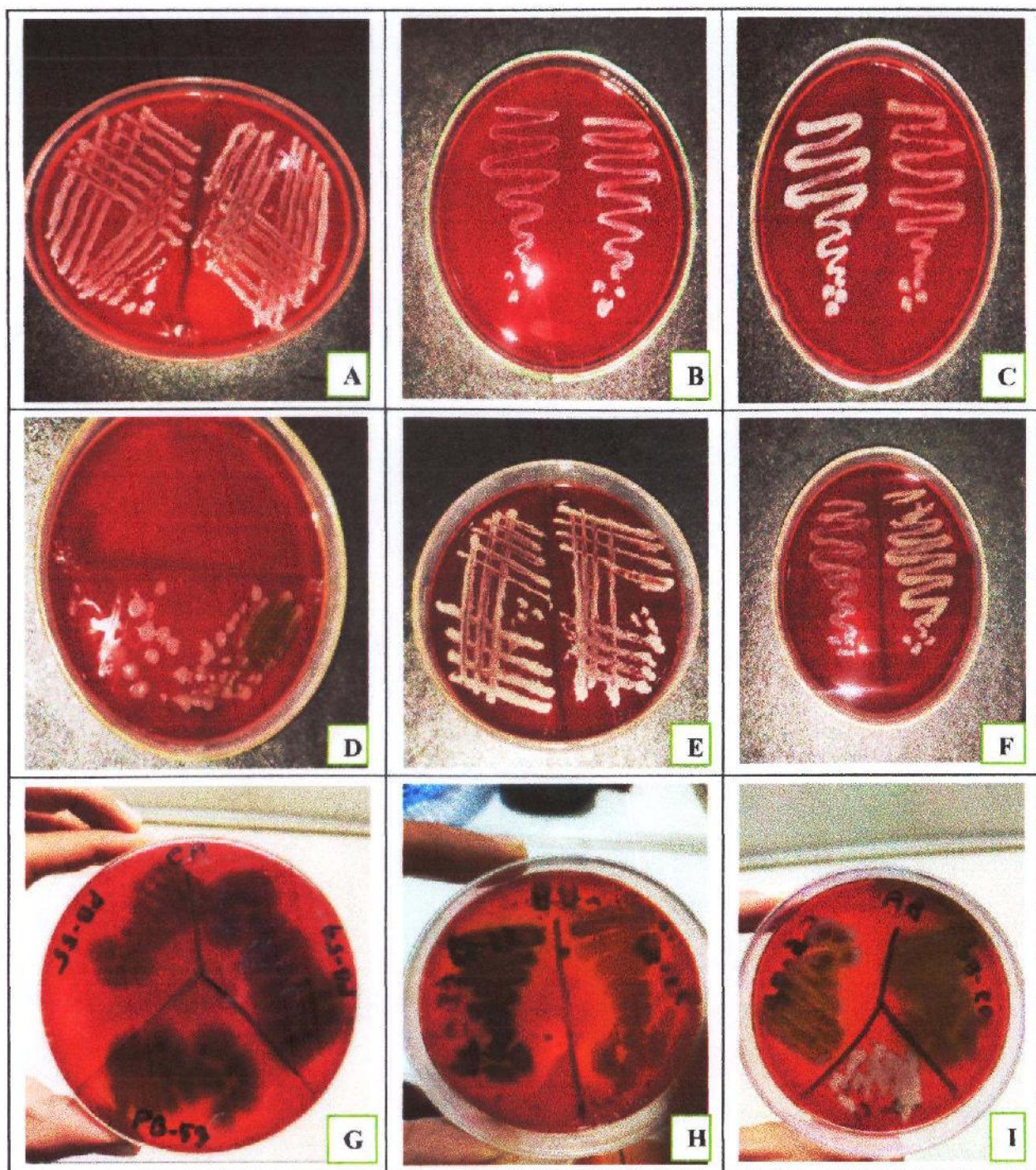


Fig 4.1.3: Growth of bacterial isolates on Blood agar media



## Chocolate Agar

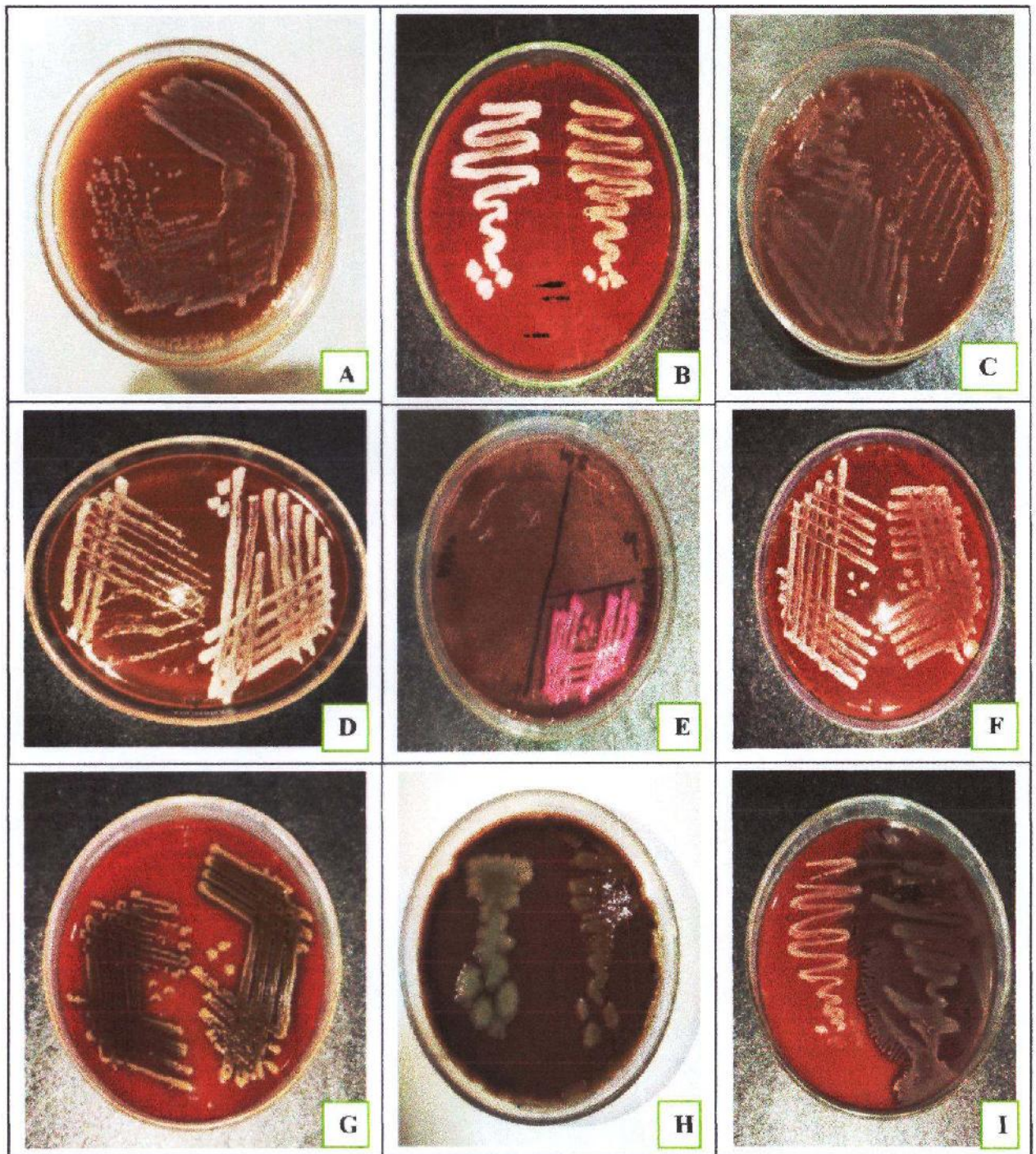


Fig 4.1.4: Growth of bacterial isolates on Chocolate agar media



### Gram Staining (Gram Positive)

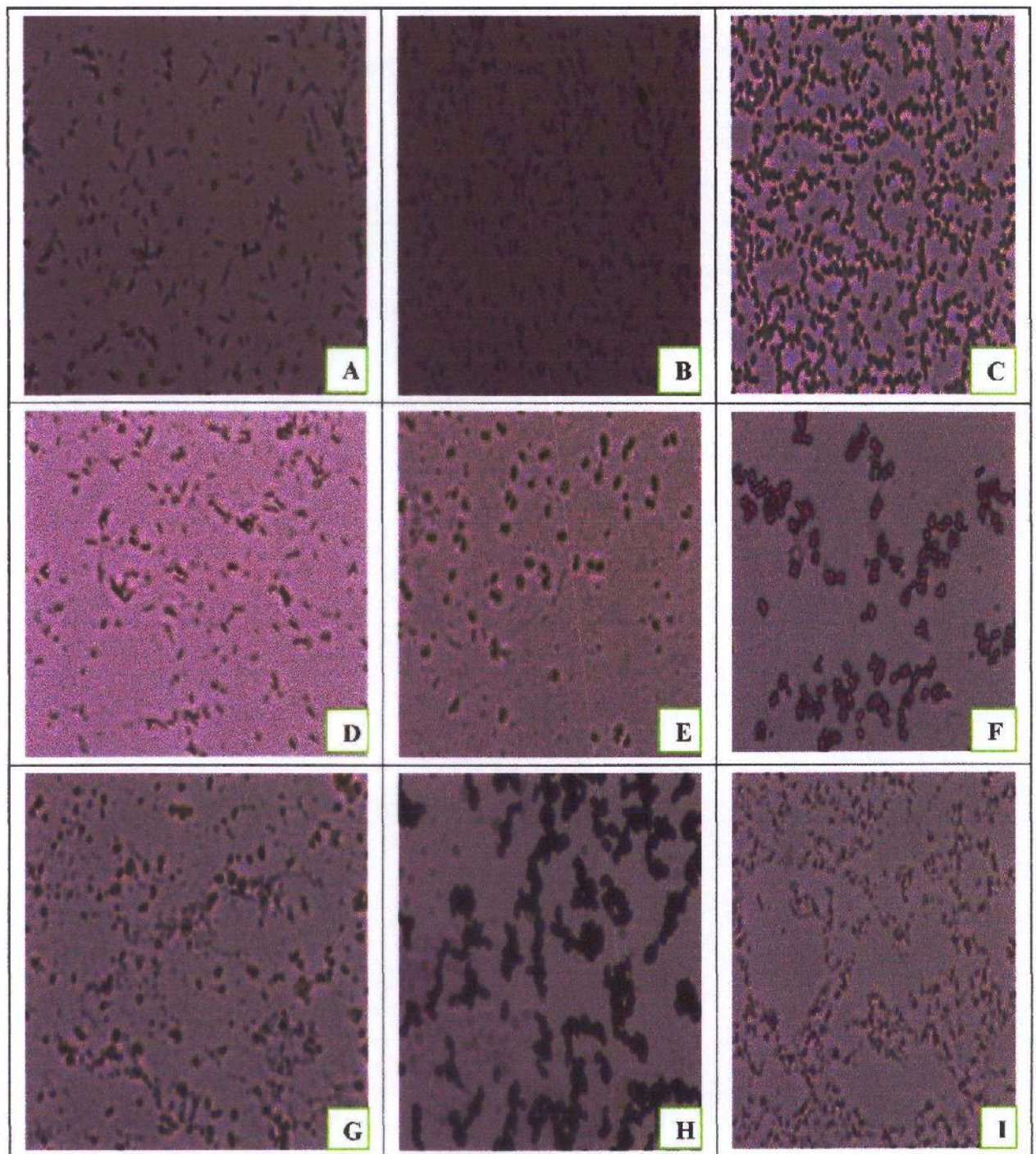


Fig 4.1.5: Gram staining (Positive) of bacterial isolates



# Gram Staining (Gram Negative)

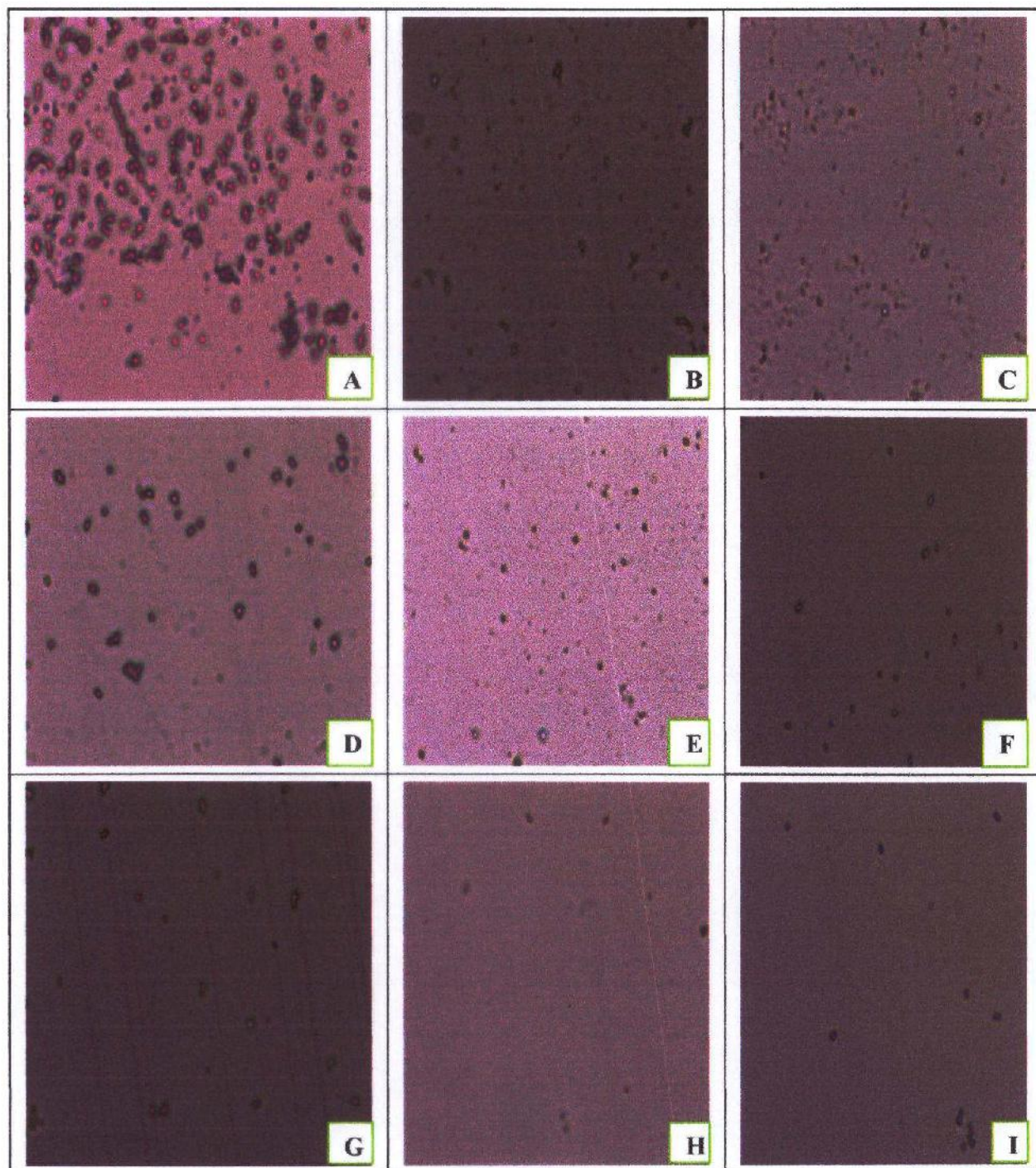


Fig 4.1.6 :Gram staining (negative) of bacterial isolates



## Oxidase Test

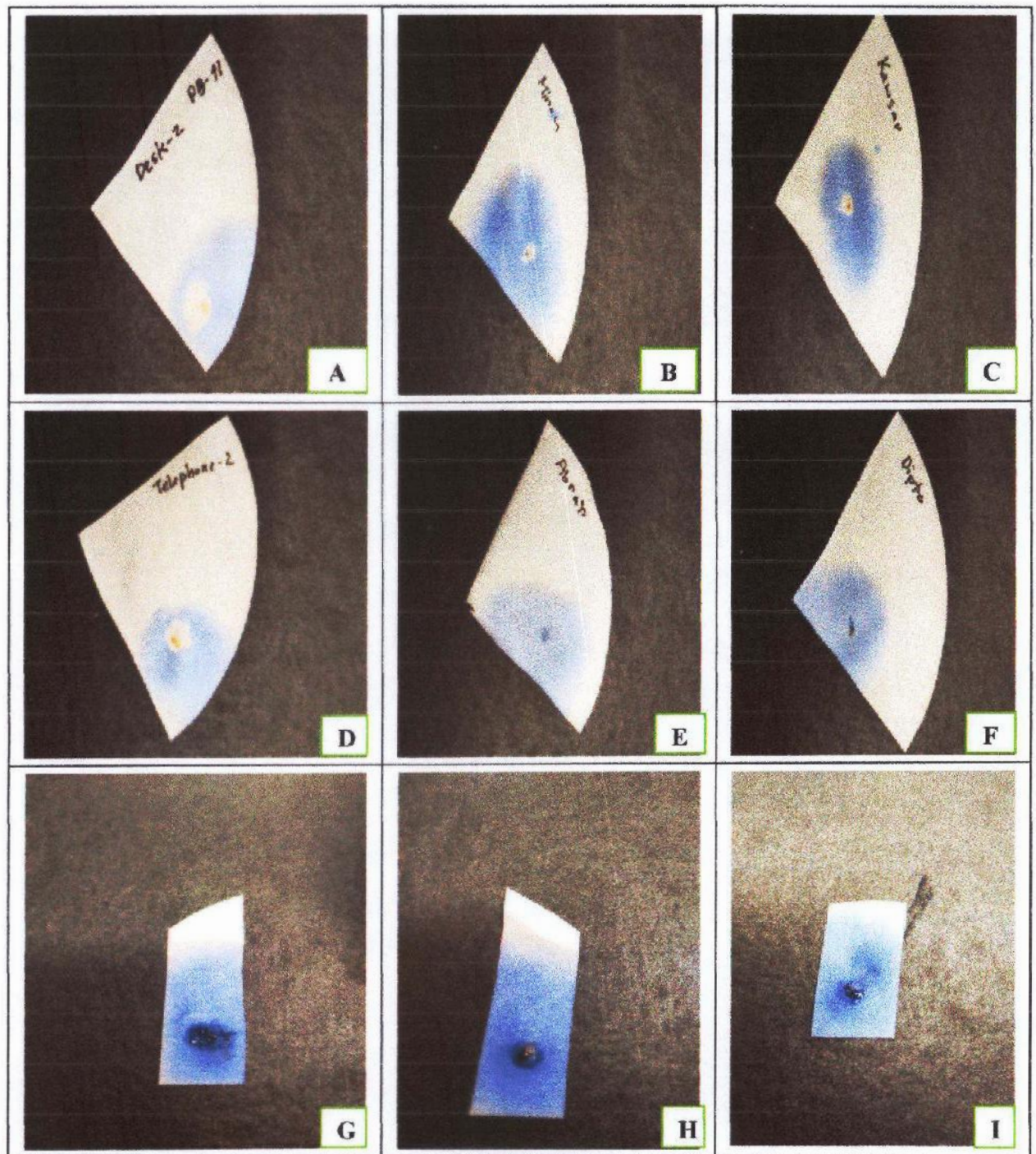


Fig 4.1.7: Oxidase test of bacterial isolates



## Catalase Test



Fig 4.1.8: Catalase test of bacterial isolates



## Urease Test



Fig 4.1.9: Urease test of bacterial isolates;(A)AAMCH (B)KSH (C)KMCH



### Citrate test



Fig 4.1.1.1 :Citrate test of bacterial isolates; (A)AAMCH (B)KSH (C)KMCH



### Triple Sugar Iron (TSI) Test



Fig 4.1.1.2: Triple Sugar Iron test of bacterial isolates; (A)AAMCH (B)KSH (C)KMC



### Coagulase Test

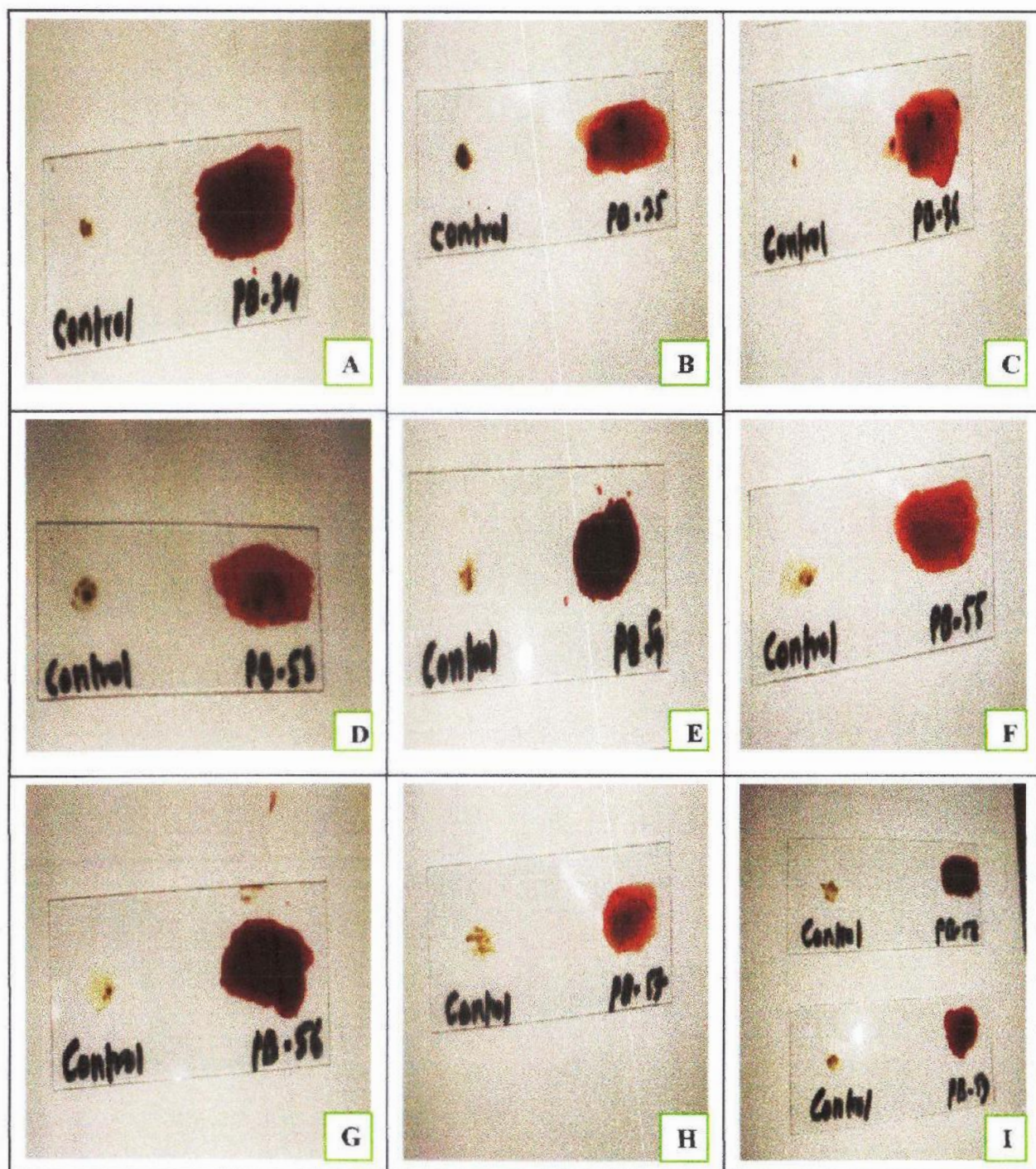


Fig 4.1.1.3: Coagulase test of bacterial isolates



### Antibiotic Susceptibility test

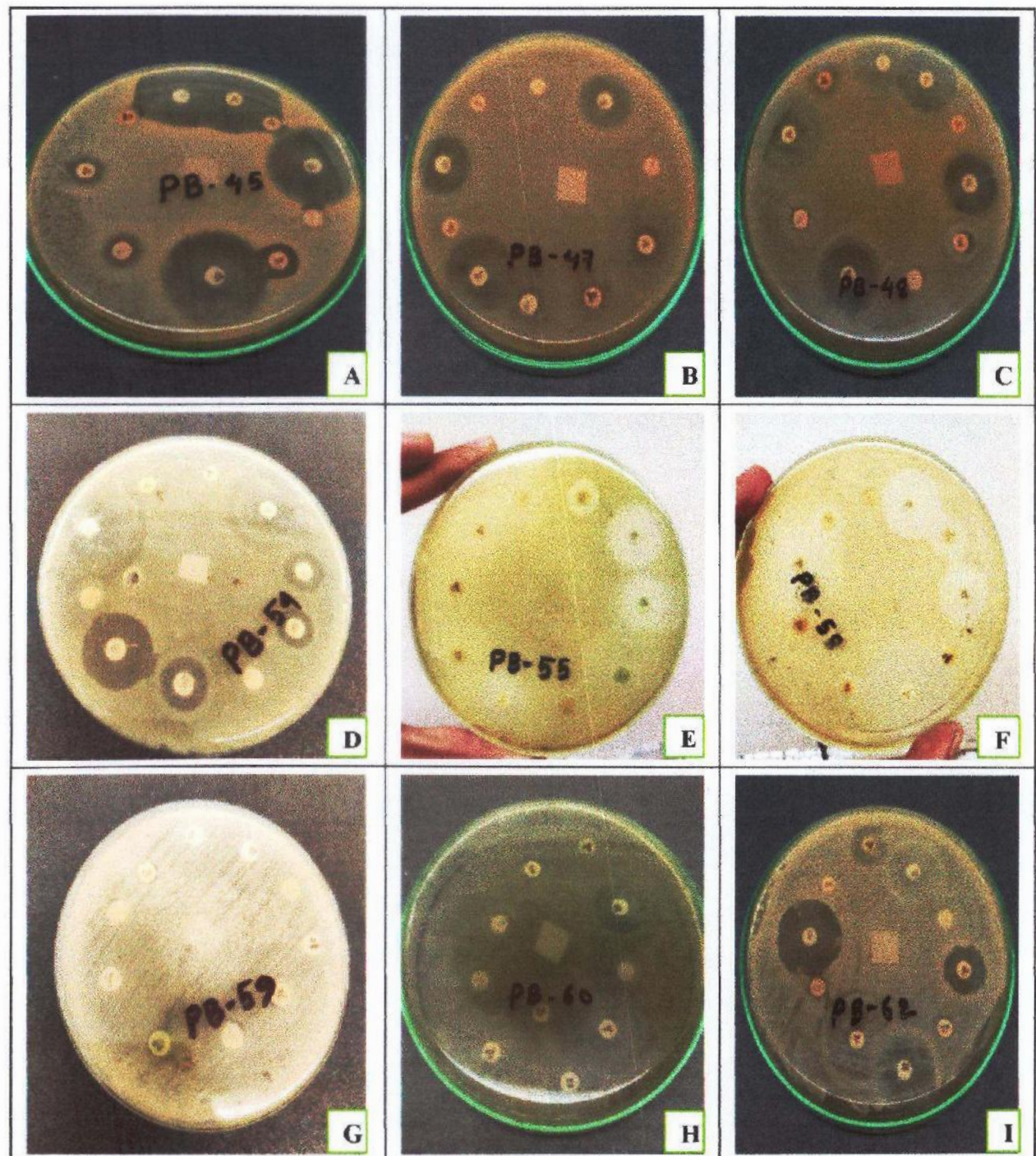


Fig 4.1.1.4: Antimicrobial susceptibility testing of bacterial isolates

# **Chapter Five**

## **Discussion**





## 5. Discussion

### A. Prevalence of isolated Bacteria

In this study, total 134 isolates from Clinical devices 81 (62%) and in-animate objects 53 (51%) on average 134 (57%) showed bacterial contamination from Pediatric Ward at 3 different hospitals in Khulna city. On the relevant study showed, Of the 99 inanimate objects and items of patient-care equipment examined contamination 66.6% from pediatric ward (Birru *et al.*,2021).

### B. Characterization of isolated bacteria

This results showed, Clinical devices and In-animate objects were mostly contaminated by antibiotic resistant bacteria as Gram-negative (55%) , Gram-positive (45%) and 9 different bacteria were identified. The other study showed almost similliar results that 10 different genera of bacteria were identified by culture. Almost all the isolates were gram-negative bacteria, while only one species of gram-positive (*Staphylococcus aureus*) was reported (Tafa *et al.*, 2023)

This work identified 9 different types of bacteria mostly followed by *Staphylococcus aureus* (36%) and perspectivevely other species were *Klebsiella pneumoniae* (30%), *Pseudomonas aeruginosa* (11%), *E.coli* (11%), *Salmonella typhimurium* (6%), *Corynebacterium spp.* (5%), *Streptococcus pneumoniae* (4%), *Acinetobacter spp.* (3%) and *Shigella spp* (1%) as well. In the other study, the case of Gram-positive bacteria, coagulase-negative staphylococci (CoNS; 52.2%) were predominant, followed by *Staphylococcus aureus* (47.7%), whereas common Gram-negative counterparts were *Acinetobacter spp.* (28.5%) and *Klebsiella spp.* (23.8%) (Birru *et al.*,2021). Another authors found that the rate of multidrug resistance (17%) in *S. pneumoniae* was much lower than in many other African and Asian countries (Radoslaw *et al.*,2024)

This study showed, mostly prevalence species were *Staphylococcus aureus* and second highest predominant bacteria were *Klebsiella pneumoniae*. The similar results were found in Pietrocola et al.,(2022) that the improvement of hygiene conditions in hospitals remain a common and serious complication in where *Staphylococcus aureus* is the most common cause of biomaterial-centered infection (Pietrocola et al.,2022). But the other study revealed that the most common bacterial contamination in pediatric ward is *Klebsiella pneumoniae* (Shirin et al.,2022) . The majority of isolated bacteria were *S. aureus* and *P. aeruginosa*. The high frequency of antimicrobial resistance, the presence of resistance genes highlight the challenge in treatment (Alshraideh et al., 2022)

Another study revealed that *Streptococcus pneumoniae* (*S. pneumoniae*) remains one of the most important pathogens causing childhood infections. The spread of antibiotic-resistant bacteria is a leading cause of treatment failure in children. The purpose of this investigation is to report the antibiotic and multidrug resistance (MDR) of *S. pneumoniae* strains isolated from healthy children throughout the years 2020–2022 (Amari et al.,2023)

### C. Antibiotic resistant bacteria

In this study, Kirby-Bauer Disk assays showed that all 9 identified bacteria were mostly resistant against Penicillin (88%), Cefixime (75%), Colistin (72%), Ciprofloxacin (71%) and Imipenem (62%). The similar results showed, Penicillin become apparent to the general public, then the antibiotic started to be overused. This triggered selective pressure for the emergence of penicillin-resistant strains mostly, which over a few years spread across different countries (Lobanovska et al.,2017). Other results revealed in Kon et.,al (2023), Colistin showed a high prevalence (67.1%) resistance to examine evolution of strains into full in colistin-containing broth (Kon et al.,2023). Besides, Antibiotic resistance/sensitivity tests revealed resistance patterns commonly against cefixime (51%) (Samanci et al., 2023).



Besides, more or less dissimilarities were found in Neyestani et al., (2023) that *E. coli* isolates were phenotypically resistant to ciprofloxacin. Based on sources of isolation, 64.63% resistant, of isolates from clinical specimens in Iran.( Neyestani et al., 2023)

This study also showed that Amikacin (58%) and Gentamicin (50%) were more or less Sensitive to all 9 types of identified bacteria . Similar results were found in other study that the sensitivity rates for Amikacin ranged from 72% to 100% (Firissa et al., 2023)

The result demonstrates that overall prevalence of multidrug-resistant bacteria was 57% . The similar result has been noticed in Birru *et al.*, (2021) that the prevalence of multidrug-resistant bacteria at Arba Minch General Hospital, Ethiopia were 57.7% (Birru *et al.*,2021). But a little dissimilarities were found in Tafa et al., (2023) that the overall detected multidrug resistance (MDR) was 64.29% (Tafa et al., 2023).

# **Chapter Six**

## **Conclusion**



## 6. Conclusion

In recent decades, due to irrational and inappropriate usage of antibiotics, Antibiotic-resistant bacteria are gradually emerging in hospital setups. Besides, Clinical devices and inanimate objects are being heavily contaminated with different species of bacterial pathogens. Encompassing 9 bacteria: *Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhimurium*, *Streptococcus pneumoniae*, *Acinetobacter spp.*, *Corynebacterium spp.* and *Shigella spp.* with growing antibiotic resistance and showed it's virulency most of the study period in pediatric ward. It is important to reduce unnecessary and over-prescribing of antibiotics when antibiotics are prescribed for conditions that do not require them. Patients should have completed the entire course of any prescribed antibiotic so that it can be fully effective and not breed resistance. The findings of this study could be used by policy-makers to develop more practical protocols for hand hygiene, reducing contact with patients and using invasive devices. Staff training programmes along with continuous supervision and monitoring are also essential to prevent the spread of infection. It should be recommended that special attention to infection control policies; antimicrobial resistance screening, good clinical practice, and cleaning techniques are needed to reduce the potential risk of pathogenic bacteria.

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