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21 September University for Medical and Applied Sciences
Faculty of Laboratory Medicine
Department of Medical Microbiology

Bacterial Contamination of Neonatal Incubators in Hospitals of Sana'a city-Yemen

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Prepared by:

Abdul Jalil Ismail Angad
Abdulqadir Mohammed Al-Dhafiri
Bushra Abdu Al-Okam
Ghadah Anwar Ghallab
Hassen Ahmed Al-Amri
Hanadi Najeeb Qaaed
Khlowd Saleem Al-Masbahi
Muawiya Dhafer Al-Nomair
Reem Abdualmlek Al-Nuzili
Thoria Abdulwhab Al-Gabri
Zainab Ahmed Abdul-Moghni
Zakaria Rashad Al-Kadasi

Supervised by:

Dr. Jamil T. Abdul-Moghni

Assistant Professor of Medical Microbiology and Immunology
Faculty of Laboratory Medicine
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الاستهـــلال

أعوذ بالله من الشيطان الرجيم

قال تعالى :{وَمَا أُوتِيتُمْ مِنَ الْعِلْمِ إِلَّا قَلِيلًا}

صدق الله العظيم

(الإسراء، الآية 85)

Dedication

To the face of Him the One, the Almighty, His righteous servants turn.

To the origin to which belonging remains, a commandment in the soul of every originator so that time can return (the homeland).

To that spirit that resists, resembling Arabism, and in its steadfastness the dignity of the nation (Palestine).

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And to the sick in this world: Shine, for the light of the sun concerns you too.

To every student who seeks to gain knowledge with the goal of achieving something, you will certainly achieve it.

We dedicate this work to all of you.

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إلى وجه الذي يتوجه إليه وحده عباده الصالحين،

إلى الأصل الذي يبقى الانتماء إليه كوصيَّةٍ في نفس

كل أصيلٍ كي يعود الزمان

(الوطن)

إلى تلك الروح التي تقاوم حاملة معها العروبة وفي صمودها كرامة أمة

(فلسطين)

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List of Abbreviations

50H	Al-Khamseen Hospital
70H	Al-Sabeen Maternity & Childhood Hospital
AICU	Adult Intensive Care Unit
AJH	Al-Gumhori Teaching Hospital Authority
ATP	Adenosine Tri Phosphate
CLSI	Clinical and Laboratory Standards Institute
CoN*S*	Coagulase-Negative *Staphylococci*
CoP*S*	Coagulase-Positive *Staphylococci*
CVCs	Central Venous Catheters
DNA	Deoxy ribo Nucleic Acid
EPEC	*Entero Pathogenic Escherichia Coli*
ETEC	*Entero Toxigenic Escherichia Coli*
HAI	Healthcare-Associated Infection
HCWs	Health Care Workers
ICN	Intensive Care Nursery
ICU	Intensive Care Unit
LMICs	Low-and Middle-Income Countries
MR*SA*	Methicillin-Resistant *Staph-aureus*
NI	Nosocomial Infection
NICU	Neonatal Intensive Care Unit
PLH	Palestine Hospital
PSBI	Possible Serious Bacterial Infection
SPSS	Statistical Package for the Social Sciences
VR*SA*	Vancomycin Resistance *S. aureus*

Abstract

Objectived of the study: Assessment the bacterial contamination of neonate's incubators surface in hospitals, Sana'a city.

Methods: This a cross sectional study was conducted from 26 December 2023 to 7 February 2024 among one hundred incubators are enrolled in the study samples from Al-Sabeen Maternity & Childhood Hospital, Al-Gumhori Teaching Hospital Authority, Al–Khamseen Hospital and Palestine Hospital in Sana'a city, Yemen. The swab samples were taken and placed in a separated sterile labeled test tube and transport to university laboratory within one hour and inoculated onto pre-prepared culture plates and bacteria were identified by standard microbiological methods such as culture traits of pure colonies, Gram staining, enzyme production and biochemical tests. The collected data was analyzed by SPSS version 17.

Results: Among 100 samples, 42% yielded positive bacterial growth after 24 hours of incubation. From those that are positive bacterial growth 76,2% were gram-positive bacteria and 23,8% were gram-negative bacteria. Seven different pathogens were identified, Coagulase negative *Staphylococci* accounted for majority of the bacterial pathogens isolated 69%, followed by *Klebsiella* spp 9.5%, *S. aureus* 4.8%, *Enterobacter* spp 4.8%, *Acinetobacter* spp 4.8%, *Escherichia coli* spp 4.8% and *Pseudomonas* spp 2.4%.

Conclusions: The bacterial contamination of objects and instruments in NICU was high. This indicate that they can be vehicles for diseases transmission specially in countries that have fragile healthcare system.

Key words: Bacterial Contamination, Neonatal intensive care unit (NICU), Neonatal incubators, Coagulase negative *Staphylococci*, Sana'a city.

Chapter one

INTRODUCATION

AND

LITERATURE REVIEW

1. Introduction and Literature review

1.1 Introduction

Nosocomial infections (hospital-acquired infections) pose a significant threat to neonates (newborn babies) in healthcare facilities, especially in resource-limited settings like Yemen (Shelke *et al*., 2023). Incubators, while critical for premature and critically ill newborns, can also harbor harmful bacteria if not properly disinfected and maintained (Li *et al*., 2014). Bacterial contamination of incubators can lead to severe and potentially fatal infections in newborns, including sepsis, pneumonia and meningitis (Hadfield and Cantey, 2021).

Yemen's ongoing humanitarian crisis has resulted in a fragile healthcare system with limited access to clean water and sanitation (United Nations Office for the Coordination of Humanitarian Affairs (Butcher, 2023). This situation creates a breeding ground for hospital-acquired infections, including those from contaminated incubators. Understanding the prevalence and types of bacteria present in incubators in Sana'a is crucial for developing effective infection prevention and control strategies.

Each year 3.6 million infants are estimated to die in the first four (04) weeks of life (neonatal period) but the majority of the neonates continue to die at home and are uncounted. There are three major causes of neonatal death, mainly infections, complications of preterm birth, and intra - partum-related neonatal deaths (birth asphyxia) and they account for approximately more than 80% of all neonatal deaths globally (Lawn *et al*., 2010).

Infections associated with neonatal care present a real public health problem responsible for increased neonatal morbidity and mortality (Chiguer *et al*., 2019).

Healthcare-associated infection (HAI) is a serious problem in neonates who are admitted to neonatal intensive care unit (NICU), The HAI is associated with increases in mortality, morbidity, and prolonged length of hospital stay (Sadowska-Krawczenko *et al*., 2012).

A Healthcare-associated infection is defined as an infection not present or incubating at the time of admission, with onset after 48 hours of stay. Nosocomial infections are usually a result of pathogens that are resistant to the first-line of antibiotics (Urzedo *et al*., 2014).

Newborns admitted to intensive care units (ICUS) are at high risk for developing nosocomial infections (NI) because of the severity of their illness and exposure to invasive medical devices such as mechanical ventilators and central venous catheters (CVCs) and resistant microorganisms (Urzedo *et al*., 2014).

Both gram negative and gram-positive bacteria have been isolated from in animate surfaces and are able to survive up to months on dry surfaces, with longer persistence seen under humid and lower temperature conditions. many factors influence and affect rate of contamination this include type of organisms, source and destination surface, humidity level, and size of inoculum (Alphons *et al*., 2020).

Other factors that play a role include hand hygiene compliance, number of nurse-staffing levels, number of colonized or infected patients and ICU structural features. The isolation of bacteria from nursery incubation by swab is a commonly used method in microbiology for detecting and identifying microorganisms present in a specific environment (Russotto *et al*., 2015).

The purpose of isolating bacteria from nursery incubation is to assess the microbial load and diversity within the environment, this information is crucial for monitoring the cleanliness and hygiene practices in nurseries, especially those caring for infants and young children who may be more susceptible to infections, by identifying and characterizing the bacterial species present, measures can be taken to prevent the spread of harmful pathogens and maintain a safe environment for the children (Chiguer *et al*., 2019).

Staphylococcus aureus infection outbreaks of infection caused by *Staphylococcus aureus* have been described in neonatal nurseries since 1889, with large epidemics in the 1920s, 1950s, and early 1970s (Kilham, 1889, Shinefield, 1995). The primary mode of transmission in neonatal nurseries is thought to be on the hands of staff, although *S. aureus* can also be carried in the nose and/or rectum of staff (Shinefield, 1995). Overcrowding of neonatal nurseries has been shown to be a major factor in spread to babies (Haley and Bregman, 1982).

After birth, the process of physiological colonization for neonatal depends on delivery mode, first maternal contact, and the surrounding environmental (Chiguer *et al*., 2019).

The infection is referred as acquired when the patient admitted to hospital for treatment of specific condition, their exposure to new disease was not in mind, and the measure of precaution and prevention are insufficient to avoid bacteria (Auriti *et al*., 2003). The first

source of microbial infection of newborn is the parent strain of the mother flora (Baltimore *et al*., 2002).

Infants do not have complete immune system against microbe and gain infection from incubators of newborn (Baltimore *et al*., 2002).

The main pathways for acquiring newborn infection are airborne transmission; direct contact [direct physical transmission] from colonized person [health care worker] (Gatchalian *et al*., 1999). *Staphylococcus aureus*, found on the skin of health care worker as normal flora, may be transmitted to the newborn causing infections (Gatchalian *et al*., 1999).

Some microorganisms transmitted by secretion or indirect contact via physical transmission from in animate objects such as transducers, thermometer, Stethoscopes, manometer, section catheters and water or other carriers such as [contaminated fluids, intravenous solution, milk, blood transmission and derivatives] (Baltimore, 2002).

Exposure to these sources will contribute to establishing of an endogenous flora of newborn, in other word, the bacterial colonization of the skin, mucosa membrane, gastro intestinal tract and respiratory tract that, in turn are frequently the source of microorganisms that cause infection (Baltimore, 1998).

The main risk factors for infection of newborn can be divided in to intrinsic and extrinsic factors (Baltimore, 1998). The intrinsic factors include gestational age, sex, birth weight, severity of disease and immunological development (Baltimore, 1998). The extrinsic factors include hospital stay, use of invasive procedures such as arterial and venous catheters, tracheal cannulas, gastric or gastric duodenal probe peritoneal shunt and chest drains, exposure to hospital environment and staff (Baltimore, 1998).

Exposure to the hospital staff is the most important risk factors for new born infants admitted to neonatal intensive care units, have prolonged hospital stay, and frequent exposure to invasive procedure and to a great number of people responsible for the care of the infants (Alkharasani, 2014).

Neonatal sepsis remains an important cause of morbidity and mortality among infants in developing countries accounting for [30-40%] of total deaths each year (Bang *et al*., 2005).

The pathogens most often implicated in neonatal infection in developing countries are *Klebsiella, E. coli, Pseudomonas, Salmonella* and *Staphylococcus* (Alkharasani, 2014).

A neonatal intensive care unit (NICU), also known as an intensive care nursery (ICN), is an intensive care unit (ICU) specializing in the care of ill or premature newborn infants. The NICU is divided into several areas, including a critical care area for babies who require close monitoring and intervention, an intermediate care area for infants who are stable but still require specialized care, and a step-down unit where babies who are ready to leave the hospital can receive additional care before being discharged (Payne and Awareness, 2016).

The major cause of neonatal incubator contamination is the colonized bacterial environment, these organisms have developed increased drug resistance over last decades and management of neonates with sepsis has become a major problem (Anwer *et al*., 2000).

Since the spectrum of organisms that cause neonatal sepsis changes over time and varies from hospital to hospital through the same city or country (Ako-Nai *et al*., 1999).

It is necessary to conduct periodic surveillance to access the changing pattern of organisms causing neonatal infection (Payne and Awareness, 2016).

1.1.1 Problem of the Research

Contamination of neonatal incubators possess a significant risk to the vulnerable population in the neonatal intensive care unit (NICU). Bacterial contamination in incubators can lead to healthcare-associated infections, sepsis, and other serious complications in neonates.

1.1.2 Justification of the Research

This study, which investigates the “Bacterial Contamination of Neonatal Incubators in Hospitals of Sana’a City Yemen, 2024. Holds significant importance and justifiable reasons as below:

1. There are no studies on contaminants of neonatal’s incubators in Yemen.
2. There are external studies in Arab countries about neonatal’s incubators and the contamination rate in them was high.

1.1.3 Research hypothesis

Bacteria contamination of incubators occurs due to several factors, including insufficient hand washing practices by staff and visitors, poor cleaning and poor ventilation and length of stay.

1.2 Literature review

1.2.1 Definition of bacteria

Bacteria are ubiquitous, mostly free-living organisms often consisting of one biological cell. They constitute a large domain of prokaryotic microorganisms typically a few micrometers in length, bacteria were among the first life forms to appear on earth, and are present in most of its habitats. Bacteria inhabit soil, water, acidic hot springs, radioactive waste and the deep biosphere of earth's crust. Bacteria play a vital role in many stages of the nutrient cycle by recycling nutrients and the fixation of nitrogen from the atmosphere. The nutrient cycle includes the decomposition of dead bodies; bacteria are responsible for the putrefaction stage in this process. In the biological communities surrounding hydrothermal vents and cold seeps, extremophile bacteria provide the nutrients needed to sustain life by converting dissolved compounds, such as hydrogen sulphide and methane, to energy. Bacteria also live in mutualistic, commensal and parasitic relationships with plants and animals. Most bacteria have not been characterized and there are many species that cannot be grown in the laboratory. The study of bacteria is known as bacteriology, a branch of microbiology (Rodwell *et al*., 1988).

1.2.2 Classification of bacteria

Bacteria are classified and identified to distinguish one organism from another and to group similar organisms by criteria of interest to microbiologists or other scientists. The classification of bacteria serves a variety of different functions. Because of this variety, bacteria may be grouped using many different typing schemes (Ivkovikj and Radmanovikj, 2022).

1.2.2.1 Classification of bacteria based on shape:

In 1872, scientist Cohn classified bacteria into four major types based on their shapes, as follows:

A) *Cocci*: These bacteria are unicellular and have a spherical or elliptical shape. They can either exist as single cells or aggregate together in various configurations. The types of cocci bacteria include:

- *Monococcus*: Also known as *micrococcus*, represented by a single discrete round cell. Example: *Micrococcus flavus*.

- *Diplococcus*: The cells of *Diplococcus* divide once in a specific plane, and after division, the cells remain attached to each other. Example: *Diplococcus pneumoniae*.

- *Streptococcus*: In this case, the cells divide repeatedly in one plane, forming a chain of cells. Example: *Streptococcus pyogenes*.

- *Tetracoccus*: Consists of four round cells that divide in two planes at a right angle to each other. Example: Gaffkya tetragena.

- *Staphylococcus*: The cells divide in multiple planes, forming irregular configurations resembling bunches of grapes. Example: *Staphylococcus aureus*.

- *Sarcina*: In this case, the cells divide in three planes, forming a cube-like configuration consisting of eight or sixteen cells with a regular shape. Example: *Sarcina lutea*.

B) *Bacilli*: These bacteria are rod-shaped or cylindrical and can exist singly or in pairs. Example: *Bacillus cereus*.

C) *Vibrios*: *Vibrios* are curved, comma-shaped bacteria and are represented by a single genus. Example: *Vibrio cholerae*.

D) *Spirilla*: This type of bacteria is spiral or spring-like, with multiple curvatures and terminal flagella. Example: *Spirillum volutans*.

Others, *Actinomycetes* are branching filamentous bacteria, so called because of a fancied resemblance to the radiating rays of the sun when seen in tissue lesions (from actis meaning ray and mykes meaning fungus). *Mycoplasmas* are bacteria that are cell wall deficient and hence do not possess a stable morphology. They occur as round or oval bodies and as interlacing filaments (Fathy *et al*., 1998).

1.2.2.2 Classification of bacteria based on reaction to the gram stain:

Gram stain (Gram staining or Gram's method), is a method of staining used to classify bacterial species into two large groups: gram-positive bacteria and gram-negative bacteria. It may also be used to diagnose a fungal infection (Coico, 2006).

The name comes from the Danish bacteriologist Hans Christian Gram, who developed the technique in 1884 (Coico, 2006).

Gram staining differentiates bacteria by the chemical and physical properties of their cell walls. Gram-positive cells have a thick layer of peptidoglycan in the cell wall that

retains the primary stain, crystal violet. Gram-negative cells have a thinner peptidoglycan layer that allows the crystal violet to wash out on addition of ethanol. They are stained pink or red by the counter stain (Beveridge and Davies, 1983).

Lugol's iodine solution is always added after addition of crystal violet to strengthen the bonds of the stain with the cell membrane (Beveridge and Davies, 1983).

Gram staining is almost always the first step in the identification of a bacterial group. While Gram staining is a valuable diagnostic tool in both clinical and research settings, not all bacteria can be definitively classified by this technique. This gives rise to gram-variable and gram-indeterminate groups (Beveridge and Davies, 1983).

1.2.2.3 Classification of bacteria on the basis ability to form spore:

1. Spore forming bacteria:

- Those bacteria that produce spore during unfavorable condition.
- These are further divided into two group

i) Endospore forming bacteria:

- Spore produced within the bacterial cell.
- *Bacillus*, *Clostridium*, *Sproutarian* etc.

ii) Exospore forming bacteria:

- Spore produced outside the cell.
- Methyl sinus.

2. Non sporing bacteria:

- those bacteria which do not produce spore. Example: *E. coli, Salmonella* (Walumbwa *et al*., 2008).

1.2.2.4 Classification of bacteria based on oxygen requirements:

An anaerobic organism or anaerobe is any organism that does not require molecular oxygen for growth. It may react negatively or even die if free oxygen is present. In contrast, an aerobic organism (aerobe) is an organism that requires an oxygenated environment. Anaerobes may be unicellular (e.g., protozoans, bacteria) or multicellular.

Most fungi are obligate aerobes, requiring oxygen to survive. However, some species, such as the *Chytridiomycota* that reside in the rumen of cattle, are obligate anaerobes; for these species, anaerobic respiration is used because oxygen will disrupt their metabolism or kill them. Deep waters of the ocean are a common anoxic environment (Danovaro *et al*., 2010).

Aerobic and anaerobic bacteria can be differentiated by culturing them in test tubes of thioglycolate broth:

1. Obligate aerobes need oxygen because they cannot ferment or respire anaerobically. They gather at the top of the tube where the oxygen concentration is highest.

2. Obligate anaerobes are poisoned by oxygen, so they gather at the bottom of the tube where the oxygen concentration is lowest.

3. Facultative anaerobes can grow with or without oxygen because they can metabolize energy aerobically or anaerobically. They gather mostly at the top because aerobic respiration generates more adenosine triphosphate (ATP) than either fermentation or anaerobic respiration.

4. Microaerophiles need oxygen because they cannot ferment or respire anaerobically. However, they are poisoned by high concentrations of oxygen. They gather in the upper part of the test tube but not the very top.

5. Aerotolerant organisms do not require oxygen as they metabolize energy anaerobically. Unlike obligate anaerobes, however, they are not poisoned by oxygen. They can will be evenly distributed throughout the test tube (Hogg, 2013).

For practical purposes, there are three categories of anaerobe:

- Obligate anaerobes, which are harmed by the presence of oxygen (Beveridge and Davies, 1983).

- Two examples of obligate anaerobes are *Clostridium botulinum* and the bacteria which live near hydrothermal vents on the deep-sea ocean floor.

- Aerotolerant organisms, which cannot use oxygen for growth, but tolerate its presence (Hogg, 2013).

- Facultative anaerobes, which can grow without oxygen but use oxygen if it is present (Hogg, 2013).

However, this classification has been questioned after recent research showed that human "obligate anaerobes" (such as *Finegoldia magna* or the *Methanogenic archaea Methanobrevibacter smithii*) can be grown in aerobic atmosphere if the culture medium is supplemented with antioxidants such as ascorbic acid, glutathione and uric acid (Hogg, 2013).

1.2.2.5 Classification of bacteria based on Bacterial Nutrition:

A) Phototrophs:

These are bacteria that gain energy from light. Phototrophs are further divided into two groups based on the source of electrons.

1. Photolithotrophs: These bacteria gain energy from light and use reduced inorganic compounds such as H2S as electron sources. Examples include *Chromatium okenii*.

2. Photoorganotrophs: These bacteria gain energy from light and use organic compounds such as succinate as electron sources.

B) Chemotrophs:

These bacteria gain energy from chemical compounds and cannot carry out photosynthesis. Chemotrophs are further divided into two groups based on the source of electrons.

1. Chemolithotrophs: These bacteria gain energy from the oxidation of chemical compounds and use inorganic compounds such as NH3 as electron sources. An example is *Nitrosomonas*.

2. Chemoorganotrophs: These bacteria gain energy from chemical compounds and use organic compounds such as glucose and amino acids as electron sources. An example is *Pseudomonas pseudoflava*.

C) Autotrophs:

These bacteria use carbon dioxide as the sole source of carbon to prepare their own food. Autotrophs are divided into two types based on the energy utilized to assimilate carbon dioxide: photoautotrophs and chemoautotrophs.

1. Photoautotrophs: These bacteria utilize light to assimilate CO2. They are further divided into two groups based on the electron sources: photolithotrophic autotrophs and photoorganotrophic autotrophs.

2. Chemoautotrophs: These bacteria utilize chemical energy for the assimilation of CO2.

D) Heterotrophs: These bacteria use organic compounds as carbon sources and lack the ability to fix CO2. Most human pathogenic bacteria are heterotrophic in nature. Some heterotrophs have simple nutritional requirements, while others require special nutrients for their growth, known as fastidious heterotrophs (Lowy, 2009).

1.2.3 Types of bacteria common in neonates in hospitals

1.2.3.1 *Staphylococcus*

The *staphylococci* are Gram-positive spherical cells, usually arranged in grapelike irregular clusters. They grow readily on many types of media and are active metabolically, fermenting carbohydrates and producing pigments that vary from white to deep yellow. Some are members of the normal microbiota of the skin and mucous membranes of humans; others cause suppuration, abscess formation, a variety of pyogenic infections, and even fatal septicemia. The pathogenic *staphylococci* often heamolyze blood, coagulate plasma, and produce a variety of extracellular Enzymes and toxins. The most common type of food poisoning is caused by a heat-stable *staphylococcal* enterotoxin. *Staphylococci* rapidly develop resistance to many antimicrobial agents, which consequently presents difficult therapeutic problems. The genus *Staphylococcus* has at least 45 Species. The four most frequently encountered species of clinical importance are *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus lugdunensis*, and *Staphylococcus saprophyticus* (Riedel *et al*., 2019).

Staphylococci are non-motile, non-spore forming facultative anaerobes that grow by aerobic respiration or by fermentation. Members of this genus are catalase-positive and oxidase-negative, distinguishing them from the genus *Streptococci*, which are catalase-negative, and have different cell wall composition to *Staphylococci*. *Staphylococci* are tolerant to high concentrations of salt and show to heat (Foster, 2002). and show resistance to heat (Kloos, 1991).

Pathogenic *Staphylococci* are commonly identified by their ability to produce coagulase, and thus clot blood. This distinguishes the coagulase positive strains, *S.*

aureus (a human pathogen), and *S. intermedius* and *S. hyicus* (two animal Pathogens), from the other *staphylococcal* species such as *S. epidermidis*, that are coagulase-Negative (CoN*S*) (Kloos and Musselwhite, 1975).

1.2.3.1.1 Coagulase-Negative *Staphylococci* (CoN*S*)

Coagulase-negative *Staphylococci* (CoN*S*) form a large group of Gram-positive cocci united by their mutual lack of the virulence factor coagulase (Becker *et al*., 2020).

CoN*S*, as typical opportunists, represent one of the major nosocomial pathogens, having a substantial impact on human life and health. They are particularly associated with the use of indwelling or implanted foreign bodies, which are indispensable in modern medicine. Colonization of different parts of the skin and mucous membranes of the host is the key source of endogenous infections by CoN*S*. However, they are transmitted mainly by medical and/or nursing procedures. Once inserted, foreign bodies can become colonized by CoN*S* and the success of the respective medical procedure is significantly impaired, resulting in enormous medical and economic burdens (Taponen and Pyörälä, 2009).

Staphylococci other than *S. aureus* can cause infections in man. *S. epidermidis* is the most important coagulase-negative *Staphylococcus* (CoN*S*) species and is the major cause of infections associated with prosthetic devices and catheter (Rupp ME, *et al.*, 1994).

Approximately 75% of these infections caused by CoN*S* are caused by S. *Epidermidis*; *S. saprophyticus* is a relatively common cause of urinary tract infections in young women, although it rarely causes infections in hospitalized patients. The *staphylococci* produce catalase, which differentiates them from the *streptococci*. *Staphylococci* slowly ferment many carbohydrates, producing lactic acid but not gas (Riedel *et al*., 2019).

CoN*S* also cause peritonitis in patients receiving continuous ambulatory peritoneal dialysis and endocarditis in those with prosthetic valves. These infections are not usually nosocomially acquired. Other species such as *S. haemolyticus*, *S. warneri*, *S. hominis*, *S. capitis*, *S. intermedius*, *S. schleiferi* and *S. simulans* are infrequent pathogens. *S lugdunesis* is a newly recognized species (Rupp and Archer, 1994).

In daily clinical practice, CoN*S* are commonly regarded as less pathogenic than *Staphylococcus aureus* and other members of the *S. aureus* complex (Becker *et al*., 2019, Alhussein *et al*., 2020, Nurjadi *et al*., 2020).

CoN*S* are often considered as simple commensal bacteria due to their rarity in clinical pathology and the absence of virulence factors like those produced by *S. aureus*. However, the emergence of nosocomial infections with CoN*S* has led clinicians and researchers to reconsider the status of these bacteria (Becker *et al*., 2014).

1.2.3.1.2 Coagulase-Positive *Staphylococci* (CoP*S*)

The coagulase-positive *Staphylococcus aureus* is a major cause of human clinical disease (Holt *et al*., 2011).

A very early-branching *Staphylococcus aureus* lineage lacking the carotenoid pigment staphyloxanthin. *S. aureus* is stain purple by Gram stain that are Cocci-shaped and tend to be Arranged in clusters. It is found in the environment and also found in normal human flora, located on the skin and mucous membranes (Rasigade and Vandenesch, 2014) Other site of colonization include nitrogenous skin folds, perineum, axillae and the vagina. In human *S. aureus* colonization is mainly found in the anterior nares 40%. Nasal carriage of *S. aureus* is a potential source of infection and colonization often precedes infection). In general, nasal carrier rates among hospital personnel and patients 60-70% are much higher as compared to those among community carriers 30-50% (Moreira *et al*., 2013).

Different factors contribute to the transmission of this microorganism such as crowded living conditions, poor hygiene habits, close skin to skin contact, sharing of personal items, hospitalization, intravenous drug abuse and interference the health Staph in direct method with patient (Shibabaw *et al*., 2013).

S. aureus is a commensal bacterium that colonizes 30% of healthy individuals from different body parts (Oliveira *et al*., 2018).

It plays a significant role in causing infections in both hospitals and community ranging between simple to life threating infections. The organism causes infections by owing different virulent genes that encode different virulent factors such as toxins and enzymes among others. Further, the virulence of *S. aureus* has risen with existence of antibiotics resistance strains such as methicillin resistant *S. aureus* (MR*SA*) and Vancomycin resistance *S. aureus* (VR*SA*). The resistance strains increased the challenge in treating the infections caused by MR*SA*. The circulation of these strains in health care settings and community changed the epidemiology of their spread. Using preventive control measures are critical in controlling *S. aureus* infections (Turner *et al*., 2019).

1.2.3.1.2.1 Methicillin-resistant *Staphylococcus aureus*

Is a group of gram-positive bacteria that are genetically distinct from other strains of *Staphylococcus aureus.* MR*SA* is responsible for several difficult-to-treat infections in humans. It caused more than 100,000 deaths worldwide attributable to antimicrobial resistance in 2019 (Gurusamy *et al*., 2013).

1.2.3.2 *Escherichia coli* spp

Is a Gram-negative, facultative anaerobic, rod-shaped, coliform bacterium of the genus Escherichia that is commonly found in the lower intestine of warm-blooded organisms. Most *E. coli* strains are harmless, but some serotypes such as *EPEC,* and *ETEC* are pathogenic and can cause serious food poisoning in their hosts, and are occasionally responsible for food contamination incidents that prompt product recalls. Most strains are part of the normal microbiota of the gut and are harmless or even beneficial to humans (although these strains tend to be less studied than the pathogenic ones). For example, some strains of *E. coli* benefit their hosts by producing vitamin K2 or by preventing the colonization of the intestine by pathogenic bacteria. These mutually beneficial relationships between *E. coli* and humans are a type of mutualistic biological relationship- where both the humans and the *E. coli* are benefitting each other. *E. coli* is expelled into the environment within fecal matter (Russell and Jarvis, 2001).

The bacterium grows massively in fresh fecal matter under aerobic conditions for three days, but its numbers decline slowly afterwards. *E. coli* and other facultative anaerobes constitute about 0.1% of gut microbiota, and fecal-oral transmission is the major route through which pathogenic strains of the bacterium cause disease. Cells are able to survive outside the body for a limited amount of time, which makes them potential indicator organisms to test environmental samples for fecal contamination (Feng *et al*., 2002).

A growing body of research, though, has examined environmentally persistent *E. coli* which can survive for many days and grow outside a host. The bacterium can be grown and cultured for over 60 years. *E. coli* is a chemoheterotrophic whose chemically defined medium must include a source of carbon and energy. *E. coli* is the most widely studied prokaryotic model organism, and an important species in the fields of biotechnology and microbiology, where it has served as the host organism for the majority

of work with recombinant DNA. Under favorable conditions, it takes as little as 20 minutes to reproduce (Tortora, 2023).

1.2.3.3 *Klebsiella* spp

Is a genus of Gram-negative, oxidase-negative, rod-shaped bacteria with a prominent polysaccharide-based capsule (Donowitz *et al*., 1981).

Klebsiella is named after German-Swiss microbiologist Edwin Klebs. Carl Friedlander described *Klebsiella* bacillus which is why it was termed Friedlander bacillus for many years. The species of *Klebsiella* are all gram-negative and usually non-motile. They tend to be shorter and thicker when compared to others in the family *Enterobacteriaceae* (Donowitz *et al*., 1981).

Klebsiella species are found everywhere in nature. This is thought to be due to distinct sublineages developing specific niche adaptations, with associated biochemical adaptations which make them better suited to a particular environment. They can be found in water, soil, plants, insects and other animals including as part of the human and animal's normal flora in the nose, mouth and intestine (Donowitz *et al*., 1981).

1.2.3.4 *Acinetobacter* spp

Is a genus of Gram-negative bacteria belonging to the wider class of Gammaproteobacteria. *Acinetobacter* species are oxidase-negative, exhibit twitching motility and occur in pairs under magnification. They are important soil organism, where they contribute to the mineralization of, for example, aromatic compounds. *Acinetobacter* species are a key source of infection in debilitated patients in the hospital, in particular the species *Acinetobacter baumannii* (Bitrian *et al*., 2013).

Species of the genus *Acinetobacter* are strictly aerobic, no fermentative, Gram-negative bacilli. They show mostly a coccobacillary morphology on nonselective agar. Rods predominate in fluid media, especially during early growth (Bitrian *et al*., 2013).

The morphology of *Acinetobacter* species can be quite variable in Gram-stained human clinical specimens, and cannot be used to differentiate *Acinetobacter* from other common causes of infection (Bitrian *et al*., 2013).

Most strains of *Acinetobacter*, except some of the *A. lwoffii* strain, grow well on MacConkey agar (without salt). Although officially classified as not lactose-fermenting,

they are often partially lactose-fermenting when grown on MacConkey agar. They are oxidase-negative, catalase-positive, indole-negative, nonmotile and usually nitrate-negative (Bitrian *et al*., 2013).

Bacteria of the genus *Acinetobacter* are known to form intracellular inclusions of polyhydroxyalkanoates under certain environmental conditions (e.g., lack of elements such as phosphorus, nitrogen, or oxygen combined with an excessive supply of carbon sources About 150 million people develop a urinary tract infection in a given year. They are more common in women than men, but similar between anatomies while carrying indwelling catheters. In women, they are the most common form of bacterial infection. Up to 10% of women have a urinary tract infection in a given year, and half of women have at least one infection at some point in their lifetime. They occur most frequently between the ages of 16 and 35 years Recurrences are common. Urinary tract infections have been described since ancient times with the first documented description in the Ebers Papyrus dated to c. 1550 BC.

1.2.3.7 *Pseudomonas* spp

Is a common encapsulated, Gram-negative, aerobic–facultatively anaerobic, rod-shaped bacterium that can cause disease in plants and animals, including humans. A species of considerable medical importance, *P. aeruginosa* is a multidrug resistant pathogen recognized for its ubiquity, its intrinsically advanced antibiotic resistance mechanisms, and its association with serious illnesses – hospital-acquired infections such as ventilator-associated pneumonia and various sepsis syndromes. *P. aeruginosa* is able to selectively inhibit various antibiotics from penetrating its outer membrane - and has high resistance to several antibiotics, according to the World Health Organization *P. aeruginosa* poses one of the greatest threats to humans in terms of antibiotic resistance (Diggle and Whiteley, 2020).

1.2.3.8 *Enterobacter* spp

Is a genus of common Gram-negative, facultative anaerobic, rod-shaped, non-spore-forming bacteria of the family *Enterobacteriaceae*. Cultures are found in soil, water, sewage, feces and gut environments. It is the type genus of the order *Enterobacterales*. Several strains of these bacteria are pathogenic and cause opportunistic infections in immunocompromised (usually hospitalized) hosts and in those who are on mechanical ventilation. The urinary and respiratory tracts are the most

common sites of infection. The genus *Enterobacter* is a member of the coliform group of bacteria. It does not belong to the fecal coliforms (or thermotolerant coliforms) group of bacteria, unlike *Escherichia coli*, because it is incapable of growth at 44.5 °C in the presence of bile salts. Some of them show quorum sensing properties (Cabral, 2010).

1.2.4 Neonatal infections are primarily bacterial in origin, and include pneumonia, sepsis, and meningitis. Neonatal infections result in over 550 000 neonatal deaths every year. Most of these deaths can be averted by preventive measures, early diagnosis, timely care-seeking, treatment with appropriate antibiotics, and follow up. Early diagnosis requires early recognition of clinical signs, symptoms and syndromes. Possible serious bacterial infection (PSBI) is the most important clinical syndrome in low- and middle-income countries (LMICs). An estimated 6.9 million episodes of PSBI occur in in young infants aged 0-59 days in LMICs every year (Isaacs *et al*., 1995).

1.2.5 Neonatal bacterial infection

Infection naturally falls into two main categories: early onset (generally acquired from the mouth), and late onset (generally acquired from nursery environment). Congenital infection will be dealt with in a separate guideline. The line between Early and Late is usually taken as 48 hr. (Isaacs *et al*., 1995).

1.3 Aims of the study

1.3.1 General objectives

Assessment of pathogenic bacteria among nursery (neonate's incubators) at Al-Sabeen Maternity & Childhood hospital, Al-Khamseen hospital, Al-Gumhori Teaching hospital Authority and Palestine hospital in Sana'a city.

1.3.2 Specific objectives

The study was conducted to:

1. Isolate bacterial organism from nursery.
2. Identify the type of isolated organism.

Chapter two

MATERIAL

AND

METHODS

2. Material and Methods

2.1 Study period and area

The study was carried out from August 2023 to April 2024, during a period of 9 months, were collected from neonate's incubators at four hospitals (Al-Sabeen Maternity & Childhood Hospital, Al-Gumhori Teaching Hospital Authority, Palestine Hospital and Al-Khamseen Hospital) in Sana'a city.

2.2 Study design

A cross-sectional study was performed to assess contamination of neonatal incubators.

2.3 Sample size

A total of 100 recorded nursery swabs were included in this study.

2.4 Inclusion criteria

This study included the neonatal incubators in Al–Sabeen Maternity & Childhood Hospital, Al-Gumhori Teaching Hospital Authority, Al–Khamseen Hospital and Palestine Hospital.

2.5 Exclusion criteria

This study excluded all incubators that out of the service.

2.6. Sample collection and laboratory test

The samples were taken from surfaces of inside of incubator from Neonatal Intensive Care Unit (NICU), by using a sterile swab moistened with sterile saline were taken from each incubator one or two samples, each swab sample placed in a separated sterile labeled test tube and transport in transport swabs then received to Microbiology Laboratory at 21 September University within one hour and inoculated on to pre-prepared culture plates and bacteria were identified by standard microbiological methods. A Blood agar was used for the growth of a wide range of pathogenic bacteria. It is also required for the detection and characterization of hemolytic bacteria. After inoculation, the medium was incubated aerobic at 37 for 24-48h. Chocolate agar is a medium has been used to grow fastidious pathogens that grow best on this highly nutritious medium, inoculation, incubated at 37°C for 24-48h. MacConkey agar is a selective and differentiating agar that only grows Gram-negative bacterial species; can differentiate Gram-negative organisms

based on lactose metabolism, the plates were inoculated with the sample and then aerobically incubated at 37°C for 24h. Nutrient agar used for subculture inoculation, incubated at 37°C for 24h. Each gram-negative isolated subjected to biochemical test. (Clinical and Laboratory Standards Institute) Identification of bacteria: bacteria were identified by standard microbiological methods: culture traits of pure colonies, gram staining, enzyme production and biochemical test.

2.5 Statistical analysis

Data was processed and analyzed by using the statistical package for social science (SPSS,17.0) and the statistical significance was computed using Chi-square and the probability value.

2.6 Ethical consideration

The study was approved by ethics research committee of the Faculty of Laboratory Medicine, 21 September University, while permission to use the generated data of registration system was obtained from hospitals laboratory management. No personal identifiers were included in this study.

Chapter three

RESULTS

AND

DISCUSSTION

3. Results and Discussion

3.1 Result

This study was conducted from August 2023 to September 2023 and the following results were found:

3.1.1 Study population and hospital distribution:

A total of 100 recorded nursery swabs were collected from four hospitals in Sana'a city. The specimens are 40 from PLH, 30 from AJH, 21 from 70H and 9 from 50H.

Table (1): Distribution of Growth in each Hospital, Sana'a City

			Result of growth		Total
			Negative	Positive	
Hospital	Al-Gumhori	Count	15	15	30
		% within Hospital	50.00%	50.0%	100%
	Al-Khamssen	Count	9	0	9
		% within Hospital	100%	0.00%	100%
	Al-Sabeen	Count	18	3	21
		% within Hospital	85.70%	14.30%	100%
	Palestine	Count	16	24	40
		% within Hospital	40.00%	60.00%	100%
Total		Count	58	42	100
		% within Hospital	58.00%	42.00%	100%

-Al-Khamssen hospital has the highest percentage of negative cases (100%), indicating no positive cases at all.

- Palestine hospital has the highest percentage of positive cases (60%).

- Other hospitals show varying percentages of negative and positive growth, with Al-Gumhori hospital having an equal split of 50% for both negative and positive cases.

Table (2): Distribution of Hospitals Specimens in Sana'a City

Hospital	Frequency	Percent (%)
Al-Gumhori	30	30
Al-Khamseen	9	9
Al-Sabeen	21	21
Palestine	40	40
Total	100	100

A total of 100 recorded nursery swabs were collected from four hospitals in Sana'a city. The specimens are 40 from PLH, 30 from AJH, 21 from 70H and 9 from 50H.

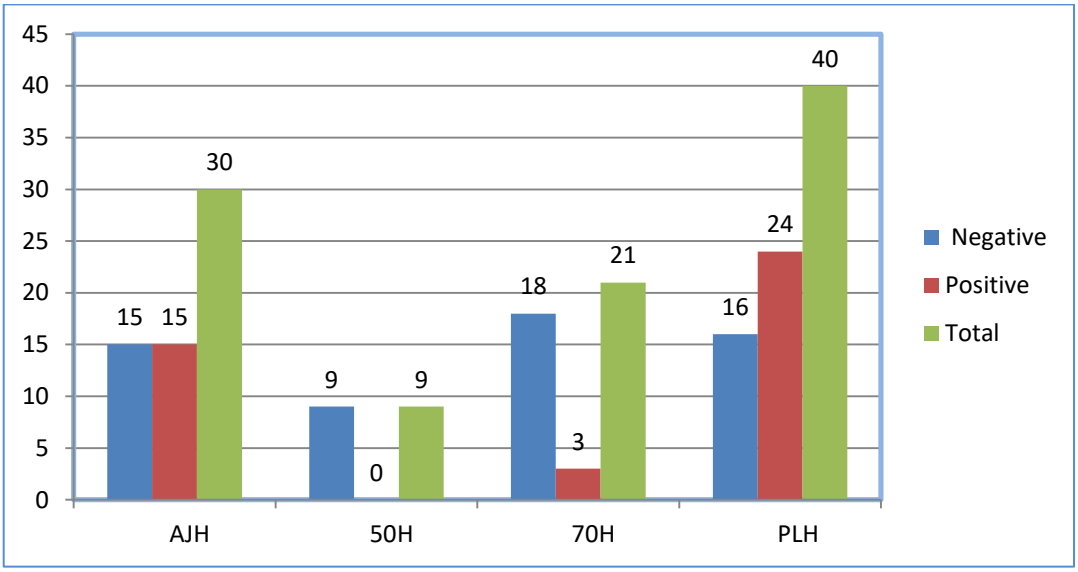

Figure (1): Distribution of Growth Rate among Hospitals in Sana'a City

The figure (1) shows that 40% of specimens were collected from PLH, while 60% from other hospital.

Table (3): Prevalence of Growth in all Hospitals, Sana'a City

Results	Frequency	Percent (%)
Non-Growth	58	58
Growth	42	42
Total	100	100

Out of 100 samples were collected and analyzed from neonatal intensive care units ,42% yielded positive bacterial growth.

Table (4): Distribution of Growth in each Hospital**,** Sana'a City

			Type of growth							Total
			Acinetobacter spp	*E. coli* spp	*Enterobacter* spp	*Klebsiella* spp	*P.aeurognosa* spp	*S.aureus*	*S.*coagulase negative	
Hospital	Al-Gumhori	Count	0	0	1	0	1	1	12	15
		% within Hospital	0%	0%	6.70%	0%	6.70%	6.70%	80%	100%
	Al-Sabeen	Count	1	0	0	0	0	0	2	3
		% within Hospital	33.30%	0%	0%	0%	0%	0%	66.70%	100%
	Palestine	Count	1	2	1	4	0	1	15	24
		% within Hospital	4.20%	8.30%	4.20%	16.70%	0%	4.20%	62.50%	100%
Total		Count	2	2	2	4	1	2	29	42
		% within Hospital	4.80%	4.80%	4.80%	9.50%	2.40%	4.80%	69%	100%

Table (5): Growth Results According to Gram-Stain in Sana'a City

Type of growth	Frequency	Percent (%)
Gram Positive	76	76.2
Gram Negative	24	23.8
Total	100	100

The data indicates that Gram-positive bacteria are more prevalent in the collected specimens than gram negative bacteria.

Table (6): Types and Frequency of Bacterial Pathogens Isolated in Sana'a City

The organism	Frequency	Percent (%)
*S.*coagulase negative	29	69
Klebsiella spp	4	9.5
S. aureus	2	4.8
E. coli spp	2	4.8
Enterobacter spp	2	4.8
Acinetobacter spp	2	4.8
Pseudomonas spp	1	2.4
Total	42	100

In this table the *S.* coagulase negative bacteria was 69% while 31% were other bacteria, Seven different pathogens were identified, CoN*S*, *Klebsiella* spp, *S. aureus*, *Enterobacter* spp, *E. coli* spp, *Acinetobacter* spp and *Pseudomonas* spp respectively. CoN*S as* presented in (Table 6) represented as majority of the bacterial pathogens (69%), while other pathogens are as follow *Klebsiella* spp (9,5%), *Enterobacter* spp (4.8%), *Acinetobacter* spp (4.8%), *E. coli* spp (4.8%), *S. aureus* (4.8%) respectively, while *psedomonas* spp represented as minority of all isolates which counted of (2.4%).

3.2 Discussion

Nosocomial infections in NICU are one of the most important causes of morbidity and mortality (Blot, 2008).

The mortality rates in NICU of resource constrained countries vary from 11.9 to 14.7%, much higher than the rates in high resource countries 6.1–7.1% (Nagata *et al*., 2002).

Microbial agents constantly inhabit the hospital environment including NICU,Bacterial contamination of NICU is one of the major factors responsible for higher incidences of nosocomial infections (Abubakar *et al*., 2014).

Higher bacterial contamination in NICU may be attributed to admission of neonates with variety of clinical conditions, overcrowded units, faecal contamination, easy access to visitors, understaffing and poor compliance to infection control practices (Gastmeier *et al*., 2007).

Most Gram-positive bacteria, such as *Staphylococcus aureus*, including methicillin resistant *Staphylococcus aureus (*MR*SA)*, survive months on dry surfaces (7 days to 7 months), Many Gram-negative species, such as *Acinetobacter* spp, *Escherichia coli* spp and *Klebsiella* spp are able to survive for up to 30 months on dry inanimate surfaces (Holma, 2015).

In this study, the swabbing method was used instead of the direct plating method to determine the diversity of bacterial species colonizing the ICU environments and HCWs' hands. The results of our study showed contamination of the inanimate environments by diverse groups of bacteria, including both Gram-positive bacteria 76.2% and Gram-negative bacteria 23.8%. This result is comparable to a study done by (Alphons *et al*., 2020).

In Namibia, were Gram positive bacteria comprised a greater percentage of the bacterial isolates 75% compared to Gram-negative bacteria 25% (Alphons *et al*., 2020).

Also in this study more Gram-positives were isolated as compared to Gram negatives and these results are not consistent with the results of a study done in Nigeria, where majority of the isolates were Gram positive organisms 52.2% as compared to the

Gram negatives 47.8%. The isolation of more Gram-positive organisms is because they are known to be members of the body flora of both asymptomatic carriers and sick persons. These organisms can be spread by the hand, expelled from the respiratory tract or transmitted by animate or inanimate objects (Maryam *et al*., 2014).

Overall the bacterial contamination rates from this study was 42%, this is similar to a study done at a tertiary hospital in Bauchi, North-eastern Nigeria in which contamination rates of 52.8% was seen in the neonatal intensive care unit (NICU) and 40.8% in the adult intensive care unit (AICU) respectively (Yusuf *et al*., 2017).

The contamination rate of 42% in this study is in line with the results of a study done in Namibia, in which a rate of 52% was reported (Alphons *et al*., 2020).

The contamination rate in this study is much lower, when compared to a study done in Morocco in which a contamination rate of 96.3% was reported (Lalami *et al*., 2016).

The high contamination rate recorded in NICU in this study and other similar studies around the globe maybe due to reasons such as high number of neonates with different clinical conditions being admitted frequently for clinical attention and evaluation. This clinical practice requires the frequent presence and attention of the mothers for breastfeeding and health care worker, thus increasing the unit occupancy density, traffic and human activities (Yusuf *et al*., 2017).

The predominant bacterial contaminants in study as presented in (Table) were coagulase-negative *Staphylococci* (CoN*S*), which accounted for 69% of all isolates, followed by *Klebsiella* spp which accounted for 9.5%, *S. aureus*, *E. coli* spp, *Enterobacter* spp and *Acinetobacter* spp each contributed 4.8%, and *Pseudomonas* spp which accounted for 2.4%. These results are in line with that of a study done in Nigeria in which the CoN*S* 19.2% were one of the predominating organisms that were isolated together with *Staphylococcus aureus* 26.8% and *Bacilli* species 33.0%, except that the study by Yusuf *et al*., (2017) did not report on *Enterobacter* spp, *Pseudomonas* spp, *E. coli* spp, *Klebsiella* spp, and *Acinetobacter* spp. The high contamination rate with CoN*S* and *S. aureus* is attributed to the fact that these pathogens are normal flora of human skin, and clothing fabrics that are continuously shed during routine activity and clothing fabrics (Yusuf *et al*., 2017).

In this study 69% of the bacterial isolated were CoN*S*, this is near to the results of a study done in an Intensive Care Unit of the University of Maiduguri Teaching Hospital in Nigeria, were CoN*S* accounted for 39.4% (15) of all the isolates (Barma *et al*., 2017).

However, result from our study is different from the study done in a teaching hospital (Abuth Zaria) in northern Nigeria, in which *S. aureus* 21.7% was the predominating isolate (Maryam *et al*. 2014).

Finally results from our study are different from that of a study done in the pediatric wards of the University of Ilorin Teaching Hospital in Nigeria, were *Staphylococcus aureus* 39.4% and *Acinetobacter baumannii* 23.8% were the two most common organisms isolated respectively.

Chapter four

CONCLUSIONS

AND

RECOMMENDATIONS

4. Conclusions and Recommendations

4.1 Conclusions

From the study results, the following can be concluded:

1. The findings of this study highlight the high level of bacterial contamination on objects and instruments, specifically neonatal incubators, within the NICUs of hospitals.
2. Out of 100 samples that were enrolled in this study, 42% had positive results approximately half of study samples and coagulase-negative staphylococci were the most commonly identified pathogens.
3. Isolation of potential pathogens like *E. coli* spp, *Klebsiella* spp and *S. aureus* that are threat to the neonates.
4. The number of bacteria that isolated from government facilities is higher than private facilities.

4.2 Recommendations

- ✓ Follow personal hygiene precautions of staff and incubators to ensure cleanliness and prevent the spread of germs inside neonatal intensive care units.
- ✓ Healthcare staff should put on gloves and wear a gown over their clothing, while taking care of neonate in intensive care unit.
- ✓ Implementation of other studies with large size of sample.
- ✓ Activating the infection control unit with periodic inspection, with focusing on the neonatal intensive care unit.
- ✓ Conduct a study on a wider scope to find out the causes of contamination in neonatal's incubators, it is also important to conduct the study with sensitivity tests.

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APPENDIXES

Appendixes

A. Study proposal

Bacterial Contamination of Neonatal Incubators in Hospitals of Sana'a city-Yemen

A Study Proposal Submitted in partial fulfillment for the Requirements of Bachelor Degree in Laboratory Medicine

Prepared by:

Abdul Jalil Ismail Angad
Abdulqadir Mohammed Al-Dhafiri
Bushra Abdu Al-Okam
Ghadah Anwar Ghallab
Hassen Ahmed Al-Amri
Hanadi Najeeb Qaaed
Khlowd Saleem Al-Masbahi
Muawiya Dhafer Al-Nomair
Reem Abdualmlek Al-Nuzili
Thoria Abdulwhab Al-Gabri
Zainab Ahmed Abdul-Moghni
Zakaria Rashad Al-Kadasi

Supervised by:

Dr. Jamil T. Abdul-Moghni

Assistant professor of medical Microbiology and Immunology
Faculty of Laboratory Medicine
21 UMAS

2024^{AD}
1445^{AH}

التلوث البكتيري لحضانات حديثي الولادة بمستشفيات مدينة صنعاء - اليمن

خطة بحث مقدمة كاستيفاء جزئي لمتطلبات الحصول على درجة البكالوريوس في الطب المخبري

إعداد/

خلود سالم المصباحي — عبد الجليل إسماعيل عنقاد
معاوية ظافر النمير — عبد القادر محمد الظفيري
ريم عبد الملك النزيلي — بشرى عبده العكام
ثريا عبد الوهاب الجبري — غادة أنور غلّاب
زينب أحمد عبد المغني — حسن أحمد العَمري
زكريا رشاد القدسي — هنادي نجيب قائد

إشراف/

د/ جميل طاهر عبد المغني

أستاذ الأحياء الدقيقة الطبية والمناعة المساعد

كلية الطب المخبري

جامعة ٢١ سبتمبر

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1. Introduction

Infections associated with neonatal care present a real public health problem responsible for increased neonatal morbidity and mortality (Maleb *et al*,2019).

Healthcare-associated infection (HAI) is a serious problem in neonates who are admitted to neonatal intensive care unit (NICU), The HAI is associated with increases in mortality, morbidity, and prolonged length of hospital stay (Krawczenko *et al*. 2012).

A Health care-associated infection is defined as an infection not present or incubating at the time of admission, with onset after48 hours of stay. nosocomial infections are usually a result of pathogens that are resistant to the first-line of antibiotics (Urzedo *et al*.2014).

Newborns admitted to intensive care units (ICUS) are at high risk for developing nosocomial infections because of the severity of their illness and exposure to invasive medical devices such as mechanical ventilators and central venous catheters (CVCs) and resistant microorganisms (Urzedo *et al*.2014).

According to dramowski *et al*. (2017) hospitalized neonates pre surceases considered a vulnerable population due to their immature immune systems and frequent infectious disease exposures through contact with healthcare staff, parents, other patients, equipment, and the hospital environment (Dramwski *et al*.2017).

Both Gram negative and gram-positive bacteria have been isolated from in animate surfaces and are able to survive up to months on dry surfaces, with longer persistence seen under humid and low temperature conditions many factors influence and affect rate of contamination this include type of organism's source and destination surface, humidity level, and size of inoculum (Kakuru *et al* ,2020).

Other factors that play a role include hand hygiene compliance number of nurse-staffing levels number of colonized or Infected patients and ICU structural features (Russoto *et al*.2015).

The isolation of bacteria from nursery incubation by swab is commonly used method in microbiology for detecting and identifying microorganisms present in a specific environment (Smith, J.*et al*.2018).

The purpose of isolating bacteria from nursery incubation is to assess the microbial load and diversity within the environment, this information is crucial for monitoring the cleanliness and hygiene practices in nurseries, especially those caring for infants and young children who may be more susceptible to infections, by identifying and characterizing the bacterial species present, measures can be taken to prevent the spread of harmful pathogens and maintain a safe environment for the children by Okokn ,Sosundi, JDibal.T.(2012)

Staphylococcus aureus infection Outbreaks of infection caused by *Staphylococcus aureus* have been described in neonatal nurseries since1889, with large epidemics in the 1920s, 1950s, and early 1970s (Kilham, 1989 & Shinefield, 1995).

The primary mode of transmission in neonatal nurseries is thought to be on the hands of staff although *S aureus* can also be carried in the nose and/or rectum of staff (Shinefield, 1995). Overcrowding of neonatal nurseries has been shown obeah major factor in spread to babies (Haley,1982).

E. coli, a member of the bacterial family of *Enterobacteriaceae*, is the most prevalent commensal inhabitant of the gastrointestinal tracts of humans and warm-blooded animals as well as one of the most important pathogens (Kaper *et al* ,2004).

As a commensal it lives in a mutually beneficial association with hosts, and rarely causes disease. It is however, also one of the most common human and animal pathogens as it is responsible for abroad spectrum of disease (Environ,2013).

After birth the process of physiological colonization for Neonatal depends on delivery mode, first maternal contact, and the surrounding environmental [Maqsoodetal, 2019; Shaoetal, 2019; Dominguez-Bello, 2010].

A low birth weight or need care for neonatal health tissues [Website]. In the respect the aim of the past studies was to evaluated health care the disinfectant in NICU, so there is no previous study about this topic in Yemen.

2. Aims of the study

2.1 General objectives

Assessment of pathogenic bacteria among nursery (neonate's incubators) at Al-Sabeen Maternity and Childhood hospital, Al-Khamseen hospital, Al-Gumhori Teaching hospital Authority and Palestine hospital in Sana'a city.

2.2 Specific objectives

The study will be conducted to:

1. Isolate the bacterial organisms from nursery.

2. Identify the type of isolated organisms.

3. Material and Methods

3.1 Study Area

In Al-Sabeen Maternity & Childhood hospital, Al-Khamseen hospital, Al-Gumhori Teaching Hospital Authority and Palestine hospital incubators.

3.2 Study Design

A cross-sectional study will be performed for incubational neonatal.

3.3 Sample Size

One hundred incubators area enrolled in the study samples.

3.4 Sample collection and laboratory tests

Sample collection will be include surfaces such as the inside of nurseries, under aseptic techniques from each nursery will be take one or two samples, each swabs sample will be place in a separate sterile labeled test tube and transport to the laboratory within one hour swabs will be streak on to blood, mannitol salt and MacConkey agar then incubate at 37°C for 24 – 48 hours. Bacterial isolates from culture –positive plates will be identify by gram staining, and biochemical characteristics. All the isolates will be test for their antibiotic susceptibility according to standard methods, all specimens will proceed according to the procedures of clinical microbiology laboratory standard operating procedures.

4. Milestone

Plan	Start date	End date
Title	13August 2023	11September 2023
Research proposal writing	10 November 2023	29 November 2023
Specimen collection	26 December 2023	7 February 2024
Laboratory test	26 December 2023	9 February 2024
Data analysis	19 April 2024	21 April 2024
Final report	30 May 2024	
Presentation	1 June 2024	

5. Budget

Items	Quantity	Cost RY
Blood agar	250mg	12500
Chocolate agar	250mg	12500
MacConckey agar	250 mg	12500
Gloves	3packets	8800
Mask	1packet	400
Distal water	5 liters	700
Petri dish one room	60units	3300
Petri dish three room	100units	5500
Normal Saline	1500ml	1800
Gram stain	Kit4*100 ml	10000

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Arabic Summary

الملخص العربي

أهداف الدراسة: الهدف الرئيسي لهذه الدراسة هو تقييم التلوث البكتيري لأسطح حاضنات الأطفال حديثي الولادة في مستشفى السبعين للأمومة والطفولة، ومستشفى الخمسين، ومستشفى الجمهوري، ومستشفى فلسطين بمدينة صنعاء.

المواد و الطريقة: أُجريت هذه الدراسة المقطعية بمدينة صنعاء من تاريخ ٢٦ ديسمبر لعام ٢٠٢٣ م وحتى تاريخ ٧ فبراير لعام ٢٠٢٤ م ، لتقييم التلوث البكتيري لأسطح حاضنات الأطفال حديثي الولادة بين مائة حاضنة مسجلة في عينات الدراسة من المستشفيات المذكورة سابقًا حيث تم أخذ عينات المسحات و وضعها في أنبوب اختبار معقم منفصل ونقلها إلى مختبر الجامعة خلال ساعة واحدة و زراعتها بعد ذلك على أطباق الزراعة المجهزة مسبقًا من ثم تحديد نوع البكتيريا بواسطة طرق مكروبيولوجية قياسية مثل سمات زرع المستعمرات النقية وصبغة القرام وإنتاج الإنزيمات والاختبارات الكيميائية الحيوية.

النتائج : من إجمالي ١٠٠ عينة تم جمعها ، أظهر ٤٢% منها نموًا بكتيريًا إيجابيًا ، بينما نسبة ٥٨% لم تظهر أي نمو ، حتى بعد ٢٤ ساعة من عملية التحضين ، ومن تلك التي أظهرت نموًا بكتيريًا إيجابيًا ، كان ٧٦.٢% من البكتيريا موجبة لصبغة القرام و٢٣.٨% كانت سالبة لصبغة القرام و من خلال ذلك تم تحديد سبعة مسببات مرضية مختلفة، وهي: المتكورات العنقودية السالبة لإنزيم التخثر، و سلالات الكليبسيلا، والمتكورات العنقودية الذهبية، ومجموعة البكتيريا المعوية، و المكورة الراكدة، ومجموعة الإشريشية القولونية، و السلالات الزائفة على التوالي حيث شكلت المتكورات العنقودية السالبة لإنزيم التخثر الغالبية من مسببات الأمراض البكتيرية المعزولة بنسبة ٦٩%، تلتها سلالات الكليبسيلا بنسبة ٩.٥%، والمتكورات العنقودية الذهبية بنسبة ٤.٨%، ومجموعة البكتيريا المعوية بنسبة ٤.٨%، والمكورة الراكدة بنسبة ٤.٨%، ومجموعة الإشريشية القولونية بنسبة ٤.٨%، و أخيرا السلالات الزائفة بنسبة ٢.٤%.

الاستنتاجات: يمكن الاستنتاج من هذه الدراسة أن التلوث البكتيري للأجسام والأدوات في وحدة العناية المركزة للأطفال حديثي الولادة كان مرتفعًا، مما يشير إلى أنها يمكن أن تكون وسائل لنقل المرض، خاصةً في البلدان التي نظام الرعاية الصحية فيها هش.

الكلمات المفتاحية: التلوث البكتيري، وحدة العناية المركزة للأطفال حديثي الولادة، حاضنات الأطفال حديثي الولادة، المتكورات العنقودية السالبة لإنزيم التخثر، مدينة صنعاء.

الجمهورية اليمنية
جامعة ٢١ سبتمبر للعلوم الطبية والتطبيقية
كلية الطب المخبري
قسم الأحياء الدقيقة الطبية

التلوث البكتيري لحضانات حديثي الولادة بمستشفيات مدينة صنعاء – اليمن

بحث تخرج مقدم إلى كلية الطب المخبري كاستيفاء جزئي لمتطلبـات الحصول على درجة البكالوريوس في الطب المخبري

إعداد/

خلـود سـالم المصـباحي
معـاويـة ظـافـر الـنمـير
ريـم عبـد المـلك النزيلـي
ثريا عبـد الوهاب الجبـري
زيـنب أحمـد عبـد المغنـي
زكـريا رشـاد القدسـي

عبـد الجليل إسماعيل عنقاد
عبـد القادر محمد الظفيري
بشـرى عبـده العكـام
غـادة أنـور غـلّاب
حسـن أحمـد العَمـري
هنـادي نجـيب قـائد

إشراف/

د/ جميل طاهر عبد المغني

أستاذ الأحياء الدقيقة الطبية والمناعة المساعد

كلية الطب المخبري

جامعة ٢١ سبتمبر

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