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By

BENKEDADRA NASSIMA

&

BELABIOD SOUMIA

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Study of the intestinal microbiota in children suffering from respiratory pathologies

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Members of jury:

President	BENGUENEB Asma	MAB	Mostaganem University
Supervisor	BOUABSA Foufa	MAB	Mostaganem University
Reviewer	BENSLIMANE Sabria	MAB	Mostaganem University
Co-supervisor	BEKKAR Nour Elhouda	MAB	Mascara University

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Dedications:

We dedicate our modest work

To our families, whose unwavering love and support have been the guiding light throughout this journey. Your encouragement, understanding, and sacrifices have made it possible for us to pursue our dreams. This thesis is dedicated to you, with deepest gratitude and love.

Thank you all

Abstract

The Gut microbiota is a collection of diverse microorganisms that inhabit the gastrointestinal tract. They play a major role in metabolic functions, immune modulation, and inflammatory response. Multiple factors may affect the composition and diversity of the microbiota, including diet, environmental factors and drug consumption. Recent research indicates a close link between the gut microbiota and respiratory health, described as the "gut-lung axis." This study looks at the gut microbiota makeup of children who have respiratory conditions

As part of this study, 15 samples were taken, 11 from respiratory-diseased children (Group A) and 4 from healthy neonates (Group B). Each sampling was followed by a survey surrounding the patient's state. Enumeration and identification of intestinal microbiota was done by conventional methods and API systems. 16 identified strains were tested for their Antibiotic resistance.

Results from the survey and the cultures were analyzed and displayed in graphs. The results of Group A identification resulted in a high abundance of Gram + Cocci with *Enterococcus* as the most prominent, and Gram - that related to nosocomial infections. Group B identification resulted in a high abundance of Gram - Bacilli, with *Enterobacteriaceae* as the most prominent. The strains tested for antibiotic susceptibility revealed high resistance to Ampicillin (AMP) across all strains. While showing high susceptibility to Imipenem, (IPM) Gentamicin (CN), and Nalidixic Acid (NA), especially within the *Enterobacteriaceae* family. In addition to AMP resistance, other strains from *Enterobacteriaceae* revealed resistance towards CN, Nitroxoline (NTX) and Cefazolin (CZ).

The results of these experiments made us conclude that there is a huge difference between the gut microbiota of healthy children and the ones suffering from respiratory pathologies, as they are both affected by different factors that contribute to the formation of their gut microbiomes. These factors can contribute to either gut microbiota homeostasis or dysbiosis.

Keywords: Gut microbiota, children, respiratory pathologies, antibiotic susceptibility, dysbiosis.

Résumé

Le microbiote intestinal est une collection de micro-organismes diversifiés qui habitent le tractus gastro-intestinal, jouant un rôle majeur dans les fonctions métaboliques, la modulation immunitaire et la réponse inflammatoire. De multiples facteurs peuvent affecter la composition et la diversité du microbiote, notamment l'alimentation, les facteurs environnementaux et la consommation de médicaments. Des recherches récentes indiquent un lien étroit entre le microbiote intestinal et la santé respiratoire, décrit comme l'axe "intestin-poumon". Cette étude examine la composition du microbiote intestinal chez les enfants souffrant de maladies respiratoires.

Dans le cadre de cette étude, 15 échantillons ont été prélevés, dont 11 chez des enfants atteints de maladies respiratoires (Groupe A) et 4 chez des nouveau-nés en bonne santé (Groupe B). Chaque prélèvement a été suivi d'une enquête sur l'état du patient. L'identification des souches a été réalisée par des méthodes conventionnelles et un kit API. Seize souches identifiées ont été testées pour leur résistance aux antibiotiques.

Les résultats de l'enquête et des cultures ont été analysés et présentés sous forme de graphiques. Les résultats de l'identification du Groupe A ont révélé une abondance élevée de cocci Gram positifs avec *Enterococcus* comme le plus proéminent, et des Gram négatifs liés aux infections nosocomiales. L'identification du Groupe B a montré une abondance élevée de bacilles Gram négatifs, avec *Enterobacteriaceae* comme le plus proéminent. Les souches testées pour la susceptibilité aux antibiotiques ont révélé une forte résistance à l'Ampicilline (AMP) pour toutes les souches, tout en montrant une forte susceptibilité à l'Imipénème (IPM), Gentamicine (CN) et l'Acide Nalidixique (NA), en particulier au sein de la famille des Enterobacteriaceae en raison de différents mécanismes de résistance intrinsèques et acquis. En plus de la résistance à l'AMP, d'autres souches *d'Enterobacteriaceae* ont révélé une résistance à la CN, la Nitroxoline (NTX) et la Céfazoline (CZ).

Les résultats de ces expériences nous ont permis de conclure qu'il existe une grande différence entre le microbiote intestinal des enfants en bonne santé et de ceux souffrant de pathologies respiratoires, car ils sont tous deux affectés par différents facteurs contribuant à la formation de leurs microbiomes intestinaux. Ces facteurs peuvent soit contribuer à l'homéostasie du microbiote intestinal, soit à sa dysbiose.

Mots-clés : Microbiote intestinal, enfants, pathologies respiratoires, susceptibilité aux antibiotiques, dysbiose.

ملخص

الميكروبات المعوية هي مجموعة متنوعة من الكائنات الحية الدقيقة التي تعيش في الجهاز الهضمي، وتلعب دورًا رئيسيًا في الوظائف الأيضية، تعديل المناعة، والاستجابة الالتهابية. يمكن أن تؤثر عدة عوامل على تكوين وتنوع الميكروبات، بما في ذلك النظام الغذائي والعوامل البيئية واستهلاك الأدوية. تشير الأبحاث الحديثة إلى وجود علاقة وثيقة بين الميكروبات المعوية وصحة الجهاز التنفسي، تُعرف بمحور "الأمعاء-الرئة". تتناول هذه الدراسة تكوين الميكروبات المعوية للأطفال الذين يعانون من حالات تنفسية.

كجزء من هذه الدراسة، تم أخذ 15 عينة، 11 من أطفال يعانون من أمراض تنفسية (المجموعة A) و 4 من أطفال حديثي الولادة الأصحاء (المجموعة B). تبع كل أخذ عينة استبيان حول حالة المريض. تم تحديد السلالات بالطرق التقليدية ومجموعة اختبار API. تم اختبار 16 سلالة محددة لمقاومة المضادات الحيوية. تم تحليل نتائج الاستبيان و الاوساط الغذائية وعرضها في رسومات بيانية. أسفرت نتائج التعرف على المجموعة A عن وفرة عالية من الكريات إيجابية الجرام مع بروز بكتيريا الأمعاء *Enterococcus* ، وسلبية الغرام المتعلقة بالعدوى المكتسبة في المستشفيات.

نتائج تحديد المجموعة B أظهرت وفرة عالية من العصيات سلبية الغرام، مع بروز بكتيريا الأمعاء. أظهرت السلالات التي تم اختبارها لمقاومة المضادات الحيوية مقاومة عالية للأمبيسيلين (AMP) عبر جميع السلالات، بينما أظهرت حساسية عالية للإيميبينيم (IPM)، الجنتاميسين (CN)، وحمض الناليديكسيك (NA)، خاصة داخل عائلة البكتيريا المعوية بسبب آليات المقاومة المتنوعة الداخلية والمكتسبة. بالإضافة إلى مقاومة AMP، أظهرت سلالات أخرى من المعويات مقاومة تجاه CN، النتروكسولين (NTX) والسيفازولين (CZ).

نتائج هذه التجارب قادتنا إلى استنتاج وجود اختلاف كبير بين ميكروبات الأمعاء للأطفال الأصحاء والأطفال الذين يعانون من أمراض تنفسية، حيث يتأثر كل منهما بعوامل مختلفة تساهم في تكوين ميكروبيومات الأمعاء الخاصة بهم. يمكن أن تساهم هذه العوامل في توازن الميكروبات المعوية أو اختلالها.

الكلمات المفتاحية: الميكروبات المعوية، الأطفال، الأمراض التنفسية، مقاومة المضادات الحيوية، خلل التوازن.

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List of Abbreviation

Abbreviation	Definition
AAT	α1 antitrypsin
ADV	Adenovirus
AMBP	Antimicrobial Peptides
AMP	Ampicillin
ANTB	Antibiotic
ATBP	Antibiotic Prescription
CAP	Community-Acquired Pneumonia
CDC	Center for Disease Control and Preventic
CF	Cystic Fibrosis
COV	Coronavirus
COPD	Chronic Obstructive Pulmonary Disease
COL	Colistin
CN	Gentamicin
CNS	Central Nervous System
CZ	Cefazolin
E	Erythromycin
ENS	Enteric Nervous System
ENTV	Enterovirus
FMT	Faecal Microbiota Transplantation
GALT	Gastro-Associated-Lymphatic Tissue
GIT	Gastro-Intestinal Tract
GM	Gut Microbiota
HAP	Hospital-Acquired Pneumonia
Hib	Haemophilus influenzae type b
HMOs	Human Milk Oligosaccharides
IBD	Intestinal Bowel Disorders
IEC	Intestinal Epithelial Cells
INF	Influenza Virus
IPM	Imipenem

List of Abbreviation

Abbreviation	Definition
LD	Lung Diseases
LRS	Lower Tract Respiratory System
LRTI	Lower Tract Respiratory Infections
LMICs	Low-and Middle-Income Countries
MDR-TB	Multidrug resistant Tuberculosis
NA	Nalidixic Acid
NCDs	Non-communicable diseases
NTX	Nitroxoline
NP	Nasopharyngeal
PAMPs	Pathogen Recognition Molecular Pattern
PIV	Parainfluenza Virus
PMNs	Polymorphonuclear Cells
PRRs	Pattern Recognition Receptors
PTB	Pulmonary Tuberculosis
RD	Respiratory Diseases
RHV	Rhinoviruses
RI	Respiratory Infections
RSV	Syncytial Virus
SCC	Stool Consistency Qualification
SCFA	Short Chain Fatty Acids
SHE	Second Hand Exposure
TB	Tuberculosis
TE	Tetracycline
TLR	Toll-Like Receptors
TS	Tobacco Smoking
TST	Tuberculin Skin Test
UFC	Unity Forming Colony
URS	Upper Tract Respiratory System
URTI	Upper Tract Respiratory Infections

List of Abbreviation

Abbreviation	Definition
URSM	Upper Respiratory System Microbiome
US	United States
VAP	Ventilator-Associated Pneumonia
VPH	Viral Pharyngitis
WHO	World Health Organization

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Introduction

Introduction

The human body is an ecosystem that hosts diverse microorganisms, in what is called the microbiota. It influences a variety of physiological processes such as digestion, metabolic regulation, immunological function, and overall health. The intestinal microbiota in children matures and shapes their health trajectory into puberty. These complex connections between the intestinal microbiota and the host highlight the need for a well-balanced microbial community for good health.

Recent studies presented a strong link between the gut microbiota and multiple organs, including the brain in the brain-gut axis and the lung in the gut-lung axis. In the lung, it highlights a connection between the gut microbiota and respiratory disease development.

Respiratory infections like Bronchitis, Pneumonia or chronic conditions like asthma or chronic obstructive pulmonary disorder (COPD) are common in pediatric cases, leading to high morbidity and healthcare costs. These illnesses are connected to changes in the gut microbiota, the loss in diversity, decrease in beneficial bacteria or increase in pathogenic bacteria can lead to dysbiosis and therefore lung disease.

Understanding these relations entails investigating how dysbiosis can influence immune and inflammatory responses in the respiratory tract. Environmental factors, Diet, Antibiotic use, interspecies and interkingdom links, and birth factors can lead to the potential exacerbation of respiratory diseases. Vice-versa, respiratory diseases affect the gut microbiota.

This study aims to explore the composition of the gut microbiota in children with respiratory diseases and pathologies, their sensitivity to antibiotic treatment, and how the alteration of the microbiota interacts with health outcomes. By understanding the links between gut microbiota and respiratory illnesses, this study hopes to contribute to the development of novel therapeutic techniques that use gut microbiota to promote respiratory health in infants.

In order to achieve this main objective, we examined the composition of intestinal microbiota in healthy and diseased children.

1) Realizing a survey to gather necessary information about respiratory health status of the 15 volunteers.
2) Enumeration and characterisation of intestinal microbiota in diseased and healthy children aged between 1 day and 4 years old.
3) Investigate if there is a difference between microbiota of healthy children and those suffering from respiratory pathologies by conducting a statistical analysis.
4) Testing strains susceptibility for antibiotics.

This work is structured into two main sections. The first section addresses the literature review of our study, divided into two chapters: chapter one introduces the intestinal microbiota, chapter two focuses on respiratory diseases. The second section; experimental section outlines the materials and methodology used in this study, culminating in a result, discussion and conclusion.

The study faced difficulties in regard to the thigh time schedule, space, and the lack of materials.

Chapter One

The intestinal microbiome

Chapter one: The intestinal microbiome

1. The intestinal microbiome

The intestinal microbiome and the human body share a complex relationship that affects aspects of health, from metabolic processes to immune system development. In this chapter, we delve into the microbial landscape and the factors that shape its evolution, to shed light on the interplay between the gut microbiota (GM) and disease development in children.

1.1. Overview on the gut microbiota

The gastrointestinal tract (GIT) harbours a diverse array of microorganisms, with an estimated 10^{14} unity forming colony (UFC), encompassing fungi, archaea, viruses, protozoans, and bacteria (Fig.1). The GM varies according to cell types, with different microhabitats found on the mucus layer, intestinal lumen, and epithelial cells, this diversity and interspecies balance are governed by multiple factors, to create eubiosis. The deregulation in balance between pathogens and commensals, also known as dysbiosis, affects various processes and eventually makes the host vulnerable to a plethora of diseases (Pathak, 2021).

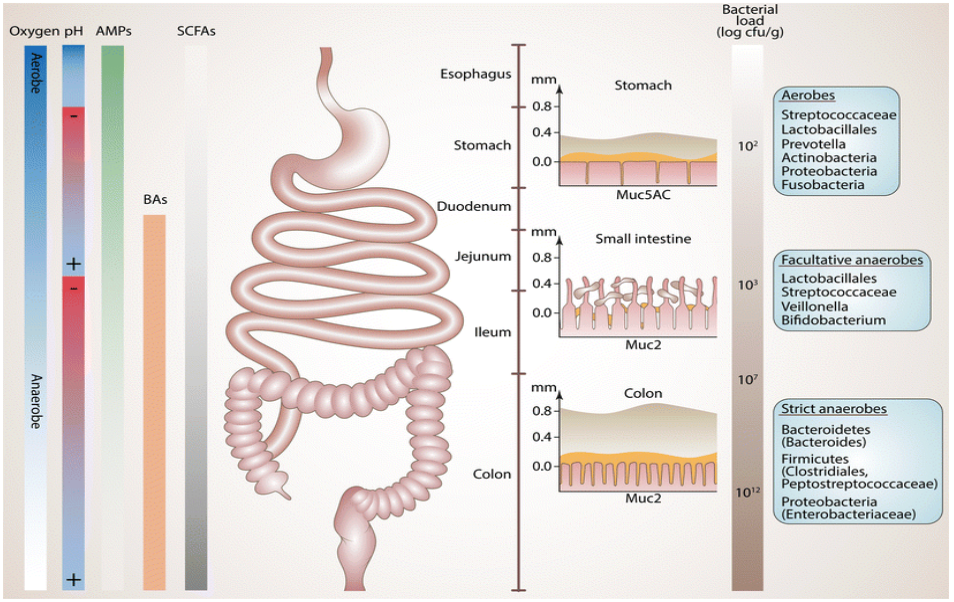


Figure 1: The gut microbiota composition and bacterial load in different segments, and the factors affecting it: Oxygen, PH, Antimicrobial Peptides (AMBP), BileAcids (BA), Short Chain Fatty acids (SCFA), mucin produced (MUC) (Gorkiewicz *et al*., 2017).

Environmental conditions influence the colonization of the GIT in each segment. In the intestines, the small intestine presents the lowest populations, with 10^3 viable bacteria in the duodenum, mostly being acid-tolerant due to the incoming stomach content. The jejunum hosts a similar microbiota. While the reduced mobility and neutral pH in the ileum help elevate the diversity and numbers of microbiota, mostly aerobes and facultative anaerobes. The colon houses a vibrant and diverse microbial ecosystem, with 300-1000 bacterial species, mostly cultivable obligate anaerobes and facultative anaerobes (Nimmy, 2023).

The constant changes from birth, infancy to adulthood, cause a shift to a more complex and stable flora in ecological succession, creating a GM that is unique to each individual (Nimmy, 2023).

1.2. Colonization and development of gut microbiota in children

The first microbial colonization is a subject of debate in the scientific community; certain scientists believe that the first colonization is in the uterus, in what is known as the in-utero colonization hypothesis. Others disregard the proof due to evidence of contamination during clinical procedures or low biomass in utero (Kennedy *et al*., 2023).

Infants' predominant microbiota rapidly changes within the first 3 years, following birth. While the GM reaches an adult-like composition by one year of age, it may take 2.5 to 3 years to form a stable adult-like community (Lv *et al.*, 2022).

Actinobacteria and *Bifidobacteriales* were the predominant species in the one-year-old infant's gut, whereas the *Firmicutes* phylum, which includes *Lactobacillales* and *Clostridiales*, dominated the adult gut. Following weaning at the age of three years, the microbiota undergoes another transition. Throughout adulthood, the GM that was formed in infancy remains intact (Akagawa and Kaneko, 2022). Furthermore, its colonization and development depend on neonatal and postnatal factors. Such as the mode of delivery, dietary habits, and environmental factors (Pola *et al*., 2021).

1.2.1. Delivery mode

The delivery can be either vaginal or caesarean. Vaginal delivery shows high resemblance in the faecal microbiota of the newborn and mother, with an abundance of *Lactobacilli* and the colonization of early facultative anaerobes (*Staphylococcus*,

Streptococcus, *Enterococcus*, and *Enterobacter*) and obligate anaerobes (*Bifidobacterium*, *Bacteroides*, and *Clostridium*) (Nimmy *et al.*, 2023). On the other hand, C-section is associated with lower bacterial diversity, a community reflecting that of the hospital settings and the skin (Fig.2), as well as an increase in *C. difficile,* and only after the use of therapeutic strategies such as synbiotics or vaginal seeding can there be improvements (Tonon *et al*., 2021).

1.2.2. Dietary Habits

The first source of nutrients for a neonate is either breastfeeding, the consumption of commercial milk, or mixed feeding. The difference in each composition affects the organization of colonizing microbiota. Biological components can be found in breast milk, namely Human Milk Oligosaccharides (HMOs), which promotes the colonization of *Lactobacillus*, *Staphylococcus*, *Streptococcus*, and *Bifidobacterium* genres (Nimmy *et al*., 2023).

Formula milk, on the other hand, is composed of different oligosaccharides to help replicate the effect of HMOs on GM, in promoting *Bifidobacterium*. The introduced microbiota is equipped to metabolize plant-derived products, such as starch, as well as lactate. Few studies on the introduction of solid foods (weaning) show a rise in metabolic pathways related to vitamin biosynthesis, and an abundance of starch-degrading *Bacteroids* (Muriel *et al.*, 2019) but, a decrease in the proportion of *Actinobacteria* (Fig.2) (Akagawa and Kaneko, 2022).

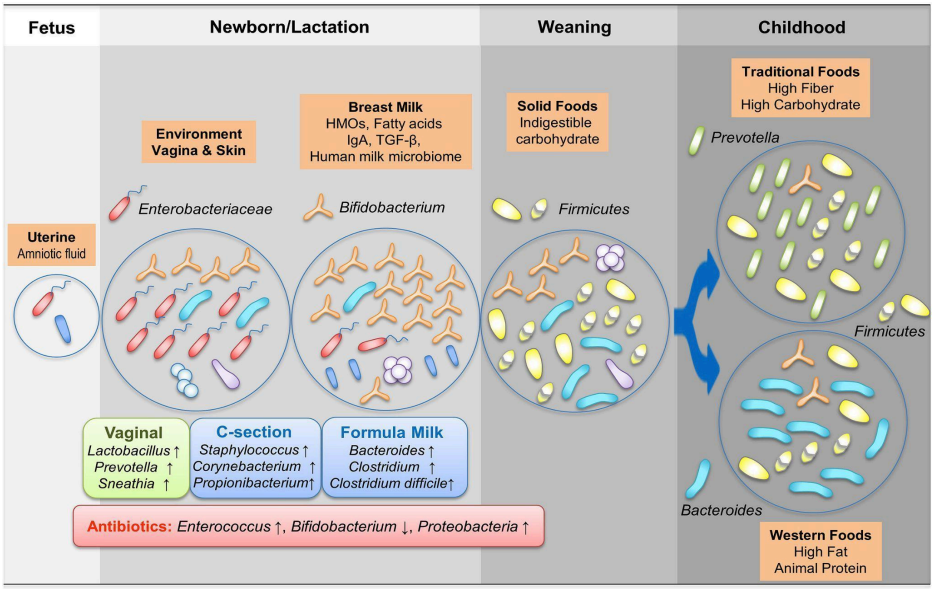


Figure 2: Factors affecting gut microbiota colonization from fetal to childhood status (Tanaka and Nakayama, 2017).

1.2.3. Antibiotic Consumption

Numerous researchers have examined the potential impact of antibiotic (ANTB) usage on the GM. There are no consistent findings regarding the properties of the GM since the effect varies depending on the type of study subject, ANTB type, duration of administration, and route of administration. ANTB use, however, has been linked to a decrease in the diversity of the GM (Akagawa and Kaneko, 2022).

With a focus on children under three who were diagnosed with and treated for upper urinary tract infections, a second study was carried out to investigate the effects of ANTB(s) , ceftriaxone and cefditoren peroxyl, on GM. Findings revealed a significant reduction in microbial diversity, *Lactobacillales* made up roughly 80% of the total abundance after ANTB usage. Nevertheless, variety started to increase 1-2 months after cessation, and by 6 months, it had reached pre-treatment levels once more. Complete recovery though, might not always be possible (Akagawa and Kaneko, 2022).

In 2020, Elvers et al, analyzed data on the effects of ANTB(s) prescribed for respiratory tract infections, to understand the changes in the GM's diversity and composition. The data are summarized in the table below (Tab.1):

Table 1: The antibiotics used in cases of lower and upper respiratory infections and their effects on the gut microbiota (Elvers *et al.,* 2020).

Antibiotic	Case	Effects
Amoxicillin	Lower tract respiratory infection	Significant increase in Anaerobe *Bacteroid*, *enterobacteria*, anaerobic Gram-positive *Lactobacilli*, *Bifidobacteria* and *Eubacteria*. No change in the abundance of *Enterococcus spp*, *Staphylococcus spp* or *Streptococcus spp*. Four patients were colonised by *P.aeruginosa*.
	18-month old patients with Bronchitis	No change to *Bifidobacteria* numbers but a decrease in species diversity, a decrease in *B. bifidum* and a Complete disappearance of *B.adolescentis*.
	Respiratory tract infection	Decrease in *Bacteroides spp*, *Bifidobacterium spp* and *Lactobacillus spp*. Recovery after 30 days of cessation.
Amoxicillin + Clavulanic acid	Acute sinusitis	ATB-associated Diarrhoea, No butyrate-producing *Clostridium,* but after 2 weeks of secassion they were detected once more.

In another data analysis by Lunchen et al., (2023), several studies on children under 2 in low to middle-income countries uncovered insights into the presence of antimicrobial resistance genes in the gut, post-antibiotic usage. The results for Sulfonamide (Cotrimoxazole), Macrolide (Azitromycin), and Beta-Lactam (Amoxicillin) all displayed an increase in gene expression of the resistance gene. However, the use of Azithromycin demonstrated a consistent alteration in the microbiota's composition with immediate reduction after use (Lunchen *et al.,* 2023).

The resistance gene in the human body poses a great risk. A study on an ICU patient, treated for urinary tract infection using Meropenem (ANTB) with genetic tests performed on the gut and lung microbiota. Results showed the apparition of Meropenem-resistant *P. aeruginosa* in the gut, which later migrated to the lung with the resistance gene, elevating the risk of Pneumonia. This was the first recorded incident of gut-to-lung translocation and an important indicator of the risk of ATB use on gut health (Wheatley *et al*., 2022)

1.3. Gut Mycobiome

Albeit most of the research regarding the development of GM has been mainly focused on the bacterial community, fungi play a vital role within the commensal flora, despite their lower abundance. Recent studies to understand gut mycobiome development showed that infants delivered vaginally exhibited a prevalence of *Trichosporon* and

Saccharomyces, whereas those born via caesarean mainly carried *Saccharomyces*. Notably, the *Candida* genre predominated at birth, and then transitioned to *Malassezia* and other genres by six months of age (Turunen *et al*., 2023).

The gut flora presents bidirectional interactions between the microbial and fungi communities to maintain host homeostasis. Fungi such as *Saccharomyces* and *Candida* species were found to inhibit the growth of certain intestinal pathogens as well as inactivate toxins from *E. coli* and *C. difficile*. In cases of microbial dysbiosis, fungi commensals prevent mucosal tissue damage and uphold immune modulation. While the microbial community modulates fungi through the production of SCFA (Enaud *et al.,* 2020).

1.3. The role of gut microbiota

The GIT and the GM engage in bilateral interactions. The microbiota helps with metabolism, nutrition regulation, and the development and maturation of the immune system. While the GIT balances between immune response and tolerance to the GM by the production of a mucosal layer and antimicrobial substances, in spatial segregation. These interactions are the primary components in the intestinal immune system's function or the Gastro-Associated Lymphatic Tissue (GALT) (Pola *et al.,* 2021). GALT comprises epithelial cells, Peyer's Patches, and Lamina Propria; each, with an important role in immune function (Aparna *et al.,* 2021).

1.3.1. Metabolic function

The body's metabolic function centers around the GM and intestinal epithelial cells (IECs) metabolic exchanges. These cells compose the gut lining, providing a barrier and a place for nutrient absorption. The microbiota's metabolites are categorised into three types: microbial-produced metabolites from the diet, including SCFA and indole derivatives; microbial-modified metabolites generated from the host, such as secondary bile acids; and de novo-produced metabolites, such as Polysaccharide A. Others include gases like CH4, H2S, and NO, vitamins, lipids, neurotransmitters, and more (Liu *et al*., 2022)

SCFA are fermented from undigested carbohydrates or protein-derived branched-chain amino acids (Liu *et al*., 2022). They can help regulate mucus production, pH, and cell function. Butyrate, a type of SCFA, regulates cell factor and enzyme expression. It also

promotes protein production for tight junctions, preventing pathogen penetration. Propionate can promote IEC migration, cell repair, and resistance (Zhou *et al*., 2022).

Primary bile acids from cholesterol help in lipid digestion and cholesterol metabolism. When unabsorbed, they are converted into secondary bile acids and serve as gut microbial substrates. Secondary bile acids may have contradicting effects; some can prevent cell repair, causing IBD and neonatal necrotizing enteritis, while others promote IEC proliferation (Zhou *et al*., 2022).

While most research is centered on SCFA, bile acids, and indole, produced gases as if H_2S can cause changes to proteins, epithelial secretion, and gut motility, or mediate mucosal protection in the case of NO. Vitamins B and K can be biosynthesized and supplied to the host by de novo synthesis. The GM can also influence minerals; the degradation of SCFA and changes in acidity levels in the colon can affect the physiological structure of the epithelia and alter transport proteins, thereby affecting mineral absorption levels (Liu *et al*., 2022).

1.3.2. Immune function: Development and modulation of the immune system

The development of the immune system begins during gestation. The maternal microbiota's signals and substances (SCFA, lipopolysaccharides (LPS), etc.) are transmitted through the placenta to reach the bone marrow via circulation, shaping and influencing the immune response. Soon after birth, the installed microbiota help degrade nutrients such as fibres into SCFA, by fermentation. They can be used as an energy source for epithelial cells (Enterocytes) and play a major part in signalling pathway mediation (Aparna *et al.*, 2021), As well as help inhibit pathogen growth (Nimmy *et al*., 2023).

Literature highlights the importance of the neonate diet. Human milk with various HMOs exerted different effects on immunomodulation, either by promoting *Bifidobacterium* and influencing SCFA production, or by acting on Mucosa-Associated Lymphatic Tissues such as GALT (Plaza-Díaz *et al.*, 2018). Others discussed the difference between a high-fiber diet and a high protein, lipid, or sugar diet in maintaining homeostasis (Nimmy *et al.*, 2023). Nevertheless, most of these findings strongly correlated with the high production of SCFA and immune modulation.

1.3.2.1. Innate immunity

Innate immunity includes inflammation, cellular barriers, and the mucus membrane. To reduce immune response against commensal bacteria, Peyer's Patches produce antimicrobial peptides (lectin-RegIIIγ) and create a barrier between the microbiota and the intestinal epithelia. This production is highly regulated by signalling pathways (TLR-MyD88). In certain cases, the commensal microbiota breaches the intestinal barrier and activates a specific immune response that later prevents the breaching commensals' adherence to epithelial cell surfaces (Fig.3) (Aparna *et al*., 2021).

Studies on germ-free mice demonstrated a smaller germinal center of Peyer's patches, reduced amounts of lymphocytes (T-reg cells, intraepithelial lymphocytes) and IgA-secreting plasma cells. Additionally, certain antimicrobial peptides such as defensin and RegIIIγ had a significant increase in the bacterial presence, with RegIIIγ targeting gram-positive bacteria, in mucosal immune response (Fig.3) (Shi *et al*., 2017).

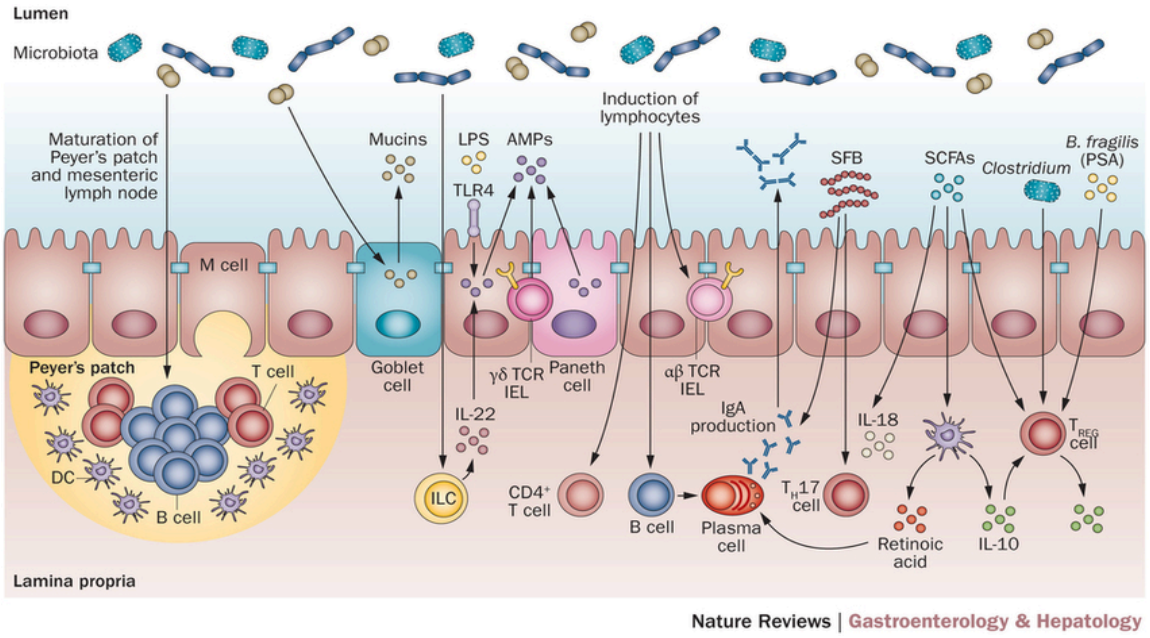


Figure 3: The Gut microbiota's role in shaping host immunity, by helping the maturation of peyer patches and mesenteric lymph nodes, the introduction of lymphocytes, the pro- and anti-inflammation response, with the different bacterial strains (SFB, *Clostridium*, *B.fragilis*) and their metabolites (Verduet *et al*., 2015).

When the GM is in a state of dysbiosis, the permeability of the gut barrier is increased, and pathogens trigger the first line of defense. Pattern Recognition Receptors (PRRs), like Toll-like Receptors (TLR), are found on the surface of different epithelial cells and help recognize Pathogen-Associated Molecular Patterns (PAMPs), including LPS, the peptidoglycan layer, and nucleic acids. The TLR-PAMP interaction activates an acute inflammatory response and signaling pathways, including Myeloid Differentiation Primary Response 88 (MyD88), for cytokine production (IL, etc.). This subsequently triggers the adaptive immune response (Nimmy *et al.,* 2023).

In this signalling, the GM modulates the process. Recent reports state that bacterial metabolites (APS, SCFA) cause an increase in TLRs, MyD88, and cell regulation. Additionally, the GM's constant signaling for the production of cytokines (Interleukins and Interferons, etc.) promotes the maintenance of selective species, preventing deregulation (Aparna *et al*., 2021).

1.3.2.2. Adaptive immunity

In the lamina propria, the uptake of antigens triggers the activation of the adaptive response. The differentiation of T cells into Th or Treg cells aids in activating B cells' IgA production, contributing to microbiota regulation (Fig.4) (Pathak *et al.*, 2021).

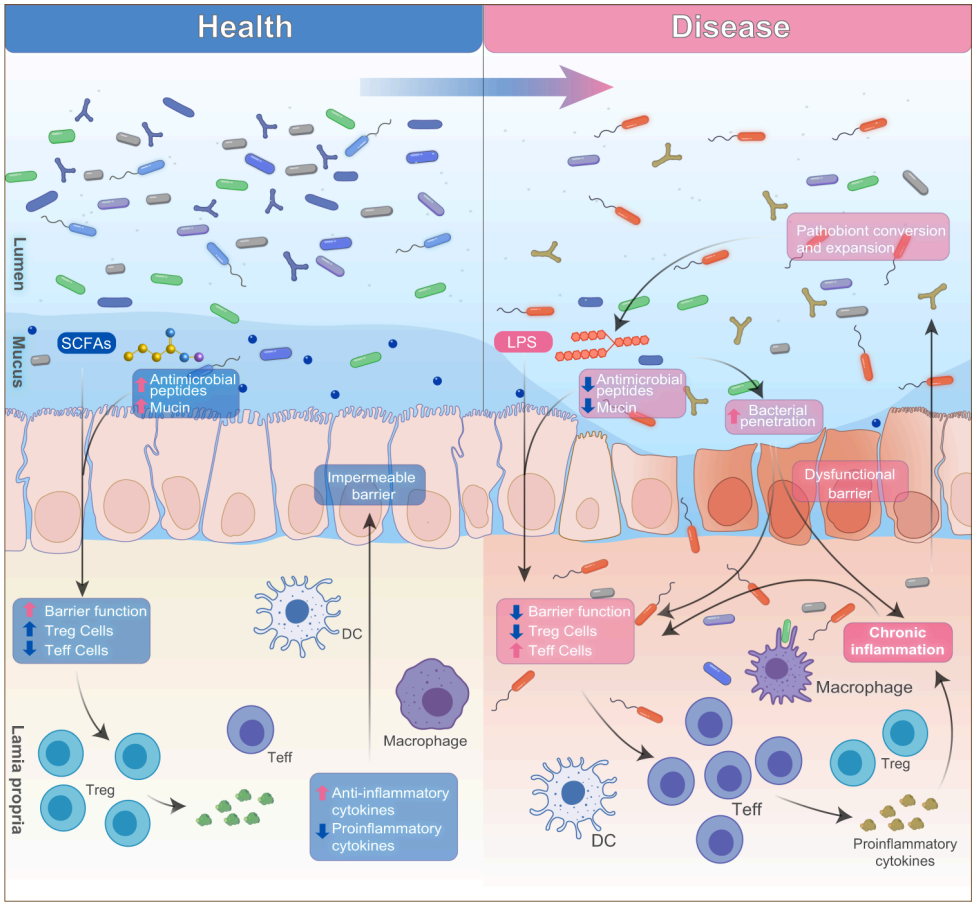


Figure 4: Schema of gut microbiota's interactions in state of health and disease. Changes in Mucin and antimicrobial peptides affecting T cells and barrier functions leading to pro or anti-inflammatory cytokine production in healthy state. Diseased state show a dysfunctional barrier, bacterial penetration, a decrease in reg cells, mucin and AMBP with a rise in Teff cells leading to a chronic inflammation (Hou K. *et al.*, 2022).

Similar to the innate response, the adaptive response is regulated by GM metabolized SCFA, such as butyrate, play a regulatory role in T cell differentiation; Low concentrations promote T CD4+ differentiation into Treg cells, while high concentrations lead to the differentiation of interferon-producing Treg cells or conventional T cells. Interestingly, certain bacteria, including *Clostridium* species, proved to influence Treg differentiation, while Segmented Filamentous Bacteria affected Th17 cells, whose dysregulation closely correlated with Intestinal bowel Disorders (IBD). Furthermore, exposure to different breaching microbial antigens elevates the diversity of Secretory IgA, allowing a better reaction to diverse microorganisms (Wang *et al.,* 2019). The regulation of T cells and microbial antigens helps avoid the progression of acute inflammation into chronic and inflammation-associated

diseases, such as autoimmune diseases, cancer (Nimmy *et al.*, 2023), and allergies (Akagawa and Kaneko, 2022).

2. Gut-brain axis

The central nervous system (CNS) is heavily influenced by intestinal microorganisms, which act through the enteric nervous system (ENS) and metabolic pathways. Microbe-produced molecules, such as secondary bile acids and tryptophan metabolites, interact with entero-endocrine cells and penetrate the CNS directly or through the vagus nerve. Similarly, the nervous system can affect the GM during Stress or immune response and regulates physiological response using neurotransmitters, including melatonin and adrenaline. Understanding the molecules and pathways that come into play can help give perspective on neuropsychiatric disorders and their correlation to GM dysbiosis (Fig.5) (Nimmy *et al*., 2023).

Different bacterial strains have been connected to different changes in behavior. The changes can either elevate the state of stress and anxiety such as in the case of *Campylobacter jejuni*, or improve certain neurological functions such as in the case of *Lactobacillus* strains. The time for the effect differs, with certain pathogens having been associated with quicker alterations than non-pathogenic microbes (Fülling *et al*., 2019).

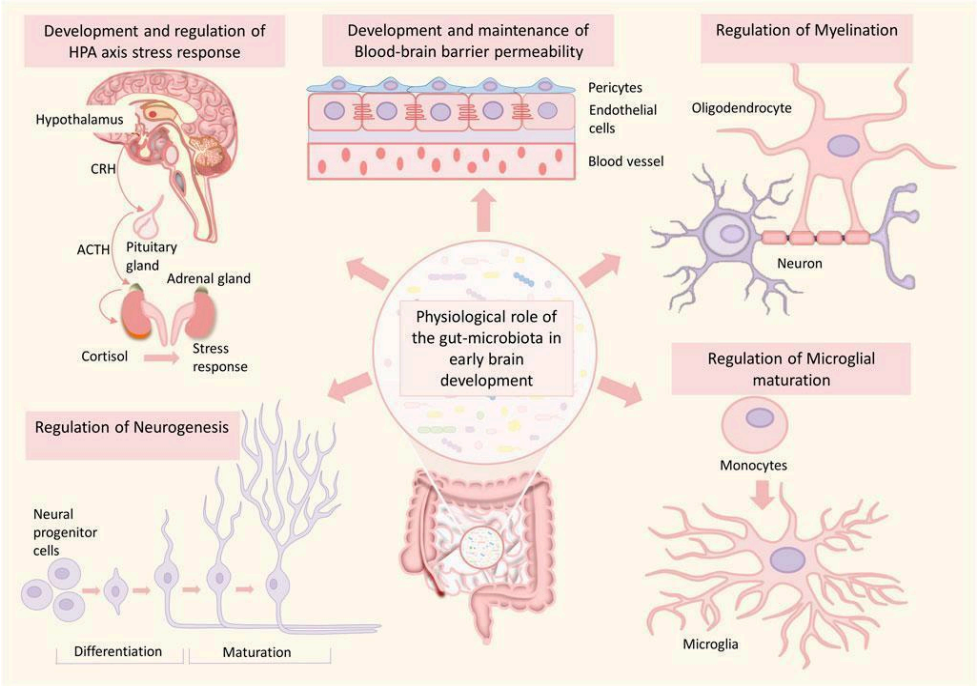


Figure 5: The different impact of the intestinal microbiota on neurodevelopment in infants thought: the regulation of stress response, maintenance of blood-brain barrier, regulation of myelination, neurogenesis, and microglial maturation (Dash *et al.,* 2022).

3. Gut-lung axis

The gut-lung axis represents an intricate connection between two systems involving the exchange of signals between microbiota, mediated by immune cells and microbial metabolites. These metabolites can travel from the gut to the lungs and regulate homeostasis and the inflammatory response. The disruption of this link can translate into disease development, bacterial diversity loss, the progression of acute infectious diseases, as well as chronic conditions like chronic obstructive pulmonary disorder (COPD) and asthma (Enaud *et al*., 2020). Changes in microbiota can be observed in cases like influenza, with an increase in *Enterobacteriaceae* and a decrease in *Lactobacillus* species (Enaud *et al*., 2020).

This transfer of microbiota from the gut to the lung can be explained by gastroesophageal reflux inhalations, sputum swallowing, or the travel of bacteria and their metabolites through the circulatory system, after epithelial disruption (Fig.6) (Enaud *et al*., 2020).

The effects of gut metabolites depend on their type and the host state. In certain cases, metabolites like trimethylamine-N-oxide are associated with higher morbidity in COPD cases. In others, the present microbiota and their metabolites can also help with immune system priming against pneumopathic cases like *Klebsiella* and *Streptococcus pneumoniae*, via interleukins and cell receptors (Fig.6) (Enaud *et al*., 2020).

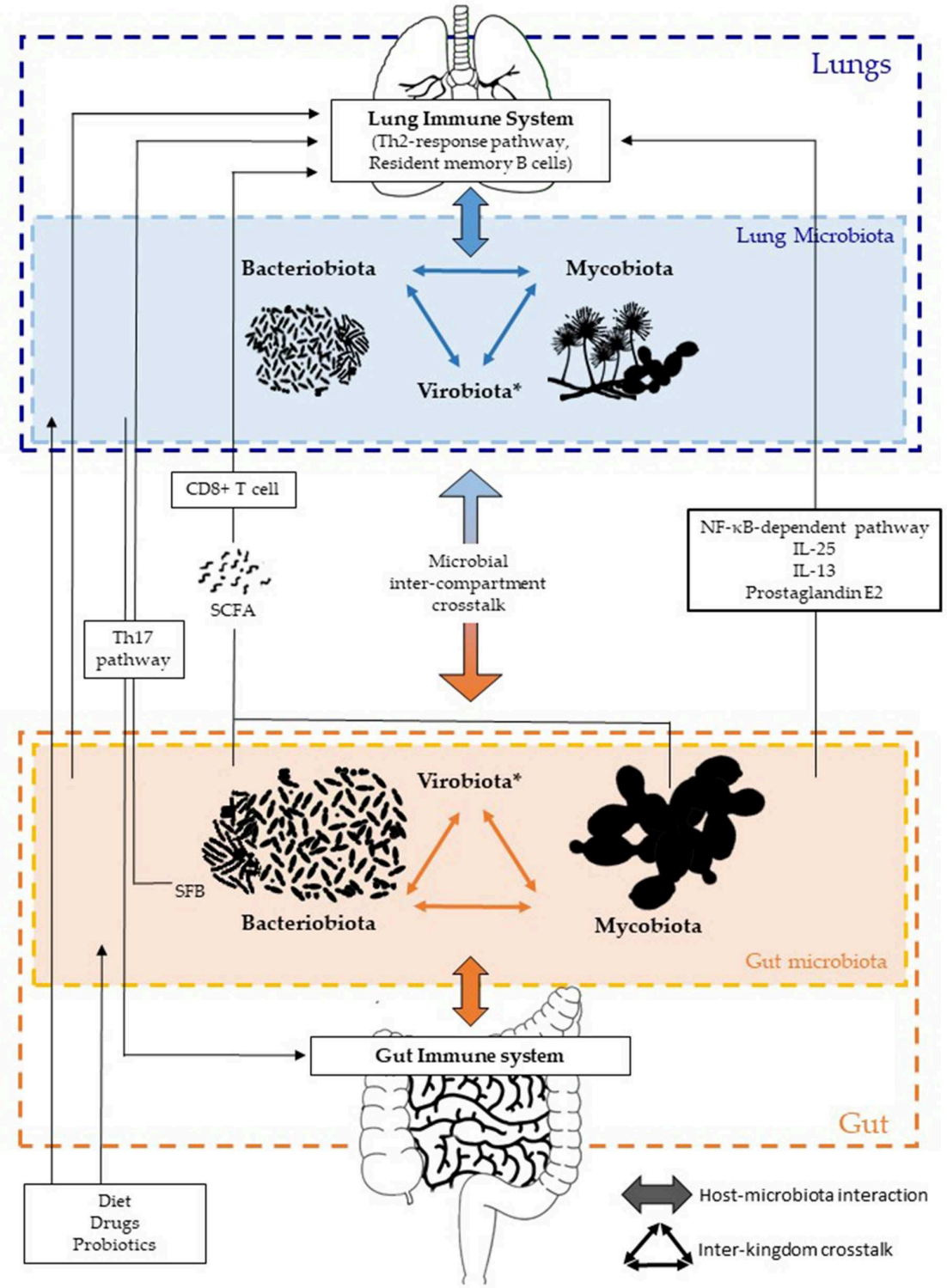


Figure 6: Inter-kingdom and inter-compartment crosstalks within the gut–lung axis (Enaud *et al.*, 2020).

From a set of data following studies on a number of patients, we were able to organize the characteristics of the GM in children with respiratory diseases; the results are presented in the table below:

Table 2: The genus types of gut microbiota present in children with different cases of respiratory diseases.

Respiratory Disease	Gut microbiota	Reference
Pneumonia	(0-3Age) *Bacilli*, *Lactobacillales*, *Enterococcaceae, Enterococcus* (4-5Age) *Scardovia*, *Actinobacteria*, *Enterococcaceae, Bacilli*	(Xiaomeng Ren *et al.*, 2020)
Acute Bronchiolitis	*Bacteroidetes, Firmicutes, Proteobacteria, and Actinobacteria*	(Wu XB *et al.*, 2023)
Recurrent Respiratory tract Infections	Abundance in *Firmicutes*, *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, *Verrucomicrobia*, *Tenericutes*	(Lei Li *et al.*, 2019)
Tuberculosis	Increased *Eubacterium rectale*, *Faecalibacterium prausnitzii*, *Roseburia*, *Butyrivibrio* and *Phascolarcto bacterium.* Fungal communities of *Ascomycota and Basidiomycota.*	(Summaya Perveen and Rashmi Sharma, 2022)
COVID-19	High in opportunistic pathogens and specifically *Pseudomonas*, *Herbaspirillum*, and *Burkholderia*	(Zama D *et al.*, 2022)

4. Therapeutic strategies to restore GM

The loss of equilibrium, microbial diversity, and increase in certain species, lead to neurological diseases, diabetes, inflammatory bowel diseases, cardiovascular diseases, cancer (Nimmy S *et al.*, 2023), and respiratory diseases. (Enaud R *et al.*, 2020). However, research to restore this equilibrium is constantly being made. Among these methods:

4.1. Probiotics, Prebiotics, and Synbiotics

The loss of flora after ANTB use puts the body in a state of immune deprivation and at risk of infection by opportunistic pathogens. To restore the lost flora, patients are given probiotics. They are beneficial bacterial strains produced from fermented dairy products such

as yoghurt. Some of the most common strains are *Lactobacillus* such as *L. acidophilus*, *Bifidobacterium* and *Enterococcus*, and were proven to help in disease management where ANTB(s) failed. The probiotics help prevent pathogen proliferation by colonization, inhibition using metabolites (acids, bacteriocins and inhibitory substances), and host deprivation by competition for nutrients (Yadav M.K *et al.*, 2022).

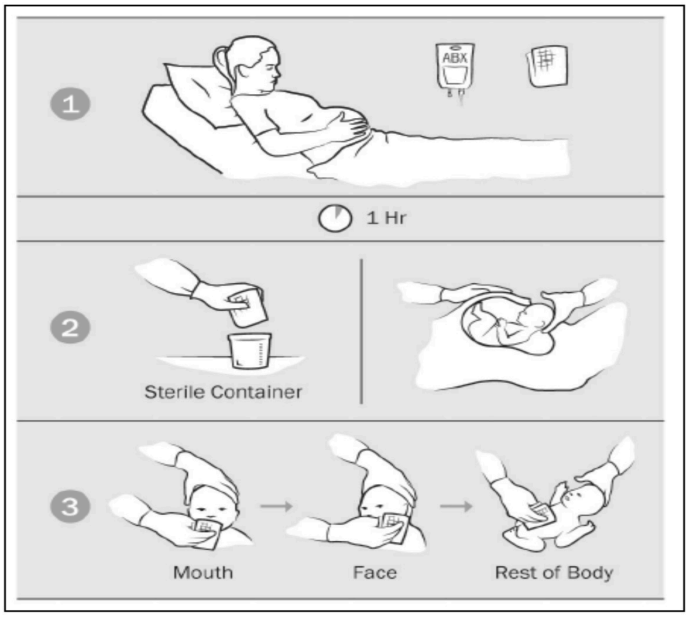
Prebiotics are indigestible substances that help stimulate the growth of probiotics and healthy bacteria, such as Fructooligosacharides that stimulate *Bifidobacterium* growth. Most Prebiotics are produced from fibres and fermented foods (Yadav, M.K *et al.,* 2022).

Synbiotics are a combination of Probiotics and Prebiotics. The relation between the probiotics and prebiotics confers the nature of the synbiotic. Complementary synbiotics are formed of pro/pre-biotics with no co-dependent nature. Synergetic synbiotics, on the other hand, are formed from dependent pre/probiotics, where the microorganism selectively uses the substrate (Yadav M.K et al., 2022).

4.2. Vaginal seeding

The hygiene hypothesis regards the introduction of microorganisms in early childhood as a way to boost immune response and prevent future inflammatory diseases (Okada *et al.*, 2010). In that regard, a method of introduction of certain bacteria is through the inoculation of vaginal fluid from the mother into the neonate's skin, mouth, and nose. This method is still under careful study and is rarely recommended by health professionals for fear of the introduction of pathogenic microbes or viral infections (ACOG, 2017).

This technique is mostly used in cases of cesarean birth to help introduce the flora necessary for the neonate's development of gut flora, and prevention of immune diseases and asthma. This method has faced different challenges and showed variable results leading health workers to only approve of it as an institutional review research protocol (Fig.7) (ACOG, 2017).



Step 1: Gauze is incubated in the mother's vagina for one hour prior to C-section and prior to the administration of peri-C-section antibiotics. Step 2: The gauze is removed and stored during the C-section procedure. Step 3: Immediately after delivery, the infant is swabbed with the vaginal secretions, starting with the mouth, then the face, and finally the rest of the body.

Figure 7: A systematic guide for the vaginal seeding method before and after the caesarean operation (Noel T Mueller *et al*., 2021).

4.3. Antibiotic treatment

Considering the risk of ANTB resistance and resistance gene transfer, ANTB treatment is being considered more cautiously. Using control measures such as swab screenings for resistance and implementing chelating or degrading agents, like Beta-lactamase enzymes or charcoal-based substances, to prevent any leftover ANTB(s) from reaching the colon without affecting bioavailability can help diminish the spread of resistance. Implementing an ANTB stewardship program for healthcare workers and specialists can assist in determining the appropriate ANTB choice, duration of use, and dose. In most cases, a narrow-spectrum ANTB is preferred over anti-anaerobic antimicrobials (Matzaras *et al*., 2022).

The duration of ANTB use differs based on age and class of ANTB . In neonates, a short duration is recommended. In cases of Gram-negative infection, a longer duration is associated with reduced resistance, contrary to ceftriaxone use. However, the use of fluoroquinolones has not been associated with resistance development (Matzaras *et al*., 2022).

The route of administration includes oral or parenteral, with conflicting reports on the better choice. Considerations include the ANTB's bile excretion, intestinal absorption, and presence in the faeces (Matzaras *et al*., 2022).

4.4. Fecal matter transplant

Faecal microbiota transplantation (FMT) is a bacteriotherapy technique involving the transfer of GM from a healthy individual to a patient with intestinal bowel disorder after ANTB treatment, aimed at restoring the flora and host function. Over the years, this method has been tested for cases of Intestinal Bowel Disease, Intestinal Bowel syndrome, and *Clostridium difficile*-caused diarrhoea. With recent advancements in sequencing technologies and treatment guidelines, the technique has shown great results without further treatment (Liu *et al*., 2017).

To understand the effect of FMT on lung dysbiosis, recent research was conducted on rats with hormonal and ANTB-induced gut and pulmonary microbiota dysbiosis using enema FMT. This resulted in the restoration of pulmonary flora at the genus and phylum levels, along with FMT-related reactions (Liu *et al*., 2017).

The effect of ANTB(s) on newborns can lead to long-term illnesses and respiratory infections. A Cincinnati Children's Hospital research group conducted a study on FMT in animals after ANTB consumption with pneumonia-causing infection, and observed microbiota restoration, leading to stronger immune system development (Stevens *et al*., 2022). While this study focused on pulmonary infections, another research on FMT in Clostridioides difficile infection followed two COVID-19-infected patients, testing their stool and nasopharyngeal samples over 40 days. They observed a rapid resolution of the COVID-19 cases without previous treatment (Biliński *et al*., 2021). These findings suggest a potential solution for treating respiratory infections and diseases.

Chapter Two

Respiratory Diseases

Chapter Two: Respiratory Diseases

1. Respiratory tract and lung microbiome

A healthy individual can breathe over 7000 L of air daily. Several microorganisms, (estimated 10^4-10^6 bacterial cells per cubic meter of air per day) are inhaled, which makes it easy for the upper tract respiratory system (URS) to be colonized with different species (Kumpitsch *et al*., 2019).

Children's upper respiratory system microbiome (URSM) is mostly dominated by six bacterial genera, which are *Moraxella*, *Streptococcus*, *Corynebacterium*, *Staphylococcus*, *Haemophilus*, and *Alloicoccus*. In early life, the URSM maturation in infancy is mainly altered by the same factors that affect the gut microbiota (such as mode of delivery, infant feeding, environmental exposures, and ANTB use…etc.). Neonates are primed to be exposed to a wide variety of microorganisms after birth, in their first moments of life their URS microbiome is strongly influenced by maternal microorganisms. After a week, diversification and abundance of more species is the predominant case. Healthy infants born through normal birth were noted to have *Corynebacterium* and *Dolosigranulum spp* in their nasopharyngeal (NP) niche unlike infants born through C-section. The oropharynx (OP) has more diverse and abundant microbiome species that belong to the streptococcal species, such as *Neisseria spp*., *Rothia spp*. and anaerobes, including *Veillonella spp*., *Prevotella spp*. and *Leptotrichia spp* (Fig.8) (Man *et al*., 2017; Durack and Christophersen, 2020).

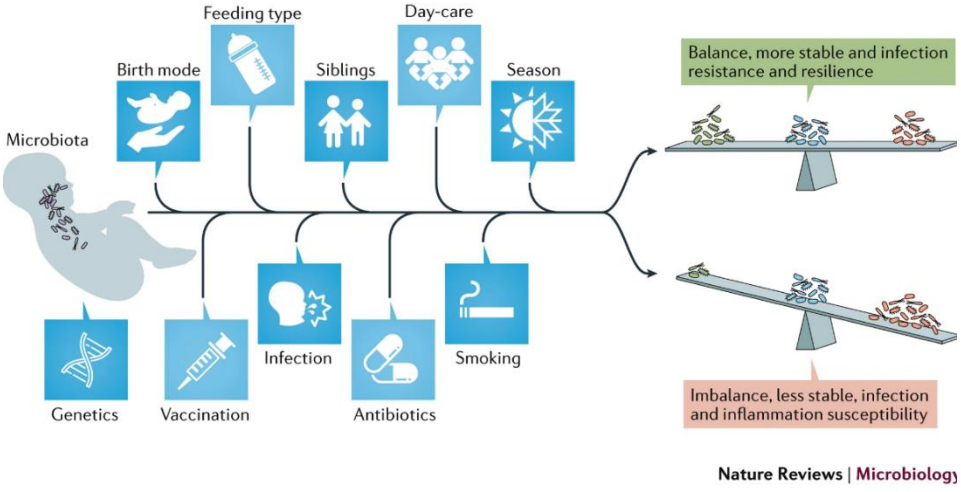


Figure 8: Host and environmental factors have ambivalent influence on the respiratory microbiota of a newborn baby (Man *et al*., 2017).

Unlike the URS, the lung was previously believed to be a sterile organ of the body due to several factors; one suggested that the high air pressure and limited nutrients create an unfavorable environment for microbes' survival. However, recent evidence shows that a healthy individual airway harbors diverse microorganisms and bacterial species, primarily anaerobes, such as gram-negative *Prevotella* and *Veillonella spp*., and gram-positive *Coprococcus* and *Dorea spp.* (Zhao *et al*., 2023).

In a case-control study in China, children's flora showed a strong association between upper respiratory tract microbiota and the presence of childhood lower respiratory tract infection (LRTI), since the URS is considered the gatekeeper of the lower tract of the respiratory system (LRS). This concluded that the slightest unbalanced URS microbiota could lead to dysbiosis and eventually to respiratory illnesses (Fig.9) (Wang *et al*., 2018).

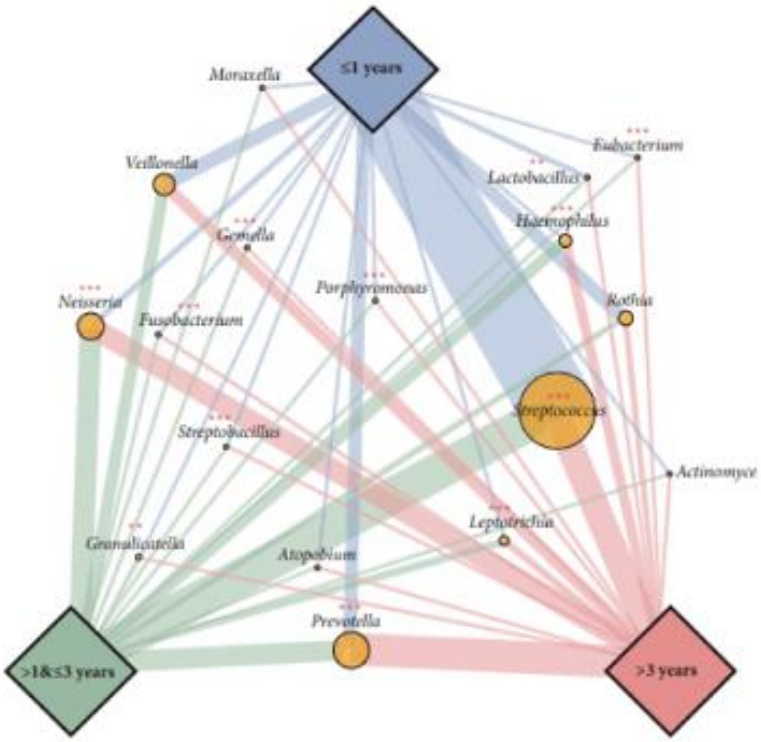


Figure 9: A Structure represents the dominant genera in the oropharynx in different ages based on relative abundance. Each circle represents the genus with total relative abundance in the three age groups, and the width of the line represents the genus with the relative abundance in each group (Wang *et al*., 2018).

2. Dysbiosis and the effect of the gut microbiota in pulmonary immunity and host defence

The establishment of the lung microbiome is influenced by microaspiration, bacterial immigration and growth rates, and elimination processes. Similar to the gut microbiota, the absence of a balanced lung microbiota disrupts certain metabolic functions, leading to bacterial overgrowth or a decrease in commensal species. In a healthy state, the low density of microbes allows for diversity and prevents pathogen overgrowth by modulating the inflammatory response. A decrease in myeloid cell differentiation is observed in cases of dysbiosis and lack of immune modulation. This results in prolonged inflammatory responses and increased mucus production, altering oxygen tension and temperature, and promoting anaerobic microbial growth (Fig.10) (Li *et al*., 2024).

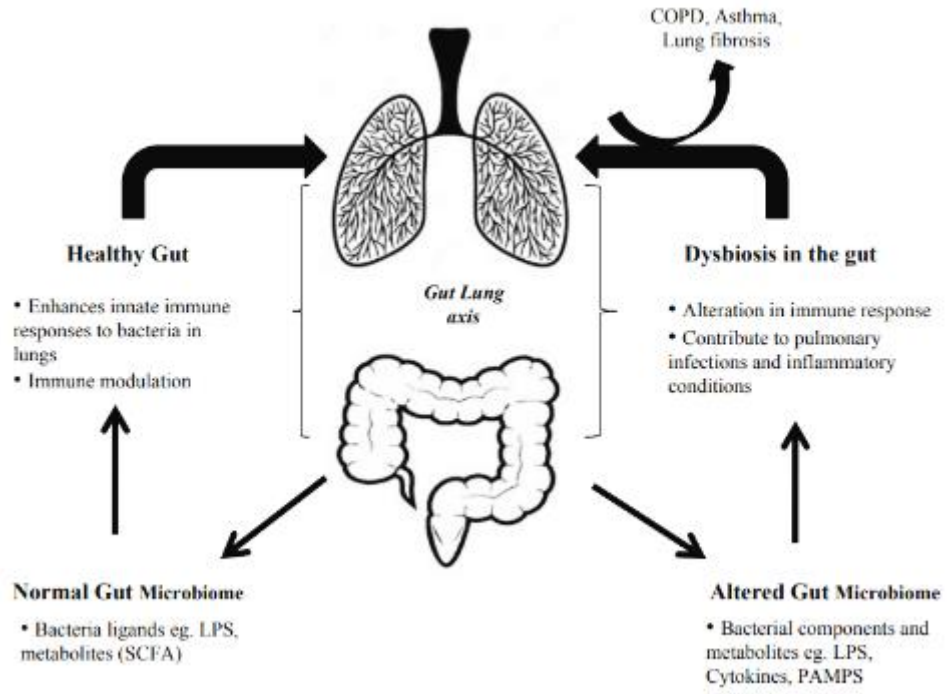


Figure 10: The Gut-Lung Microbiome's normal and abnormal function in the pathophysiology and changes presence leading to different lung disorders (Venma *et al*., 2022).

It is well known that gastrointestinal bacteria are involved in nitric oxide cycles and denitrification, with these gases playing an important role in metabolic functions. However, this cycle is also observed in the lung microbiota during dysbiosis and respiratory pathologies. For example, in cystic fibrosis patients infected with *Pseudomonas aeruginosa***.** The infection creates anaerobic zones within the endobronchial mucus, providing the appropriate conditions

for denitrification cycles. This is evidenced by a decrease in NO_3^- and NO_2^-, and an increase in N2O. The production of such gases promotes the proliferation of pathogenic bacteria and rapid anaerobic growth (Soodaeva *et al*., 2020).

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

Figure 11: The reduction formula of nitrates and nitrites by Prokaryotes (Soodaeva *et al*., 2020).

While *P. aeruginosa* is known to proliferate in aerobic conditions, evidence suggests that it can grow in microaerophilic and anaerobic conditions. When it infects the lungs, it causes the depletion of other microbes until it is the sole bacterium present, forming a biofilm in the thick mucus. The inflammation leads to an immune response and the intervention of neutrophils, which produce ROS such as O2- and RNS such as NO. The reaction between these molecules creates NO3-, supporting anaerobic respiration by denitrification. This process can be observed not only in *P. aeruginosa* infections but also in infections caused by *E. coli*, *S. typhimurium*, *K. pneumoniae*, and most *Gammaproteobacteria*. This underscores the complex interplay between lung microbiota and host environments, especially under pathological conditions (Scales *et al*., 2016).

3. Lung and respiratory diseases

Lung disorders are significant respiratory illnesses caused by exposure to various environmental factors such as smoking, pollens, dust, and chemicals. These factors increase the likelihood of acquiring various illnesses and conditions, which are the third leading cause of death in the US and account for most baby mortality. LD can affect the entire respiratory system, including the trachea, bronchi, alveoli, pleurae, and pleural cavity (Jadhav *et al*., 2021).

Respiratory diseases (RD) can be roughly categorized into two, based on disease pathology and transmission mode: communicable or infectious diseases, like tuberculosis and pneumonia, and non-communicable diseases (NCDs), such as asthma, COPD, cystic fibrosis, interstitial pulmonary fibrosis, and lung cancer (Shukla *et al*., 2020).

3.1. Non-infectious respiratory diseases

A confluence of physiological, behavioral, and environmental factors leads to chronic diseases, or NCDs. These cases result in respiratory diseases, diabetes, cancer, and cardiovascular illnesses. Respiratory disease is one of the top causes of death in low- and middle-income countries (LMICs), with 4.1 million deaths annually (Bhattacharya *et al*., 2023).

Among NCDs, with the sixth-highest death rating, there are lung cancer, trachea, and bronchitis (WHO, 2020). They go as follows.

3.1.1. Chronic obstructive pulmonary disorder (COPD)

COPD is a heterogeneous and preventable lung disease known for its persistent respiratory symptoms and airflow obstruction (Zatloukal *et al*., 2020; Labaki & Rosenberg, 2020b). The two major conditions associated with COPD are emphysema and chronic bronchitis, which often coexist within individuals. While chronic bronchitis leads to inflammation of the bronchial tubes, emphysema gradually destroys the air sacs (alveoli) in the lungs (Prasad, 2019). Individuals with COPD are more likely to die due to comorbid diseases, a significant cause of death worldwide (Jadhav *et al*., 2021). In 2019, COPD was ranked third at a global level out of the ten leading causes of death (WHO, 2020).

Tobacco smoking (TS) represents the most common cause, whether is active smoking or second-hand exposure (SHE) that occurs passively. Similarly, exposure to chemicals, Indoor air pollution, fumes, and dust plays a part in COPD etiologies. Cases are often linked to a rare genetic defect called α1 antitrypsin (AAT) deficiency in young people. In addition to early life events like poor utero growth, preterm birth, asthma, and frequent respiratory infections that can hinder lung expansion (Tab.3) (Jadhav *et al*., 2021; Shetty *et al*., 2021; WHO, 2023).

Table 3: COPD proposed Taxonomy (Celi *et al*., 2022; Stolz *et al*., 2022; Global Initiative for Chronic Obstructive Lung Disease., 2023)

Classification	Description
Genetically determined COPD (COPD-G)	Alpha-1 antitrypsin deficiency (AATD) Other genetic variants with smaller effects acting in combination
COPD due to abnormal lug development (COPD-D)	Early life events, including premature birth and low birthweight, among others
Environmental COPD: Cigarette smoking COPD (COPD-C) Biomass and pollution exposure COPD (COPD-P)	 - Exposure to tobacco smoke, including *in utero* or via passive smoking - Vaping or e-cigarette use - Cannabis Exposure to household pollution, ambient air pollution, wildfire smoke, occupational hazards
COPD due to infections (COPD-I)	Childhood infections, tuberculosis-associated COPD, HIV-associated COPD
COPD and Asthma (COPD-I)	Particularly childhood asthma
COPD of unknown cause (COPD-U)	

3.1.2. Asthma

Asthma or allergic Asthma is a heterogeneous, common chronic respiratory disease that mostly affects children and, in certain conditions, adults. The number of affected children reaches 6.2 million cases aged less than 18 years old. Asthma is mainly triggered by a hyperactive immune response to what is called non-harmful allergens, resulting in shortness of breath, coughing, bronchospasm and wheezing sounds caused by hyper-responsiveness and obstructed airway (Mthembu *et al.*, 2021).

Asthma endotypes differ from one patient to another, each endotype is caused by a certain factor and has its diagnosis and treatment. Several accumulating factors including genetics and environmental factors such as air pollutants, aeroallergens, dietary food, and more. While certain microbes play a part in shielding against asthma development, some infectious

pathogens such as viruses and bacteria are mostly associated with its development (Mthembu *et al*., 2021).

When the case phenotypes are well understood, the optimal treatment for pediatric asthma (PA) may be provided. The current management of asthma is based on a simple regimen of cheap and effective safe drugs; the drugs are made to improve symptoms and lung function. Inhaled corticosteroids (ICS) which are usually prescribed for children younger than 12 years old are not fully effective, especially for severe therapy-resistant asthma (STRA). ICS were only useful at managing mild asthma, which made a call for different types of medications such as Omalizumab, a biological treatment of atopic Asthma that's prescribed for ages 6 to 12, and showed great efficacy in comparison to other biological treatments (Ahmed and Turner, 2019; Pijnenburg *et al*., 2021).

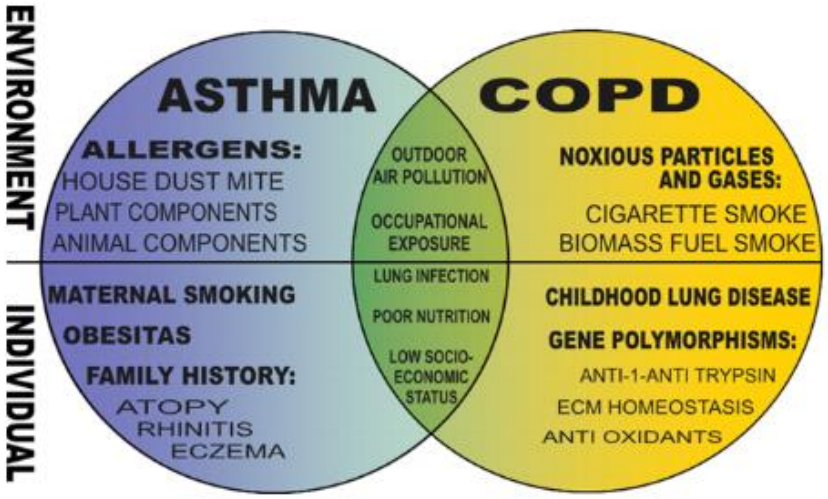


Figure 12: Difference between Asthma and COPD overlapping factors (Gillina, 2012)

3.1.3. Lung cancer

Lung cancer (LC) is a malignant illness among the most common types of cancer, and the deadliest worldwide. In recent years, Algeria saw a rise in cases between 2000 and 2019, with 3.4 cases per 100000 people. Still, these numbers are considerably lower in comparison with Europe and the USA. 80 to 85% of cases are due to a rise in tobacco use, mostly being male, and rarely in children and adolescents (Adda *et al*., 2020)

Two primary types of lung cancer exist, Small Cell Lung Cancer (SCLC) and Non-Small Cell Lung Cancer (NSCLC). NSCLC encompasses large cell carcinoma, adenocarcinoma and squamous cell carcinoma. The last two predominantly affecting smokers. SCLC is less common and challenging to treat, often because of the advanced spread upon detection (The American Cancer Society, 2024).

3.1.4. Cystic fibrosis

Cystic fibrosis (CF) is a hereditary condition (CFTR gene) resulting in respiratory and digestion issues. In normal cases, the body produces tears, sweat, and mucus in a slippery thin form. However, in CF patients a thick sticky fluid is produced that clogs the passageways carrying them out, this leads to a disruption in key physiological functions, such as breathing and digestion. The blocked ducts prevent pancreatic secretion leading to undigested foods to pass with little absorption. The unabsorbed nutrients cause bloating, gas, and abdominal pain (Stanford, 2024). In certain cases, the small intestinal microbiota reaches a staggering number due to the high mucus content causing Small Intestinal Bacterial Overgrowth (SIBO) (Dorsey *et al*., 2017).

In the lungs, the cilia cannot clear the mucus due to the stickiness, leading to an immunological response. However, since there is no clearing of the residues from the response, an infection is bound to happen again. There is presently no cure but several pulmonary treatments and physical therapies can help elevate the infections and symptoms (Stanford, 2024).

3.1.5. Idiopathic pulmonary fibrosis

IPF is a lung disease that affects elderly or middle-aged patients. The mechanism behind this disease remains under research. Nevertheless, most assume it is due to genetic factors and repetitive injury of the alveolar epithelium. The injury activates fibroblasts and myofibroblasts differentiation leading to an over-deposition of the extracellular matrix and lung remodeling. Several factors are at the base of it, such as environmental factors like smoking and pollutants that induce lung fibrosis. In other cases, the dysbiosis in the lung microbiota with a high bacterial load seems to precede a lung injury, with recorded cases of viral infections in patients. Ageing is another factor, which is why most cases are in adults and not children (Mei Qianru *et al*., 2022). With 1-4 cases among millions of children, IPF is very rare among infants, but

some theories suggest that environmental factors trigger the pathogenic mutations present; these changes affect cellular functions triggering a response and leading to IPF (Nathan *et al*., 2019).

Symptoms are a persistent dry cough, weight loss, swollen round fingertips, shortness of breath and tiredness. The most successful treatment is the immunosuppressant Pirfenidone and Nintedanib, followed by a healthy lifestyle and oxygen treatment or a lung transplant (NHS, 2024).

3.2. Infectious respiratory diseases

Respiratory infections (RI) are most likely to spread among the elderly and children in cold climates. They can be primarily spread by inhaling the aerosolized respiratory secretions from infected hosts, or spread by direct contact with mucous membranes, less commonly. Severe bacterial causes and less common ones were included, such as anthrax and pneumonia plague, zoonotic agents classified as agents of bioterrorism (Gilligan *et al*., 2014).

In general, RI can be divided into two groups, upper and lower tract infections (Gilligan *et al*., 2014).

3.2.1. Upper respiratory tract infections

Upper respiratory infections (URTI) are an acute medical condition caused by either bacterial or viral pathogens affecting URS (Guibas and Papadopoulos, 2017). The URS consists of the nose, the oral cavity, throat, pharynx, and larynx that connects the upper and lower airways (Beachey, 2022).While most URTI are acute and self-limiting, others can seriously influence the individual's daily activities (Wang *et al*., 2021).

In a study that was conducted in China on a general population between 5 months and 99 years old, suffering from divers URTI, concluded that the URS get infected mostly with viruses including syncytial virus (RSV), parainfluenza virus (PIV) and Adenovirus (ADV), or bacterial infections, such as *Mycoplasma penumoniae, Chlamydophila pneumoniae* and *Bordetella pertussis* (Tang *et al*., 2019). While some infections start with minor symptoms like a runny nose and headache, it is difficult to observe these symptoms in children younger than two years old. The URTI can be divided into two groups, according to the type of pathogen: viral infections and bacterial infections (Kharel and Bhandari, 2020).

3.2.1.1. Viral infections in children

Viral respiratory infections reach their peak in cold seasons. A study showed that rhinoviruses were behind up to 80% of common colds (Clementi *et al*., 2021; Guibas and Papadopoulos, 2017). Most symptoms can be a little self-limiting at first, such as cold and the sore throat that can transfer into a life threatening LRTI. The death cases of children from viral respiratory infections in developed countries makes fivefold of the deaths in HIC (Clementi et *al.*, 2021).

a. Common cold

Common cold also called as viral pharyngitis (VPH) is a viral infection that can be caused by various types of viruses such as coronavirus (COV), influenza virus (INF), ADV, and coxsackievirus, especially rhinoviruses (RHV). They are the most common causes for pharyngitis in children younger than 5 years old. VPH infection causes inflammation in both the nose and throat of its host. While children become immune after the first infection, they are still susceptible to variants several times a year, with up to eight recurrent occurrences (Jadhav *et al*., 2021; Kharel and Bhandari, 2020).

VPH symptoms can vary from one patient to another. Still, similarities are present in throat soreness, Rhinorrhoea or blocked nose, coughing, sneezing, and rarely a fever that causes difficulty sleeping (Kharel and Bhandari, 2020). Rhinorrhoea starts clear but may turn to green or yellow. These symptoms appear as a result of the release of cytokines from the infected nasal epithelial cells including polymorphonuclear cells (PMNs) which represent the first defense in the innate immune system (Pappas and Hendley, 2018).

b. Laryngitis

Compared to an adult's larynx, an infant's larynx is positioned higher in the neck and smaller, this makes gulping and breathing simultaneously possible. As the infant grows up, the larynx descends and transforms into that of an adult (Allen *et al*., 2019). The acute larynx inflammation is called Laryngitis, which is the most common type of larynx inflammation (Wood *et al*., 2014).

Children may have acute laryngitis from viruses, bacteria…etc. However, most commonly viruses. Although it may be difficult to distinguish between the two, the coexistence

of them both is plausible due to the virus first infection allowing for a superinfection by opportunistic bacteria. The bacteria include *S. pneumonia, S. aureus, β haemolytic streptococci, Moraxella catarrhalis,* and *Klebsiella pneumoniae* (Wood *et al*., 2014).

When children are infected, their mucous membrane of the voice box becomes damaged, caused by an increase in mucosa permeability and loss of connective tissue. Ultimately, this results in hyperresponsiveness of the vocal tract (VT) that stimulates wheezing like symptoms, dyspnea, and asphyxia (She *et al.,* 2020). Other typical symptoms include hoarseness, loss of voice, sore throat, fever, and swollen lymph nodes, lasting approximately 2 weeks (Kivekäs and Rautiainen, 2018).

c. Influenza

Influenza also known as flu is a contagious infection that affects people of different ages. It marks high rates of mortality during pandemics, epidemics, and sporadic outbreaks. A study noted that 10% of the world is affected by it annually, with half a million deaths a year. Influenza virus is categorized into 4 groups: A, B, C, and D; however, humans are predominantly affected by group A during winter, leading to flu outbreaks. Influenza is transmitted by either direct contact or inhaling the laden-virus aerosols released from a contaminated patient, or while using contaminated objects that contain viral particles (Javanian *et al.*, 2021).

While cold symptoms may appear gradually, flu symptoms can be both sudden and rough. They are represented in fever, cough, sore throat, runny nose, body aches, headaches ... etc., these symptoms are the main symptoms of every other viral respiratory illness, making it hard to diagnose. Worsening of these symptoms require emergency warning signs (Centers for Disease Control and Prevention: CDC, 2022).

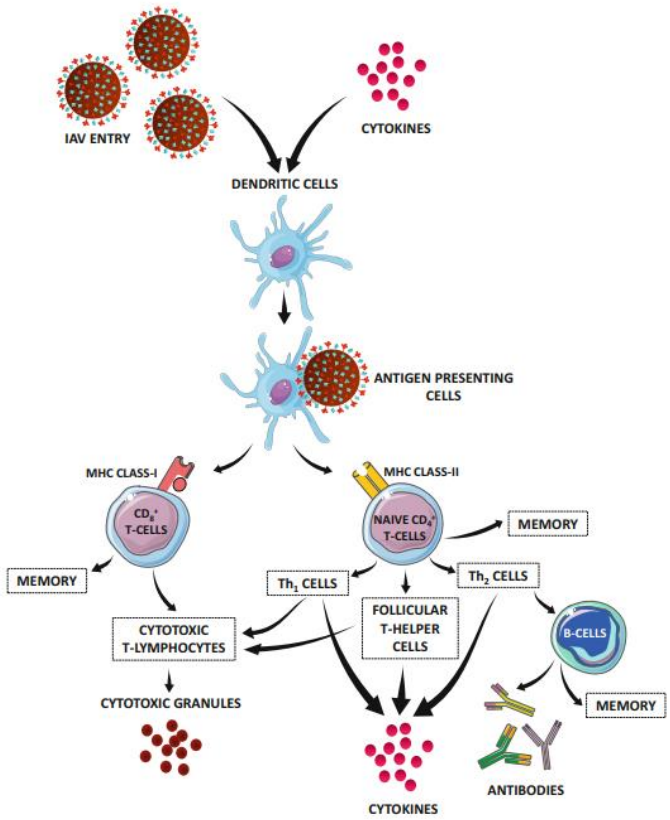


Figure 13: The responsible immune response against viral infection due to the entrance of influenza A virus (IAV) (Gopal et al., 2021).

3.2.1.2. Bacterial infections in children

Compared to all pediatric ages, neonatal period has a higher incidence of bacterial infections, especially premature newborns due to their immature immune system and other complications, including birth sophistication and congenital anomalies that are associated with infant's death. The main infection causing agents are group B *Streptococcus* (GBS) which is more prevalent in full term infants whereas *E.coli* is more common in premature infants (Cohen *et al*., 2023; WHO, 2024).

In these passages we highlight the most common bacterial infections in infants specifically and children in general.

a. Sinusitis

Sinusitis is an inflammation of the mucosal lining of the paranasal sinuses that can be categorized into acute, subacute, and chronic, based on symptoms duration. In general, sinusitis

can be caused by viral, bacterial, or fungal infections, and at times by environmental irritants and allergies. Acute bacterial sinusitis (ABS) is common in children yet it is overlooked due to its non-specific symptoms, this poses diagnostic challenges for primary care pediatricians (Leung *et al*., 2020).

Sinusitis symptoms share similarities with other conditions. The most common symptoms are a stuffy nose with thick colored drainage that goes down the back of the throat. Headache, cough, pain or soreness over sinuses, fever and loss of smell can also be presented. Diagnosing and treating sinusitis in children has its own implications for healthcare utilization and antibiotic prescription (ATBP). Culturing the discharge from the nose identifies bacterial infections, X-rays and radiologic imaging is also considered for a sinuses confirmation (Stanford Medicine Children's Health, 2024).

ANTB therapy, particularly amoxicillin-clavulanate, is the standard treatment for uncomplicated ABS. However, overprescription of ANTB(s) , especially for symptoms attributed to sinusitis, is a concern due to limited benefit and potential adverse effects (Leung *et al*., 2020).

b. Streptococcal pharyngitis

Streptococcal pharyngitis (SPH) also known as strep throat is a bacterial infection caused by the bacterium *group A Streptococcus pyogenes* (GAS). While SPH is widely spread among children, GAS infection occurs in up to 30% of pediatric sore throats (Sykes *et al*., 2020). SP can be self-limiting and accurate diagnosis is crucial, and has more severe complications than VPH in general (Norton and Myers, 2021).

Diagnosing SPH in children requires a combination of clinical assessments and laboratory testing. Overlapping symptoms, such as sore throat, fever, and odynophagia makes the clinical differentiation between VPH and bacterial pharyngitis (BPH) challenging. Yet, specific signs such as tonsillar exudates are more indicative of strep throat (Sykes et al., 2020), with the throat cultures being of high specificity and sensitivity making them a criterion for diagnosis (Miller *et al*., 2022).

Treatment of streptococcal pharyngitis in children involves antimicrobial therapy to eradicate the infection. A 6- to 10-day course of oral amoxicillin is the mainstay treatment

(Mustafa and Ghaffari, 2020). Strep A vaccines are promising burden reduction of both antibiotic use and resistance (Miller *et al*., 2022).

c. Otitis media

Otitis media (OM) stands for a range of middle ear inflammations prevalent in young children worldwide. It encompasses several types based on the disease duration and severity. Acute OM (AOM) is the easiest to identify among the three clinical presentations, which are Chronic OM (COM) and chronic suppurative OM (CSOM) (Schilder *et al*., 2016; Massa *et al*., 2021). AOM affects 80% of children under 2 years old and up to five. It is marked as the most infectious disease that implies the use of antibiotics (Jamal *et al*., 2022; Feghaly *et al*., 2023).

AOM's cause has a relation with the activation of the immune system. Precisely, of innate immunity, triggered by viral or bacterial infections of the URT, with viruses being the factor that paves the way for bacterial superinfections in cold seasons (Schilder *et al.,* 2016; Massa *et al*., 2021). AOM symptoms are ear pain with defined actions such as rubbing, tugging or holding the ear; further symptoms include fever, otorrhea (ear drainage) and vomiting. Diagnosis is by examining the tympanic membrane (eardrum) that may be bulged as a result of otorrhea, which indicates an infection (Gaddey *et al*., 2019).

Managing AOM in children requires analgesics like ibuprofen or paracetamol. As a first line treatment (FLT), Amoxicillin is recommended for uncomplicated AOMs. For second line therapy (SLT), when the FLT is not effective anymore especially against the recurrent infections like resistant *S. pneumoniae,* Amoxicillin high doses and a combination of AMX-clavulanate is recommended, however risks of ATB must be considered due to ANTB resistance and adverse effects, including disruption of gastrointestinal microbiota (Feghaly *et al*., 2023).

Table 4: The most common causing agents of respiratory infections in children (Feghaly *et al*., 2023).

Bacteria	General characteristics	Patient population	Disease manifestation
Bordetella pertussis	fastidious Gram-negative bacterium	Children, Adults	Whooping cough, chronic cough
Chlamydia trachomatis	Obligate intracellular bacterium; does not Gram stain	Neonatal	Conjunctivitis, Pneumonia
Chlamydiophila pneumoniae	Obligate intracellular bacterium; does not Gram stain	Children, adults	Pneumonia, Bronchitis
Chlamydiophila psittac	Obligate intracellular bacterium; does not Gram stain	Children,adults with exposure to birds	Pneumonia, ornithosis (psittacosis)
Group A streptococci (Streptococcus pyogenes)	Catalase-negative, Gram-positive cocci in chains	Children >2 years, adults	Pharyngitis, pneumonia with empyema
Group B streptococci (Streptococcus agalactiae)	Catalase-negative, Gram-positive cocci in chains	Neonates	Pneumonia
Haemophilus influenzae	Pleomorphic, Gram-negative bacillus	Children; adults, especially with COPD	Otitis media, conjunctivitis, epiglottitis, bronchitis, pneumonia
Moraxella catarhallis	Oxidase-positive, Gram-negative diplococcus	Children; adults, especially with COPD	Otitis media, conjunctivitis, bronchitis
Mycobacterium tuberculosis	Acid fast bacillus	Children,adults espicialls HIV infected	Tuberculosis
Mycoplasma pneumoniae	Fastidious doesn't Gram stain	Children, adolescents,adults	Walking pneumonia
Streptococcus pneumoniae	Catalase-negative, Gram-positive diplococcus	Children, adults	Otitis media, sinusitis, conjunctivitis, pneumonia
Fungi	_	_	_

Aspergillus spp	Acute-angle-branching, septate hyphae in tissue; molds	Children and adults with chronic lung disease, adults with cavitary lung lesions, neutropenic individuals	Allergic bronchopulmonary aspergillosis, aspergilloma (fungus ball), invasive pneumonia
Coccidioides posadasii/immitis	Spherules in tissue; mould with arthroconidia at 30°C	Children and adults, especially in desert	Flu-like illness with pneumonia; can disseminate
Viruses	_	_	_
Adenovirus	Enveloped, dsDNA	Children, adults	Pharyngitis, bronchiolitis, pneumonia, conjunctivitis
Coronavirus	Enveloped, ssRNA	Children, adults	Common cold, pneumonia in immunocompromised individuals
Enteroviruses	Non enveloped, ssRNA	Children,	Common cold, pharyngitis, bronchiolitis and pneumonia
Hantaviruses	Enveloped, ssRNA	Children, adults	Acute respiratory distress syndrome, pneumonia
Influenza viruses	Enveloped, ssRNA	Children, and elderly	Influenza, pneumonia
Metapneumovirus	Enveloped, ssRNA	Infants, young children, adults, immunocompromised individuals	Common cold, croup, bronchiolitis, pneumonia
Parainfluenza virus (type 1,2,3,4)	Enveloped, ssRNA	Infants, young children	Croup, bronchiolitis, pneumonia, laryngitis
Respiratory syncytial virus	Enveloped, ssRNA	Infants, young children, elderly	Cough, wheezing, bronchiolitis, pneumonia
Rhinoviruses	Nonenveloped, ssRNA	Children, adults	Common cold; pneumonia in immunocompromised individuals
COPD: Chronic Obstructive Pulmonary Disease; dsDNA: double-stranded DNA; ss-RNA: single-stranded RNA.			

3.2.2. Lower respiratory tract infections (LRTI)

LRTI are infections that affect the LRS, right below the larynx also known as the voice box. LRS includes trachea, lungs, and the bronchopulmonary tree (bronchi, bronchioles, and alveoli) (Sarfo *et al*., 2023). These infections are one of the deadliest communicable diseases in the world, they ranked fourth in 2019 with 2.6 million deaths. Although numbers have decreased significantly by 460.000 fewer than in the 2000s, still its negative impact on the children population under 5 years is comorbid (Safiri *et al*., 2023). In the same year, cases in Algeria were classified among the top ten causes of death, for both sexes, aged 1 to 4 (WHO, 2019).

LMIC has the most reported cases, the majority being from South Asia and Sub-Saharan Africa. Children under 5 years are known to be infected with one of these pathogens, Bacterial, such as *Streptococcus pyogenes*, *Pneumococci*, *Staphylococcus aureus*, *Klebsiella pneumonia*, and *Haemophilus influenzae;* and viral such as, RSV, PIV and ADV. LRTIs include pneumonia, bronchitis, bronchiolitis, and Tuberculosis, in addition to other viral infections such as INFV (Sarfo *et al*., 2023).

3.2.2.1. Pneumonia

Pneumonia is an acute respiratory infection that affects the lungs; more precisely, it affects the small sacs that form the interior of the lungs, also called alveoli. Infected individuals will have their alveoli filled with pus and fluid, which makes breathing painful, and air intake limited. The infection is characterised by its specific symptoms such as fever, chills, cough with sputum production, chest pain and shortness of breath (Stokes *et al*., 2022). It affects people of all ages causing moderate to life threatening infections. Moreover, is the leading cause of infant deaths under the age of 5 years worldwide (WHO, 2022).

There are two types of pneumonia, Community-acquired pneumonia (CAP) and Hospital-acquired pneumonia (HAP). While CAP is clinically defined as an infection acquired outside the hospital (from environment), HAP is acquired after at least 48h hospital admission (Rodrigues and Groves, 2018; Roch *et al*., 2017).

CAP is mainly caused by the main causative agent *Streptococcus pneumonia*, the most common cause, followed by *Haemophilus influenzae* type b (Hib) as well as RSV, which belongs to the virus category (WHO, 2022; Jadhav *et al*., 2021). HAP along with its subtypes

such as Ventilator-associated pneumonia (VAP), which occurs to hospitalized patients under mechanical ventilation, can be implicated by a broad variety of microorganisms. The most common are methicillin resistant *Staphylococcus aureus Pseudomonas aeruginosa* and other gram-negative bacilli such as *Klebsiella pneumoniae*, *Escherichia coli* and *Acinetobacter baumannii* (Miron *et al.,* 2024).

Infantile pneumonia infecting 29 days to 4 years old children is different from that which affects children of 5 to 12 years. The first age group is more likely to be infected by viral than bacterial agents, in which it belongs to the same group of causative agents of CAP. The second category is no different, as viruses remain the most causative agents in school-aged children. Nevertheless, bacterial agents such as *Mycoplasma* are the dominant species, including *Chlamydophila pneumonia* and *S. pneumoniae* (Popovsky and Florin, 2022).

Bacterial Pneumonia is treated following prolonged intravenous antibiotics such as amoxicillin and cephalosporins by taking in consideration the child's health, possible comorbidities, and microbial resistance (De Benedictis *et al.*, 2020); meanwhile, viral pneumonia is usually treated with antiviral medications combined with anti-inflammatory drugs.

3.2.2.2. Bronchitis and Bronchiolitis

Bronchitis is a term used to indicate an inflammation of the bronchial tubes also known as the large airways of the lower airway tract. The inflammation is a result of a protective response against infections caused by strange particles and microorganisms that may reach the airway surfaces (Markova, 2024). Bronchitis affects infants with viral or bacterial disease-causing agents, viral agents are the predominant, including RHV, enterovirus (ENTV), INFV A and B…etc. Bacterial agents for pneumonia are detected in 1-10% of cases of acute bronchitis (ÇiLekar *et al*., 2021). Acute bronchitis is frequent in pediatrics; it is characterized by coughing-like symptoms lasting for a short period. Conversely, chronic bronchitis persists longer than 1 month (Wopker *et al*., 2020 ; Gallucci *et al*., 2020).

Bronchiolitis is a similar infection to bronchitis; it affects the small airways, which are also called bronchioles. Bronchiolitis is a frequent cause for infants hospitalization and young children under 2 years old admission to hospitals seeking medical care; with males being affected more than females. Just Like influenza, bronchiolitis peak season is winter in which it

causes complications and low mortality rate; it is mainly caused by a viral infection inflicted by RSV followed by RHV and other viruses that affect the URS (Fig.14) (Tian *et al*., 2023).

Acute bronchiolitis is mainly treated by supportive measures such as adequate oxygenation and hydration, it is advised to avoid antibiotic use, bronchodilators, corticosteroids or any inflammatory mediator. On the other hand, bronchitis is initially treated with acting beta-2 agonists but for children suffering from wheezing, inhalational corticosteroids are advised (Fig.14) (Hothan *et al*., 2022).

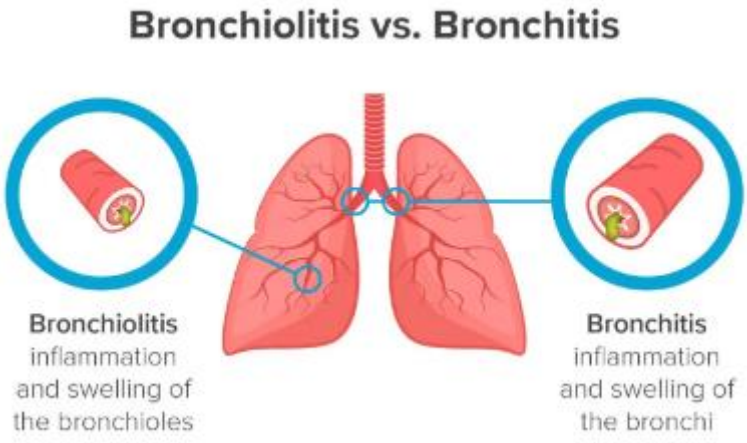


Figure 14: Comparative illustration on the difference between bronchiolitis and bronchitis in inflammation (De Pietro Crt, 2020).

3.2.2.3 Tuberculosis

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis*; a pathogenic bacterium that is transferred through droplets expelled by infected individuals making it the world's most lethal infectious disease (Boom *et al.*, 2021).

Although TB is a preventable and curable disease it still impacts the life of more than 1 million children and young adolescents aged under 15 years, representing 11% of all TB cases worldwide. Children between the age of 1 and 4 years old are more prone to getting TB especially those aged under 2 years who are vulnerable to *Tuberculosis meningitis* (TBM) (WHO, 2022).

Symptoms of PTB in children include cough, weakness, weight loss, fever and sweat at night (Gopalaswamy *et al*., 2021).

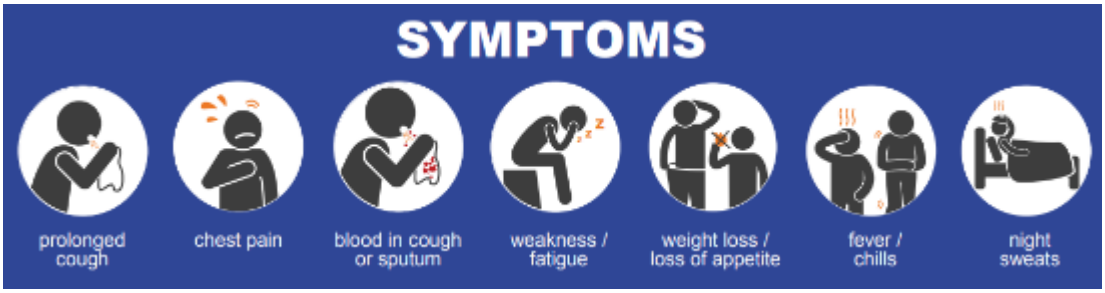


Figure 15: Symptoms of Tuberculosis infection (BCCDC, 2024).

Diagnosis is made based on chest radiography, symptoms and tuberculin skin test (TST) results. Screening methods are recommended for early detection of PTB, in order to initiate treatment since TB cases become more exacerbated by any lethal partnership with AIDS, and multidrug resistance TB (MDR-TB) (Acharya *et al*., 2020).

Treatment of TB in children is based on the same treatment prescribed for adults with the same disease; even so, children require specific treatment as their illness may present unique challenges. Rifampicin and isoniazid have been around for a long time as the most used medication for treating TB however the emergence of MDR threatens achieving cure. Combination of Medicines were taken orally for children with MDR-TB for a duration of 15-20 months. Bedaquiline is prescribed for children >6 years old while Delamanid is for children between 3-6 years old. Children younger than 3 years old consume para-amino salicylic acid or linezolid, which are alternatives to previous injectable agents (Huynh *et al*., 2020).

Experimental study

Materials and Methods

Experimental study: Materials and methods

1. Study objective

The main objective of our study was to answer the question; does the gut microbiota in children with respiratory pathologies differ from that in healthy children? Moreover, to understand the link between gut microbiota and respiratory pathologies.

In order to achieve this main objective, we examined the composition of intestinal microbiota in healthy and diseased children.

1. Realizing a survey to gather necessary information about respiratory health status of the 15 volunteers.

2. Enumeration and characterisation of intestinal microbiota in diseased and healthy children aged between 1 day and 4 years old.

3. Investigate if there is a difference between microbiota of healthy children and those suffering from respiratory pathologies by conducting a statistical analysis.

4. To test strains susceptibility for antibiotics.

Our study took place at the Microbiology laboratory of the Faculty of Natural and Life Sciences at Abd Elhamid Ibn Badis-Mostaganem University during the period from February to May 2024.

2. Study Material

During our study, we used several instruments, apparatus, and different selective and differential media. The list is mentioned in the appendices section 2 and 3.

3. Stool sampling

Sampling is the first step in any analysis. It is a very important act, which determines the quality of the diagnostic results. It must therefore be done under conditions of rigorous asepsis.

Before taking the sample, a survey was given to the parents of each volunteer. The survey contained a set of questions concerning respiratory health status of the volunteer (Appdx 1).

During the period of this study, 15 samples were taken from hospitalized volunteers children at Mostaganem University Hospital Center "Al-Mujahid Dr. Ben Samaine Boumediene" and EHS Complex Maternity-Children in Mostaganem.

Fresh fecal samples were collected using sterile spatula, placed in sterile specimen collection containers (each container was labelled with a code) and immediately transferred from hospitals to the university microbiology laboratory in an airtight transport bag.

Stool samples must be fresh. If it is not, the bacteria in it can multiply. This means the levels of bacteria in the stool sample will not be the same as the levels of bacteria in the digestive system. Once arrived at the laboratory, samples were handled out and prepared for analysis. However, the samples that could not be analyzed on the same day were kept in the fridge at 4°C.

The samples collected from diseased and healthy individuals (Number, Age, Sex, and Health status) are displayed in the table below (Tab.5).

Table 5: Different samples collection.

Sample	Age	Sex	Health status	Source
E1	5M	♂	COPD, suspected TBC Pneumonia.	Mostaganem University Hospital Center "Al-Mujahid Dr. Ben Samaine Boumediene"
E2	11M	♀	Diagnosed Untreated Asthma.	
E3	6M	♂	Diagnosed Asthma (First episode recorded).	
E4	4Y	♂	Immunodeprived patient with Bronchiolitis and Asthma, which was developed post-cancer recovery.	
E5	5M	♂	Suspected Acute bronchitis.	
E6	2M	♂	Diagnosed Whooping cough (Caused by *Bordetella pertussis*).	
E7	1M	♂	Suspected Whooping cough (Caused by *Bordetella pertussis*).	
E8	6M	♂	Diagnosed asthma (Repetitive exacerbations).	
E9	2M	♂	Diagnosed Acute bronchitis.	
E10	6M	♂	Suspected Meningitis (presented with respiratory exacerbations).	
E11	38D	♂	Diagnosed Whooping cough (Caused by *Bordetella pertussis*).	
EA	2D	♀	Healthy Neonate	EHS Complex Maternity-Children in Mostaganem
EB	1D	♀	Healthy Neonate	
EC	6D	♂	Healthy Neonate	
ED	1D	♂	Healthy Neonate	
D: Days; M: Months; Y: Years; ♂: Male; ♀: Female.				

4. Isolation and enumeration of intestinal microbiota

4.1. Stool examination

The samples were first diagnosed macroscopically using a medical tool called Bristol stool scale in order to evaluate and classify human faecal samples into seven different groups (Fig.16). This step is very important in deciding how much the length of the serial dilutions must be.

Samples from 6-7 types of the Bristol stool chart were less diluted as the microbial concentration was less in numbers in comparison to samples of type 4-5.

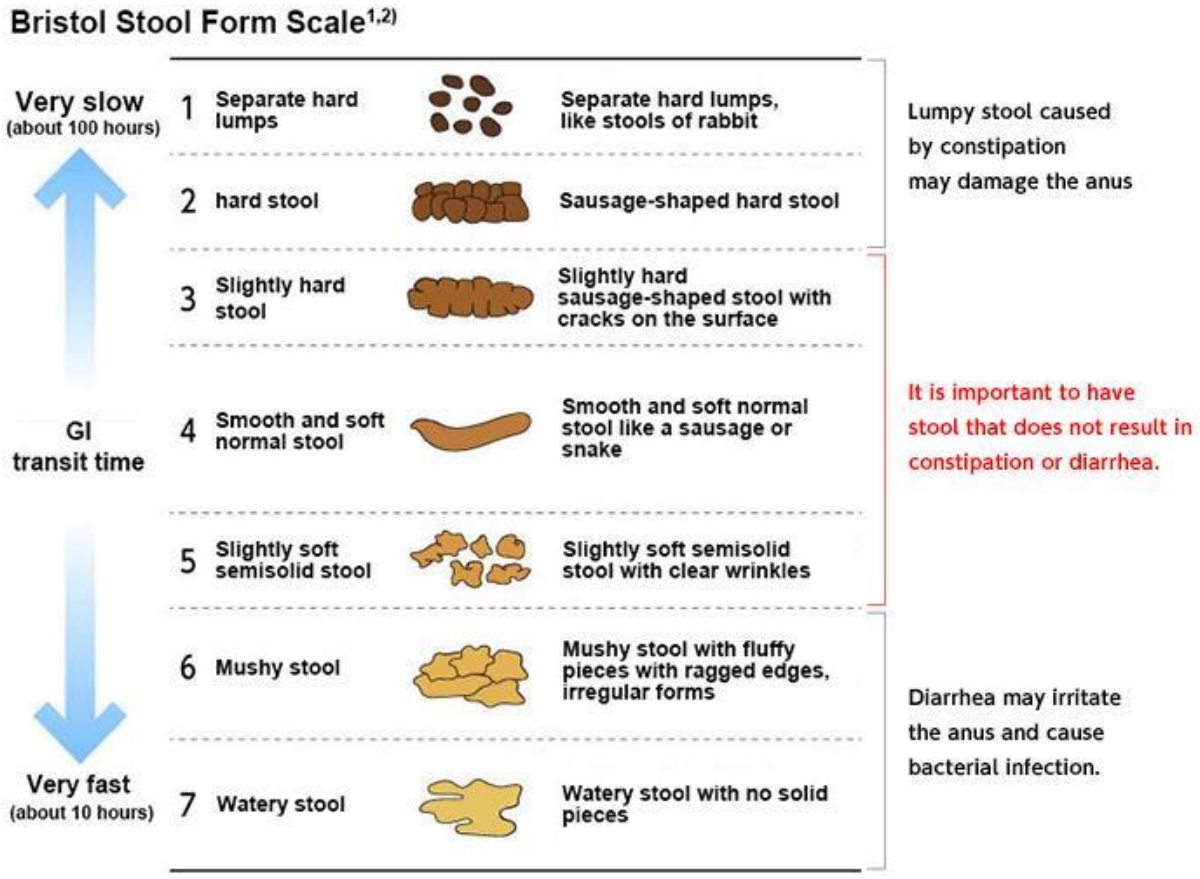


Figure 16: Bristol stool scale representing the seven types of stool (*BORRALABO on Buttocks Health Problems*, n.d.)

The samples were also examined according to stool consistency qualification (SCC) criteria based on mucus presence; consistency and colour (Tab.6).

Table 6: The evaluation of stool samples macroscopically (Kasırga, 2019).

Color	Consistency	Mucus presence	Form
Tawny : normal color in adults due to the presence of bilirubin and bile	Smooth and soft	Small amount of mucus	Normally type 3 and 4
Green: normal color in infants	Watery or pasty	Small amount of mucus	Normal stools according to SCC
Clay/Putty colored: abnormal color indicate biliary obstruction	N/A	N/A	N/A
Black: if more than 100ml blood is lost from the upper gastrointestinal system	N/A	N/A	N/A
Red: indicates lower gastrointestinal tract bleeding	N/A	N/A	N/A
N/A : Not Available			

4.2. Serial dilution and cultivation

For the microbiota cultivation, each specimen was prepared by performing serial decimal dilutions from an aliquot of 1g of stool to obtain concentrations of 10^{-6}, 10^{-7} and 10^{-8}. We used Ringer and PBS (phosphate buffer saline) solutions as diluent (Appdx 3).

The last two dilutions of each sample were cultured in several selective and differential media using the surface spreading method.

The culture media used were: Columbia was used to cultivate different aero anaerobes generally the total flora; Chapman agar was used to isolate *Staphylococcus*; *Enterococcus* and *Micrococcaceae* bacteria. MRS agar was used for the isolation of *Lactobacillus*. *Enterobacteriaceae* were cultivated on Hektoen agar and finally yeasts such as *Candida* were isolated using Sabouraud Dextrose Agar. Other mediums have also been used to assess further identification such as EMB, Desoxycholate, Schaedler, M17, blood agar and Chromagar orientation. Mediums composition is mentioned in the (Appdx 2).

The cultures were incubated at 37 C° for 1 to 5 days according to the strains being searched for.

Table 7: The different culture media used for germs isolation.

Culture media	Principal characteristics	Germs
Chapman	Hypersale medium with the presence of sodium chloride and phenol red.	***Staphylococcus***
Schaedler	Schaedler Agar supplemented with Vitamin K1 and 5% sheep blood is used for the recovery of fastidious anaerobic bacteria such as Bacteroides.	***Anaerobes***
EMB	Eosin and methylene blue inhibit most of the Gram + flora (except Streptococcus D). this medium contains a differentiation criterion, lactose.	**Gram- Bacilli**
Hecktoen	Bile salts as inhibitors. Sugars and sodium thiosulfate as differentiating factors.	**Gram- Bacilli; *Enterobacteriaceae*: *E. coli, Klebsiella***
Desoxycholate	Sodium desoxycholate, ferric citrate and sodium citrate inhibit growth of gram-positive bacteria. Differentiation of enteric bacilli is based on fermentation of lactose.	**Gram- Enteric Bacilli**
Sabouraud	The high concentration of dextrose and the acidic pH of the medium permit selectivity of fungi. The medium can be supplemented with chloramphenicol to increase bacterial inhibition.	***Candida***
M17	Sodium glycerophosphate increases the buffering capacity of the medium and maintains the pH above 5.7 guaranteeing the growth conditions of the lactic *streptococci*. Ascorbic acid stimulates the growth of lactic *streptococci.*	***Streptococci***
MRS	Polysorbate 80 supplies fatty acids required for the metabolism of lactobacilli, Ammonium citrate and sodium acetate inhibit growth of unwanted germs.	***Lactobacillus***

Bacterial counts were expressed as logarithms of colony-forming units (CFU) per gram of tissue (CFU/g log). As follows:

$$\text{Log CFU/g of sample} = \frac{N}{d\ x\ V} \quad \text{(Béraud, 2001)}$$

N: the number of colonies.

d: the inverse of the first dilution.

V: inoculated volume.

4.3. Purification

After 24 hours of culture, we reseat different colonies of each sample in selective and differential new culture media using a platinum loop or Pasteur pipette to obtain completely pure bacteria. Two streaking methods were used; the quadrant method and the T shape method. We then read the data of the various culture media based on their specific characteristics.

5. Strains identification

The determination of the type of microorganism was done by examining the gross morphological/macroscopic features on agar culture. Moreover, we identified the bacterial strains microscopically and we realized different biochemical tests. Finally, we tested bacteria susceptibility to antibiotics.

5.1. Macroscopic observation

Macroscopic aspects refer to a microorganism's overall appearance, such as form, size, color, and smell.

After the incubation period, we observe the plates for bacterial growth by the naked eye and record colony morphology, size, shape, margins, and colour

5.2. Microscopic observation

Once we identified the purified colonies, a microscopic observation was performed using a light microscope. In order to make cells visible, two staining methods were applied on each smear prepared from the purified cultures. Gram stain was applied on bacteria while simple coloration with methylene blue was applied on yeasts. The process is in the (Appdx 4).

Gram staining is the foremost commonly utilized recoloring in clinical microbiology research facility. It classifies microbes in one of two primary bunches: gram-positive (blue or purple) or gram-negative (pink). A few organisms are gram-variable or do not color at all. Gram

recoloring comprises of a slight warm obsession of the spread and the expansion of four successive components: purple gem (primary staining, 1 minute), iodine (mordant or fixator, 1 minute), alcohol (fading, flushing is quick), and saffron (the counter-dye, 30 seconds). Timelines are not exact and vary by body; a water rinse between each step is vital (Connie and Donald, 2019).

Each stained smear was observed under x40 and x100 magnification. The cells Gram, form and arrangement were recorded.

5.3. Biochemical identification

Based on the microscopic observation catalase test and oxidase test have been performed in order to detect the respiratory enzymes.

5.3.1. Catalase test

Catalase is an enzyme found in most strict aerobic and anaerobic optional bacteria. The main function of catalase in cells is to prevent the accumulation of toxic levels of hydrogen peroxide (H_2O_2) formed as a byproduct of metabolic processes. It catalyzes the conversion of hydrogen peroxide into water and oxygen that is released.

Place a few drops of H_2O_2 on the blade. Then, using the platinum handle, take a few colonies from the box and place them on the blade. Observation is immediate (highlighted by the formation of bubbles).

5.3.2. Oxidase test

This test consists in highlighting the ability of the bacterium tested to oxidize the reduced colourless form of methylated derivatives of paraphenylene diamine, in their semi-quinonic form purplish pink. Oxidase or cytochrome oxidase is an enzyme present in certain bacterial respiratory chains; it is an enzyme that catalyzes an redox reaction by involving an oxygen molecule as an electron acceptor (Vezina, and Lacroix, 2000).

To determine the oxidase activity, a sampled colony is placed on the oxidase disk. The development of a purple colour means that the test is positive and the isolate has the oxidase enzyme (Bekada, 2019)

5.3.3. Miniaturized biochemical galleries (API)

API is a standardized system for the identification of bacteria and fungi (yeast), including various miniaturized biochemical tests, as well as a database. The complete list of bacteria that can be identified with this system is present in the Identification Table at the end of the leaflet. In our study we used two types of API; API 20E and API Staph.

API 20 E is a standardized system for the identification of Enterobacteriaceae, including 21 miniaturized biochemical tests, as well as a database.

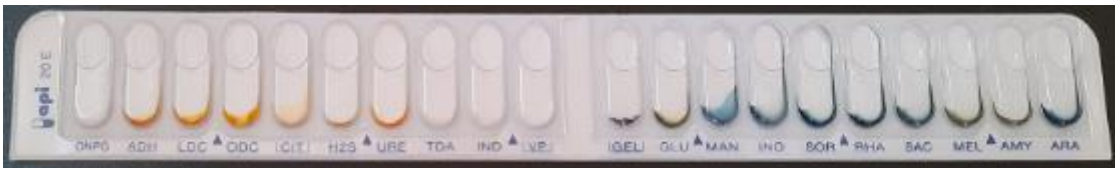


Figure 17: API 20E gallery from Biomerieux before inoculation.

API Staph is a standardized system for the identification of *Staphylococcus*, *Micrococcus* and *Kocuria* genera including miniaturized biochemical tests and a database.

Figure 18: API Staph gallery from Biomerieux before inoculation.

The API 20E and API Staph gallery have 20 microtubes containing dehydrated substrates. The microtubes are inoculated with a bacterial suspension (API Staph Medium for API Staph) which reconstitutes the tests. The reactions produced during the incubation period result in spontaneous colour turns or revealed by the addition of reagents. The reading of these reactions is done using the Reading Table and the identification is obtained using the Analytical Catalog or identification software.

Other biochemical tests were also done, such as the mannitol motility test, Nitrate reduction test and respiratory type test.

For the identification of other groups of bacteria, a classical biochemical gallery was performed. Seeding methods and expected results are presented in Tab.8.

Table 8: Different biochemical tests used for bacterial identification.

Test	Researched character and Method	Result
Simmons Citrate	Use of citrate as the only carbon source. Seeding by serialized streaks the agar, then incubate at 37°C/24h at 7 days.	A turn to blue indicates a positive test so alkalization of the medium.
Nitrate reductase test	Production of nitrate reductase. Inoculation of medium with bacterial suspension and incubation at 37°C/24h.	Nitrate reductase+: red turn of the media after the addition of the two reagents NR1 and NR2 Yellow medium: zinc powder is added. NR- : turn to red. NR+: the media remains yellow.
Clark and Lubs	Determine the fermentation pathways. Seed the medium by adding a few drops of bacterial suspension, then incubate at 37°C/24h.	VP+: turn to cherry red after adding VP1et VP2 reagents. RM+: red coloring after addition of RM reagents.
Urea-indole	Research of indole and urease production. Inoculation of medium with bacterial suspension and incubation at 37°C/24h.	Urease+: turn from media to red/pink. Indole+: appearance of a red ring on the surface after the addition of a few drops of kovacs reagents.
LDC, ODC and ADH tests	Detect the ability of a microorganism to produce decarboxylase, enzymes that decarboxylate the amino acids arginine, lysine and ornithine, respectively in agmatin, cadaverine and putrescine. Seed the medium by adding a few drops of bacterial suspension, then incubate at 37°C/24h.	Yellow media: test- (acidification of the media) Purple media: test + (alkalization of the media)
Mannitol mobility	Mannitol is a mannose reduction derivative. This polyalcohol can be fermented by the bacteria with the release of acidic products that cause the pH indicator to shift from red in basic medium to yellow in acid medium.	Red media: Mannitol - Yellow media: Mannitol + Motile bacteria show a diffuse turbidity away from the inoculation line, while non-motile organisms only grow along the stab line.

5.3.4. Other tests

5.3.4.1. Coagulase test

The identification of free coagulase allows the differentiation of species of the genus *Staphylococcus*. Indeed, only the species *Staphylococcus aureus* can possess this enzyme which plays an important role in the pathogenicity of the bacterium than other species of the genus *Staphylococcus* coagulase negative (S.*epidermidis and S.saprophyticus*) (Couderc *et al*., 2014).

The technique involves seeding a tube of cerebral heart broth (BHIB) through suspect *Staphylococcus aureus* colonies, incubated at 37°C for 24 hours. After incubation, aseptically transfer to a sterile tube a volume of broth and a volume of plasma, mix and incubate at 37°C for 4 to 24 hours. The positive result is expressed by the formation of a coagulum.

5.3.4.2. Haemolysis test

According to Lamouri A, 2020, the fresh blood agar haemolysis test is a very important clinical selection criterion (Fig.19). It is used to differentiate strains of *Streptococcus* and *Enterococcus* and look for haemolytic activity. After a 24-hour incubation at 37°C, there are three types of haemolysis (Gillespie and Hawkey, 2006):

a. Haemolysis α: in case of partial haemolysis.

b. Haemolysis β: in case of complete haemolysis.

c. Haemolysis γ: characterized by the absence of haemolysis, which distinguishes two strains either *Enterococcus faecalis* group D or *Enterococcus faecium*.

Figure 19: Différents types d'hémolyse (α, β et γ) sur gélose au sang frais (Lamouri, 2020).

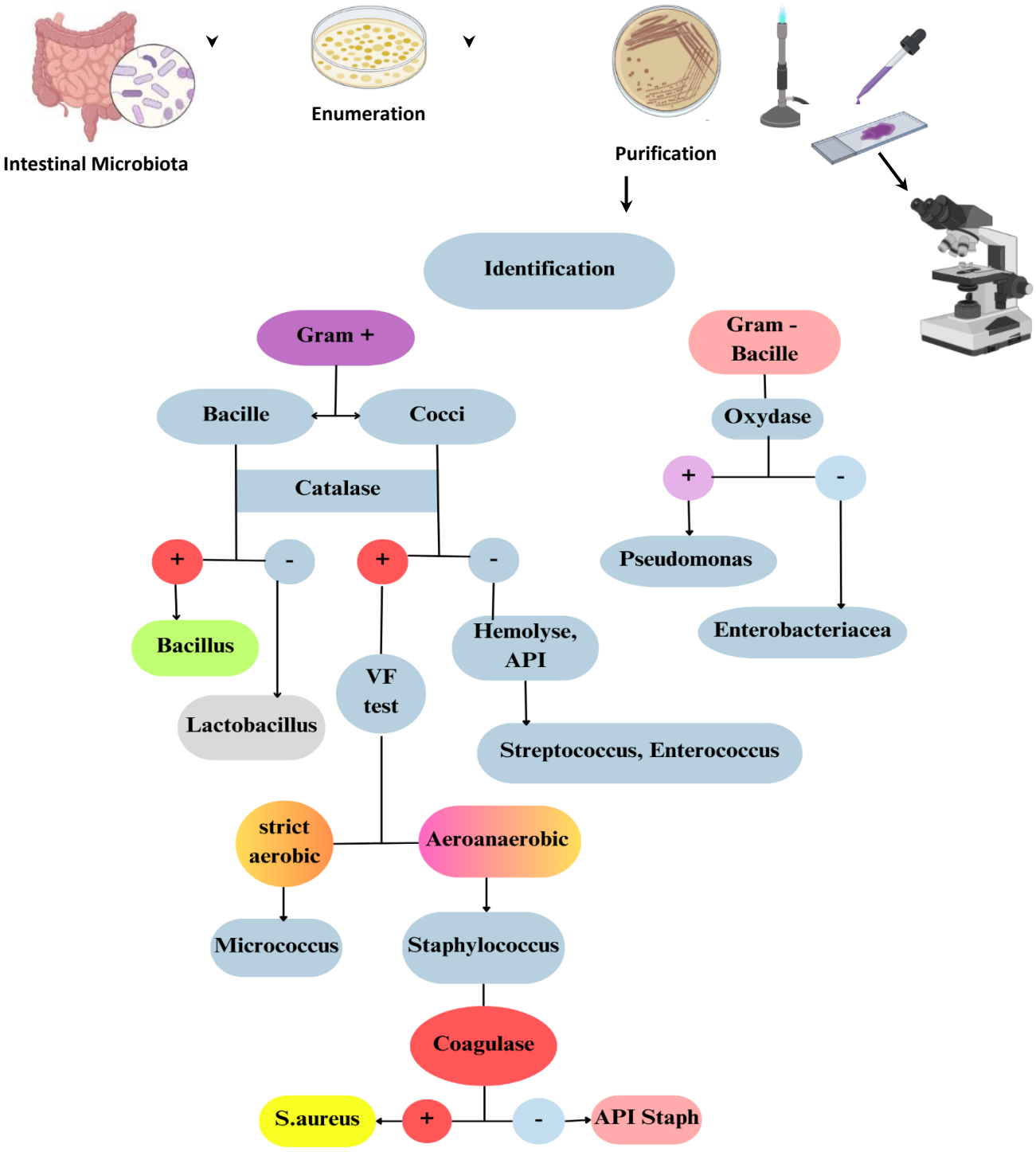


Figure 20: Schematic of the Gram-positive Cocci and Gram-negative Bacilli identification protocol.

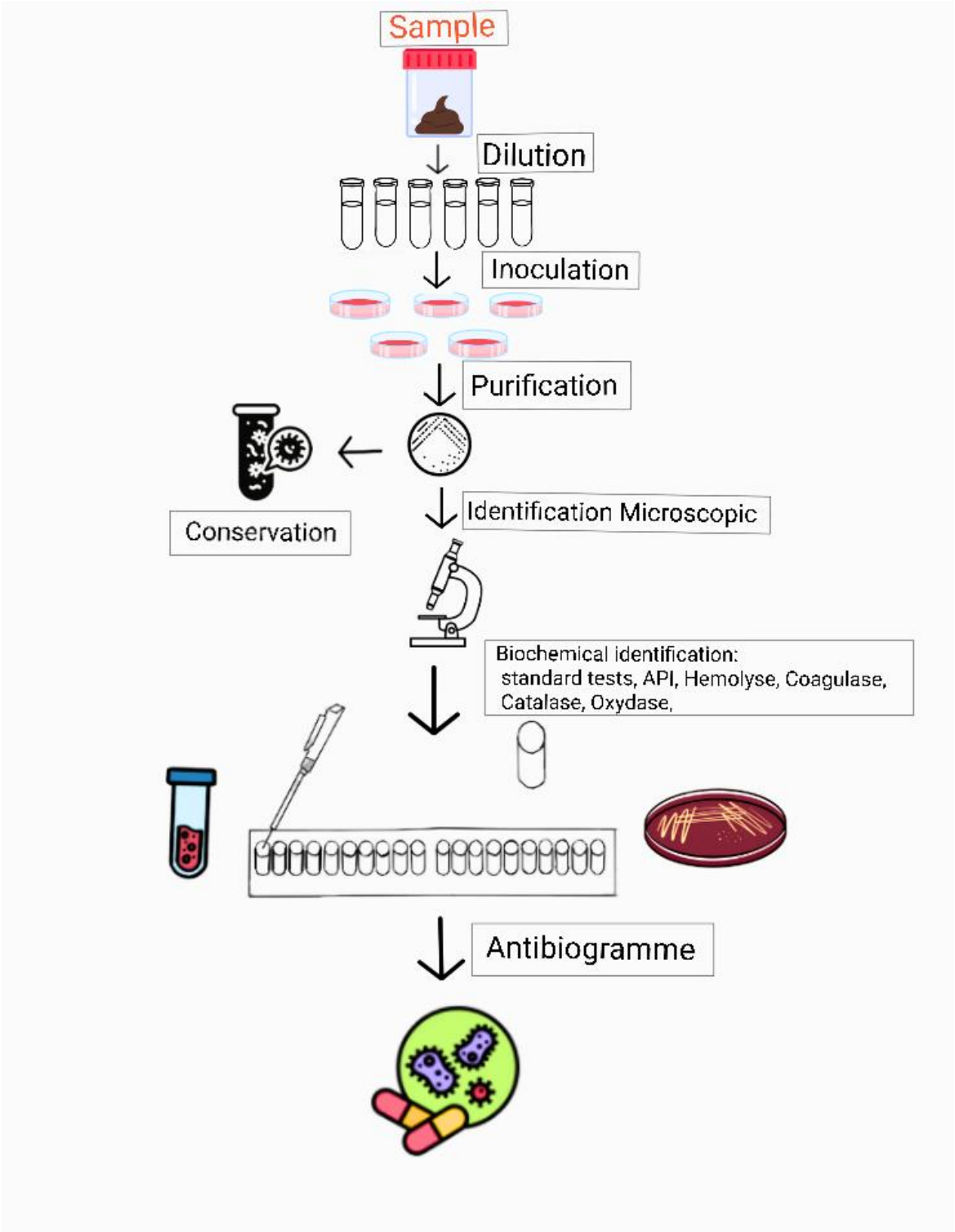


Figure 21: Schematic of different steps of this study protocol.

6. Antibiogram: susceptibility testing

Antibiogram also known as antibiotic susceptibility testing is a technique used to test resistance and susceptibility of each identified strain.

The procedure entails placing antibiotic-impregnated discs at a predetermined concentration on the surface of an agar medium. Once the discs are inserted, the antibiotic diffuses evenly into the agar. After incubation, the discs form circular inhibition zones that indicate the lack of culture.

6.1. Preparation of agar

Mueller-Hinton agar was melted in a water bath and then poured into petri dishes with a thickness of about 4 mm.

6.2. Inoculum preparation and adjustment

The inoculum is prepared from a 24-hour bacterial strain. Colonies of the bacterium to be studied were collected with the platinum loop and were introduced into a tube containing 10 ml of sterile distilled water by forming a suspension. Then the inoculum is adjusted to a DO of 0.08 to 0.1 using 0.5 McFarland standard. This prepared suspension will be used for seeding (CASFM, 2020).

6.3. Seeding

Seeding was done by the swabbing technique; the entire surface of the agar was swabbed with the bacterial suspension ensuring a good distribution of bacteria. Finally, we allowed the petri dishes to dry for a few minutes at room temperature (CASFM, 2020).

6.4. Arrangement of Antibiotic Disks

After drying, the discs are deposited on the agar with sterile tweezers, gently pressing each disc to ensure uniform contact with the medium. The petri dishes are then left at room temperature for 30 minutes on the bench to allow the spread of the antibiotic in the agar (CASFM, 2020).

6.5. Incubation

The petri dishes were incubated in an incubator at 37°C for 18 to 24 hours.

6.7. Interpretive reading

The inhibition diameters around the discs were measured and then compared to the critical diameters. CASFM 2023 standards (Antibiogram Committee of the French Society of Microbiology) and Pasteur institute standards were used. It should be noted, however, that a strain whose sensitivity to antibiotics is so assessed might be declared "sensitive, intermediate or resistant".

Table 9: Antibiotics used to test bacterial susceptibility (CASFM, 2020-2023).

Bacterial family	Antibiotic class	Antibiotics used	Antibiotics acronyms	Discs charge µg
Enterobacteriaceae	Penicillin	Ampicillin	AMP	10
	Cephalosporin	Cefazolin (instead of Cephalexin)	CZ	30
	Carbapenem	Imipenem	IPM	10
	Fluoroquinolones	Nalidixic Acid	NA	30
	Aminosides	Gentamicin	GEN /CN	10
	Macrolides	Erythromycin (instead of Azithromycin)	E	15
	Others	Colistin	COL	10
Pseudomonadaceae	Cephalosporin	Cefazolin (instead of Cefepime)	CZ	30
	Carbapenem	Imipenem	IPM	10
	Aminosides	Gentamicin	GEN /CN	10
	Others	Colistin	COL	10
Staphylococcaceae	Aminosides	Gentamicin	GEN /CN	10
	Macrolides	Erythromycin	E	15
		Clindamycin	CD	2
	Tetracyclines	Tetracyclines	TE	30
	Others	Fusidic Acid	FA	10
	Betalactamines	Ampicillin	AMP	10 (2)

Enterococcaceae		Imipenem	IPM	10
	Aminosides	Gentamicin	GEN /CN	10
	Glycopeptides	Vancomycin	VA	10 (5)
	Macrolides	Erythromycin	E	15
		Clindamycin	CD	2
	Tetracyclines	Tetracyclines	TE	30
Streptococcaceae	Penicilines	Ampicillin	AMP	10
	Cefalosporinas	Cefazolin (instead of Cefepime)	CZ	30
	Carbapenem	Imipenem	IPM	10
	Aminosides	Gentamicin	GEN /CN	10 (500)
	Glycopeptides	Vancomycin	VA	10 (5)
	Macrolides	Erythromycin	E	15
		Clindamycin	CD	2
	Tetracycline	Tetracycline’s	TE	30
	Others	Fusidic Acid	FA	10
Bacillaceae except ***B.anthracis***	Carbapenem	Imipenem	IPM	10
	Glycopeptides	Vancomycin	VA	10 (5)
	Macrolides	Erythromycin	E	15
		Clindamycin	CD	2
Bacteroidaceae	Carbapenem	Imipenem	IPM	10
	Penicillin	Amoxicilline	AMP	10
	Macrolides	Clindamycin	CD	2

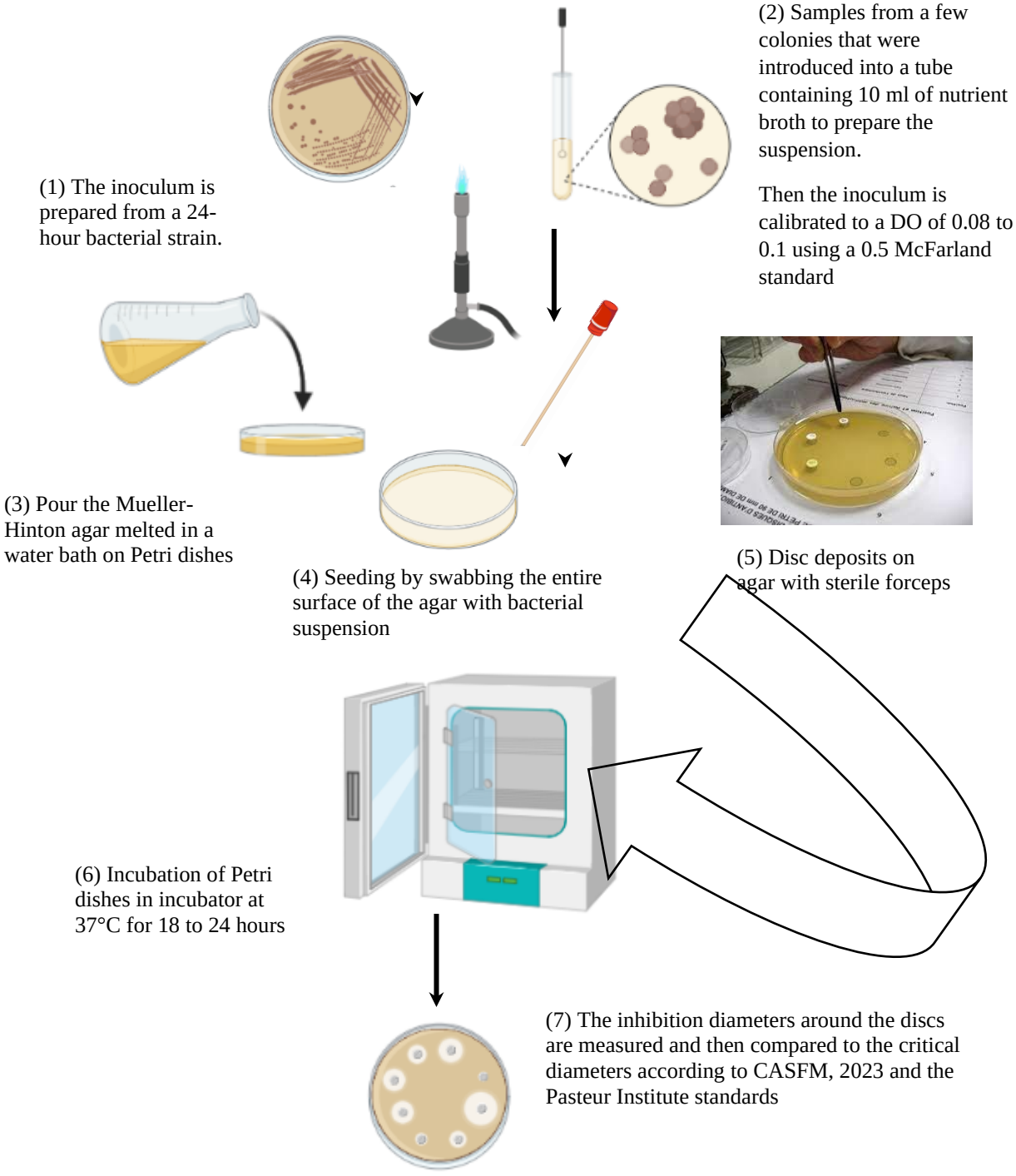


Figure 22: Stages of achieving the antibiogram.

7. Statistical analysis

Data of the present study are expressed as means ± standard deviation (SD). To compare between the microbiota counts of children suffering from respiratory diseases and healthy ones, a statistical analysis was done using the Student's *t*-test. Statistical analysis was performed with the Excel program. A $p\ value < 0.05$ was considered significant.

Results and discussions

Experimental study: Results and Discussion

1. Survey results

The information gathered from the population concerned to carry out this study survey on respiratory pathologies is presented in the graphs below. We surveyed all 15 children based on age, gender, delivery mode, feeding method, duration of disease, encountered symptoms and treatment.

A total of 11 cases diagnosed with respiratory diseases were included in the study, with the majority (90%) of the male children aged between 30 days and 4 years (Fig.23). The survey estimated that 9% of the children were newborns, 73% were between 1 and 4 years of age and 18% were infants aged more than 1 year.

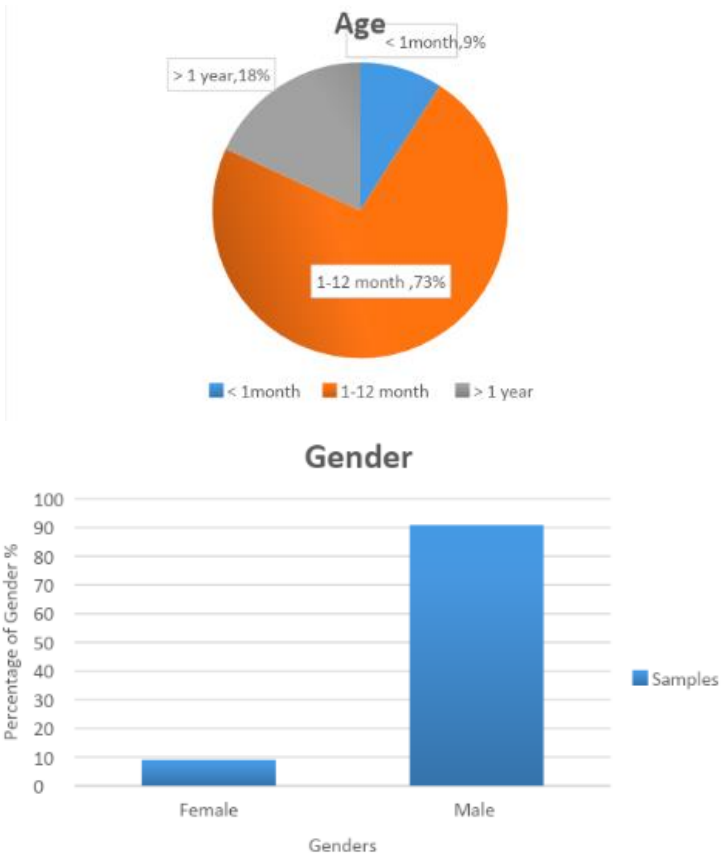


Figure 23: Gender and Age distribution of patients.

Our results showed that 45% of studied subjects were delivered naturally (vaginal) while 36% were delivered by C-section (Fig.24). 27% were fed with natural breastfeeding, 18% with artificial formula and 36% were fed with both natural and artificial formula (Fig.25).

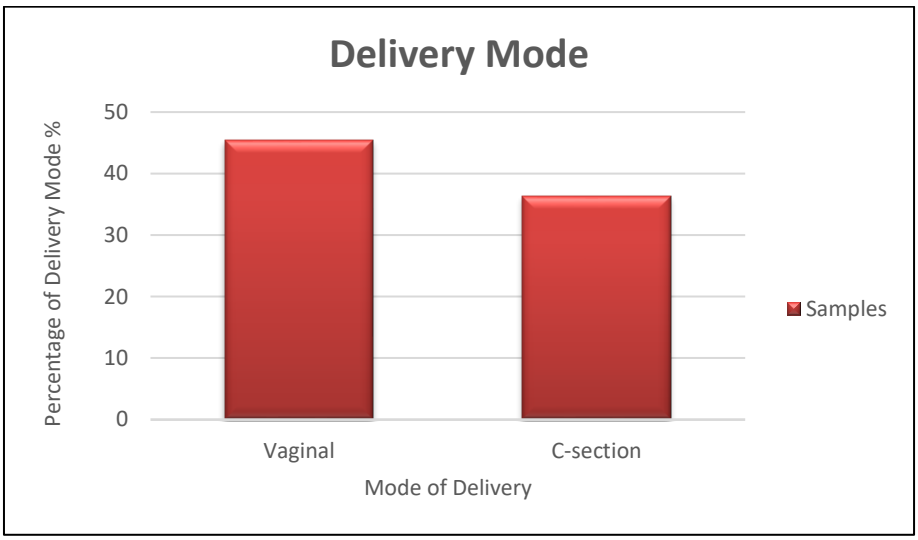


Figure 24: Distribution of hospitalized patients based on delivery mode.

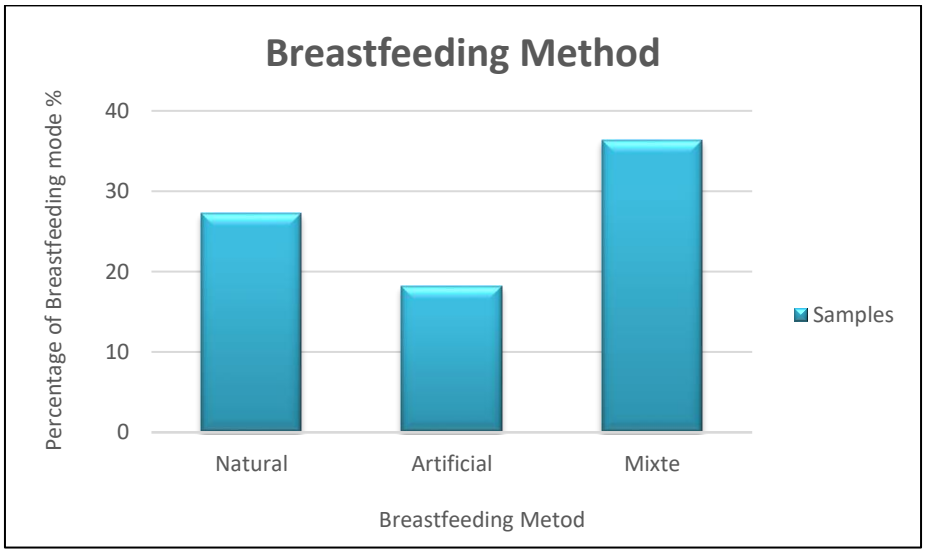


Figure 25: Distribution of hospitalized patients based on breastfeeding method.

Whooping cough and asthma were the most common (27.2%) respiratory disease, followed by COPD, acute bronchitis, acute bronchiolitis, meningitis and mild allergy (9%) (Fig.26).

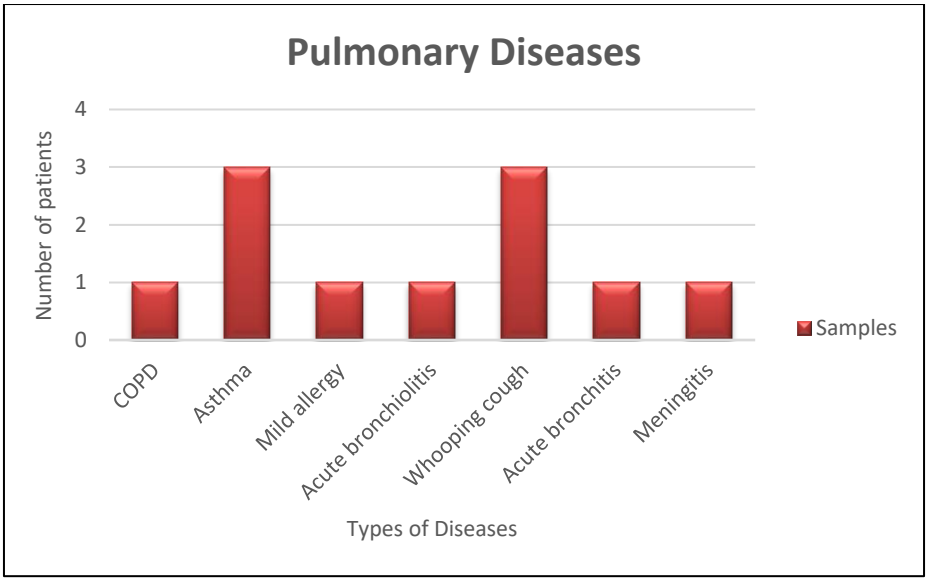


Figure 26: Distribution of respiratory diseases in patients.

Most often, these children had an illness of recent onset and were likely to recover. Half of the patients, though, were either infants who had been ill since birth or older children who were chronically ill (Fig.27).

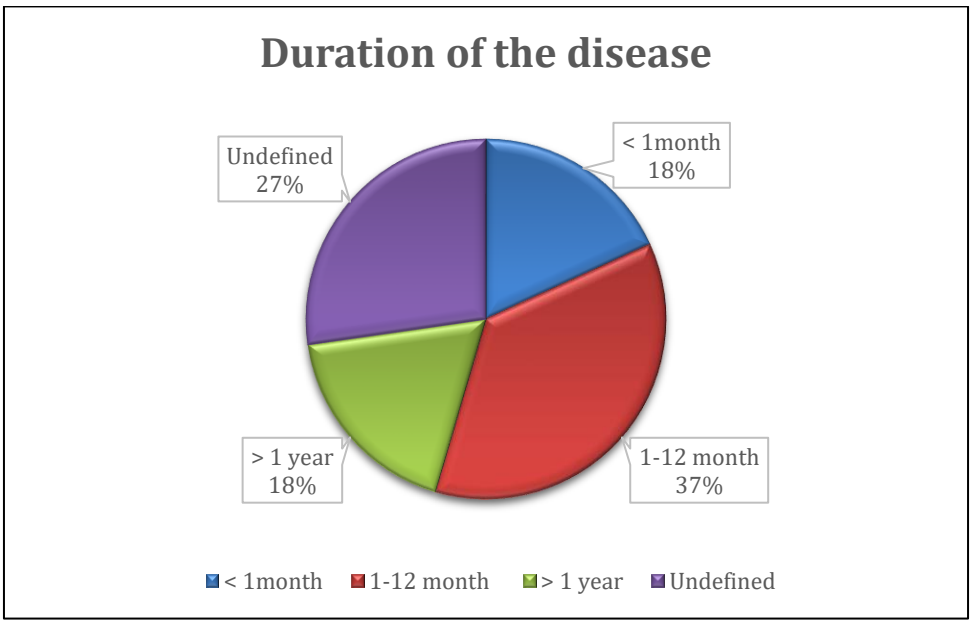


Figure 27: Distribution of duration of the disease among patients.

The clinical manifestations collected from the medical record showed all of the study population presented cough (100%). 45.4% suffered from dry cough while 54.5% had productive cough. Moreover, difficulty in breathing and chest tightness were the most

encountered symptoms in 81.1% and 54.5% cases, respectively. Beyond that, fever, vomiting, allergy, and diarrhea were also observed sickness besides respiratory diseases (Fig.28).

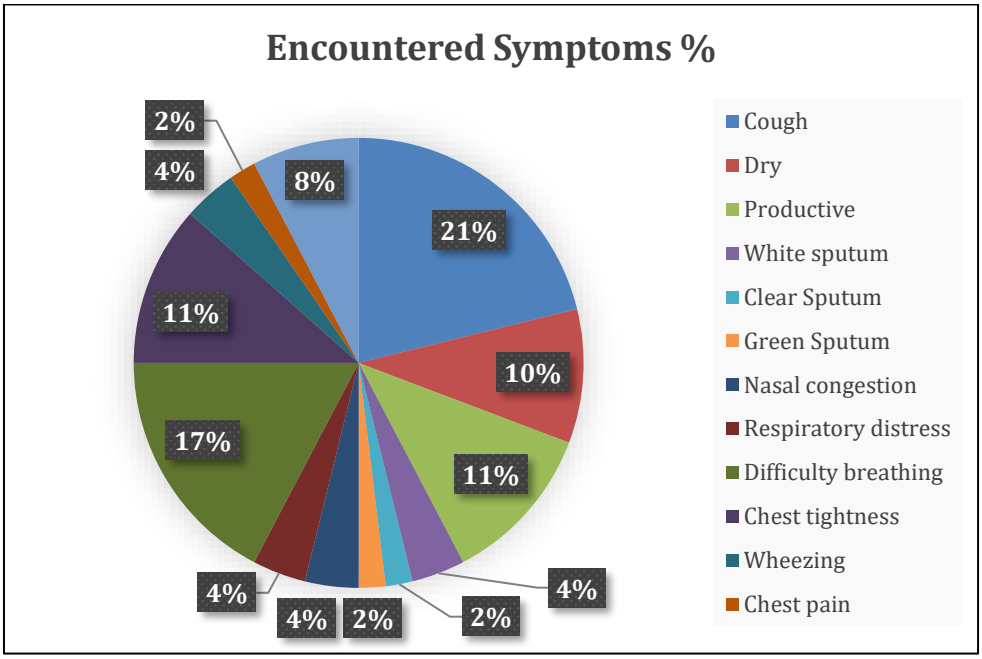


Figure 28: Distribution of different symptoms in the studied cases.

As shown in (Fig.29), different treatments were prescribed to all children diagnosed with respiratory diseases. Antibiotics were prescribed to 45.5% of the study population, followed by corticosteroids (27.2%) and antibiotics plus corticosteroids combinations (18.2%).

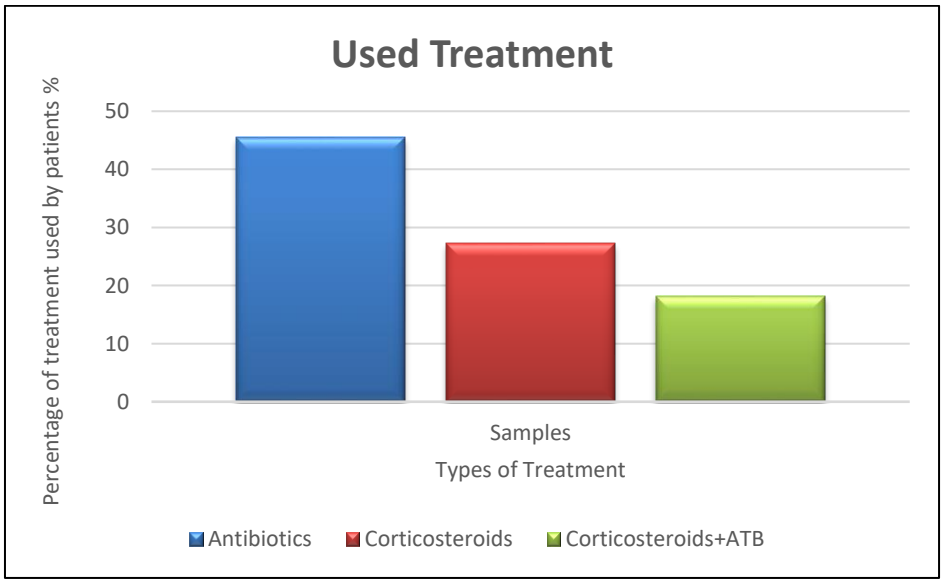


Figure 29: Distribution of Treatments used by the patients suffering from respiratory pathologies.

2. The Bristol Stool scale results

The results from Group A and Group B stool characterization were organized in the table below:

Table 10: Results of the Bristol stool chart examination.

Samples	BSC Levels	Color	Texture
E4, E9, E10, E11, EC	Level 7	Yellow	Watery with mucus
E1, E2, E5, E7	Level 6	Yellow Greenish	Fluffy with lumps
E3, E8	Level 2	Dark brown	Lumps and undigested bits of food
EA, EB, ED	Meconium	Greenish black	Mucus only

The majority of samples were brought from hospitalised children that were born through the vaginal canal, as for the breastfeeding mode, the mixte mode is the dominant one due to extended hospitalisation. Cough, difficulty breathing and chest tightness were the most common symptoms that implied the use of ANTB and corticosteroids in order to ease symptoms.

The macroscopic observation of stool samples from healthy and sick candidates suggested that healthy neonate stool is in the form of greenish-black mucus, also called ‘meconium’. A level 2, Dark brown lumps indicate a disruption in the gastrointestinal microbiota and inflammation that lead to a state of Constipation, which may be due to illness or drug consumption. The patients who exhibited pulmonary symptoms were prescribed the use of corticosteroids, antibiotics or both. Yellow watery stools suggest a severe case of diarrhea that is mostly tied to antibiotic consumption.

3. Microbiota enumeration in respiratory pathologies suffering and healthy children

3.1. Group A: Children with respiratory pathologies

3.1.1. Enterobacteriaceae

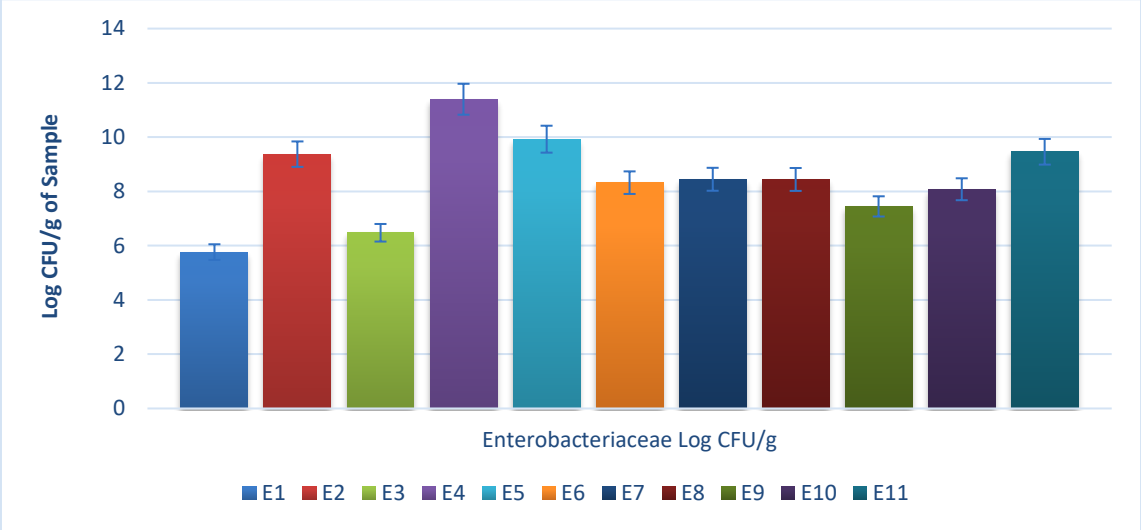


Figure 30: *Enterobacteriaceae* enumeration in diseased children.

3.1.2. Total Flora

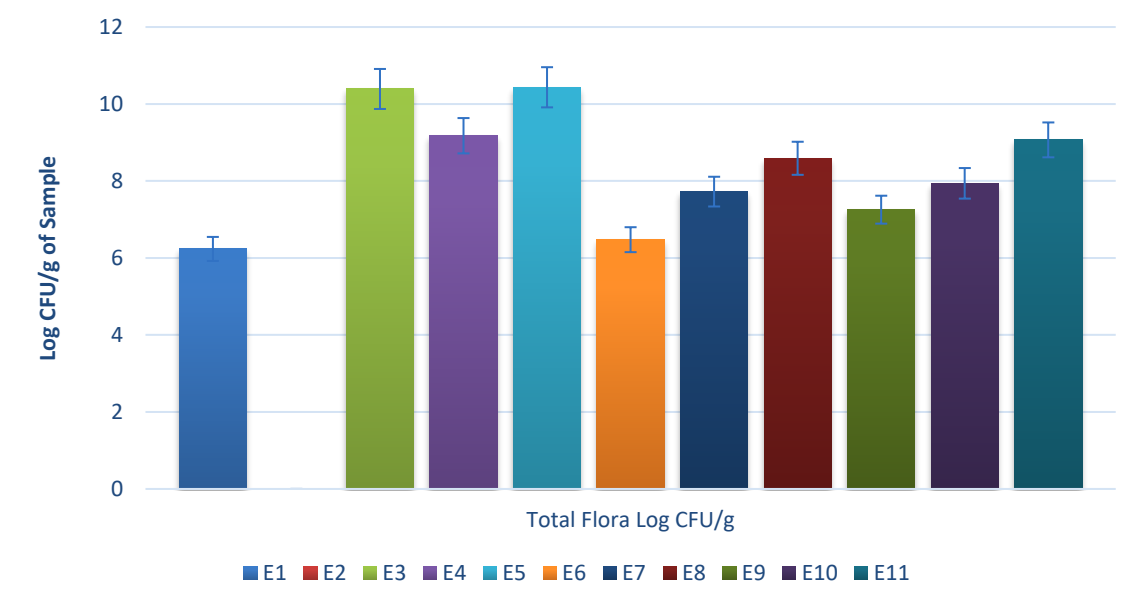


Figure 31: Total flora enumeration in diseased children.

3.1.3. Staphylococcus

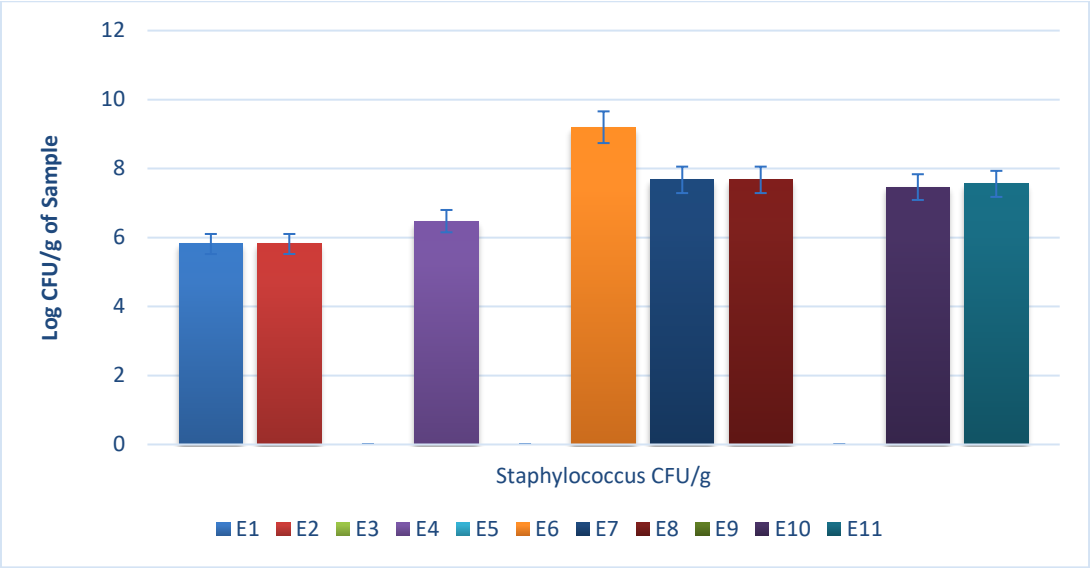


Figure 32: *Staphylococcus* enumeration in diseased children.

3.1.4. Lactobacillus

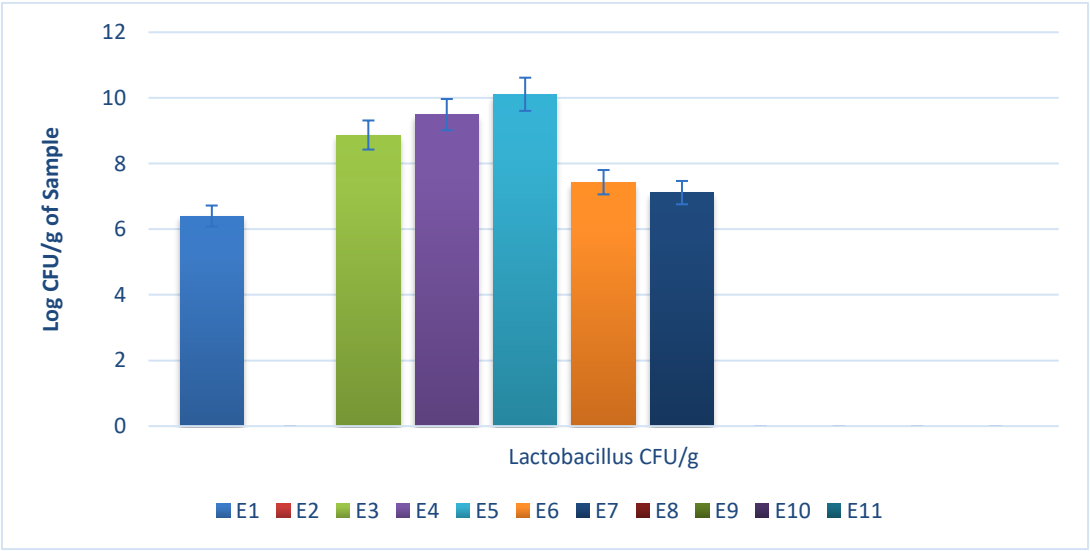


Figure 33: *Lactobacillus* enumeration in diseased children.

3.1.5. Streptococcus

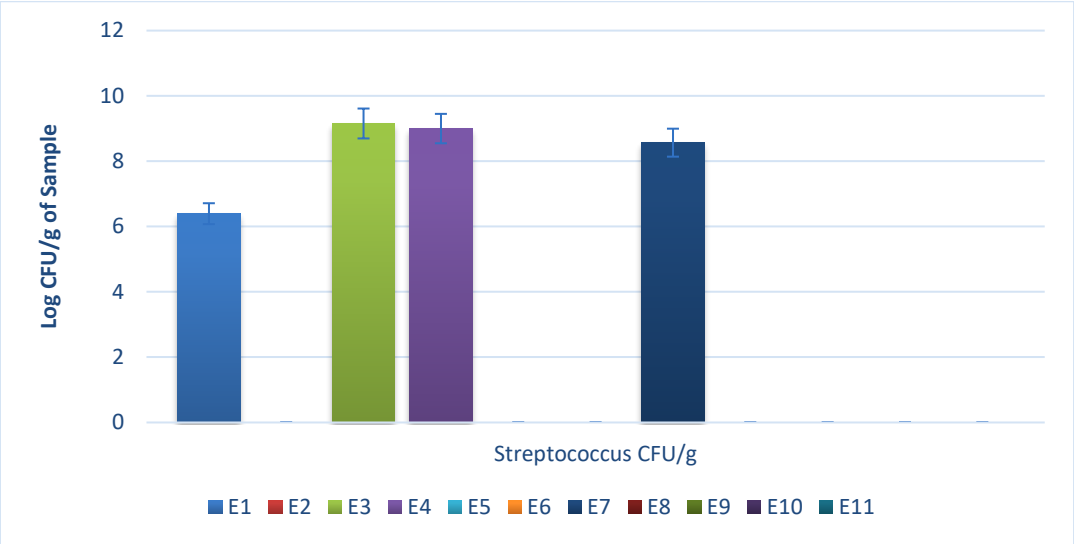


Figure 34: *Streptococcus* enumeration in diseased children.

3.1.6. Yeast

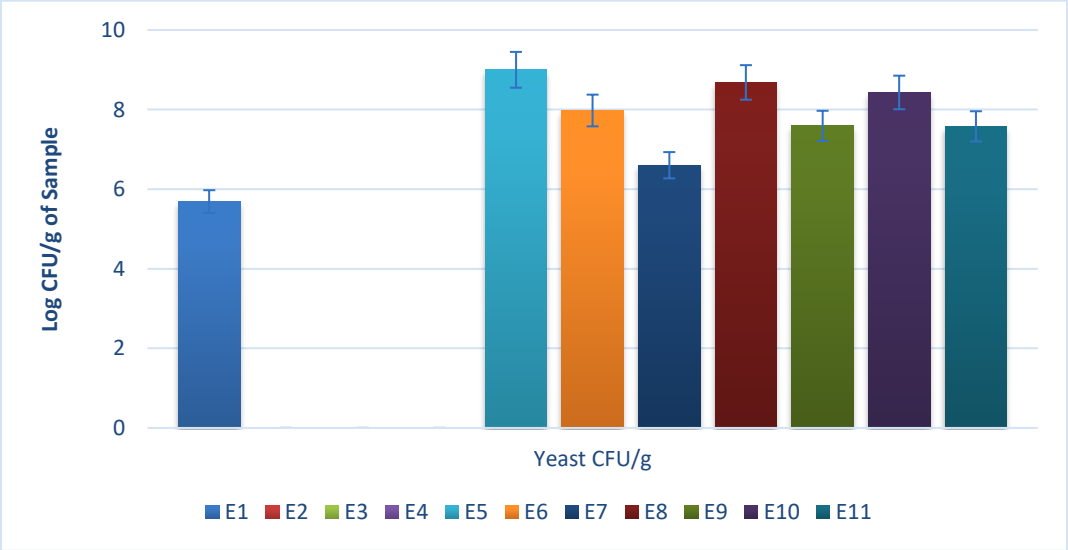


Figure 35: *Yeast* enumeration in diseased children.

3.2. Group B: Healthy Children

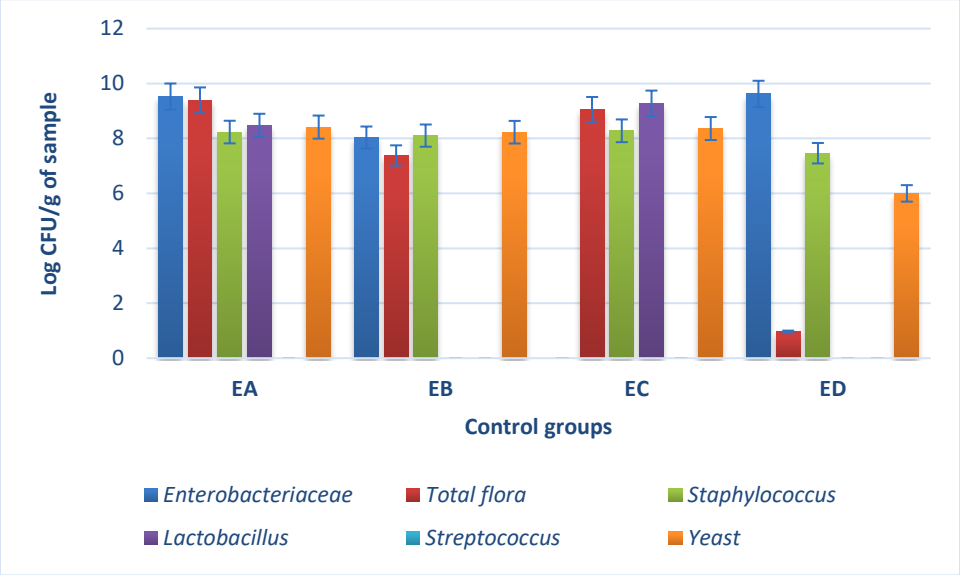


Figure 36: graph of Controls intestinal microbiota enumeration.

Intestinal bacterial distribution in the diseased and healthy children showed the constant presence of aerobic Gram-negative bacteria (*Enterobacteriaceae*). Indeed, *Escherichia coli* has been systematically isolated while other species only. Quantitatively, *Enterobacteriaceae* levels were 8 to 9 $x10^9$/g of faeces. In addition, strictly anaerobic bacteria of the genus *Bacteroides* were isolated (7-9 10^8/g tissue).

3.3. Statistical data analysis results

We would like to know if there is a difference in the intestinal microbiota between healthy individuals and individuals with respiratory diseases.

Our aim is to compare the intestinal microbiota between the two groups.

We know that bacterial counting is a continuous scale and that the study design is unpaired since the individuals in the two groups are not the same and have not been paired.

Note that it is impossible to randomly assign who should be in a healthy group and who should be in the disease group out of the 15 participants.

Given that the assumption for the unpaired t-test are fulfilled, the most appropriate t-test for this study would be an unpaired t-test, also called an independent samples t-test.

Background information:

1. Your question: compare difference
2. The scale of your data: continuous
3. The experimental design: unpaired
4. Number of groups: 2
5. Assumptions for parametric test: unpaired t-test

Null hypothesis (H0): there is no difference between the intestinal microbiota of healthy individuals and individuals with respiratory pathologies.

(mean of diseased individuals = mean of controls)

Alternative hypothesis (Ha): there is a significant difference between the intestinal microbiota of healthy and diseased individuals.

(mean of diseased individuals ≠ mean of controls)

Table 11: Results of statistical analysis.

	Diseased individuals			Healthy Controls			*p* value	α
Intestinal microbiota count Log CFU/g	Mean	SD	SE	Mean	SD	SE		
Enterobacteriaceae	8,46636485	1,581125729	0,476727345	6,794390473	4,587459889	2,29372994	0.52	**0.05**
Total flora	7,571818	2,87350743	0,866395089	6,695815496	3,92562152	1,96281076	0.70	
Staphylococcus	5,242095564	3,498021876	1,054693279	8,019487858	0,378709553	0,18935478	**0.02**	
Lactobacillus	4,492535526	4,424623134	1,33407407	4,438606197	5,135736577	2,56786829	0.98	
Streptococcus	3,010726313	4,235729793	1,277120585	0	0	0	**0.04**	
Yeast	5,595930789	3,710947697	1,11889283	7,750728576	1,169765091	0,58488255	0.11	

When the *p* value is less than α (0.05), we reject the H0 and we conclude that there is a significant difference between the means.

For example:

Staphylococcus: *p* value < α (0.02 < 0.05)

Streptococcus: *p* value < α (0.04 < 0.05)

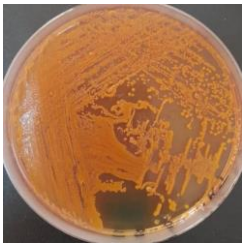
In this case, we reject the H0 and we accept the alternative hypothesis that there is a statistically significant difference in *Staphylococcus* and *Streptococcus* counts between the diseased and healthy individuals.

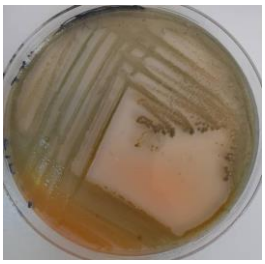
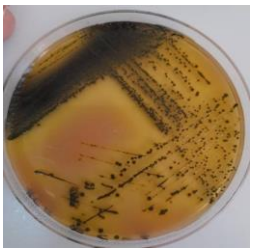
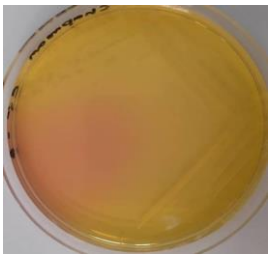
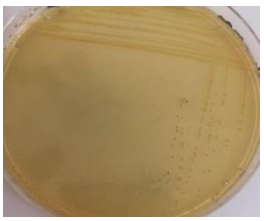
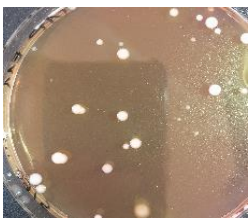
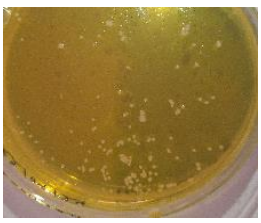
4. Micro–Macroscopic Identification

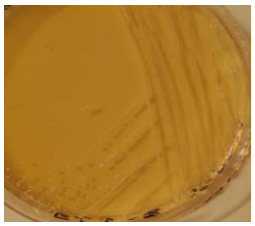
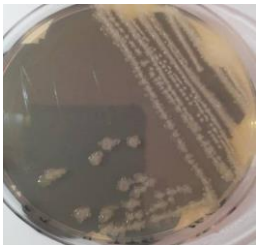
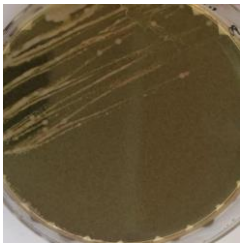
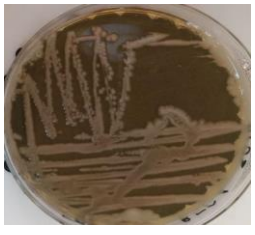
4.1 Macroscopic Identification

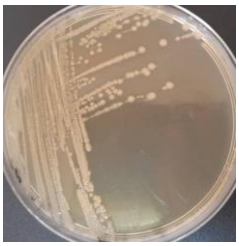
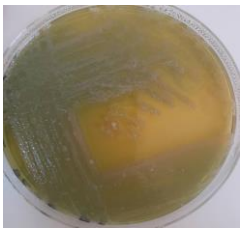
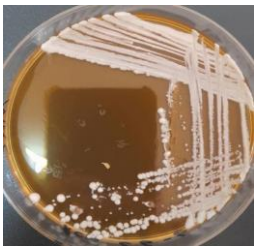
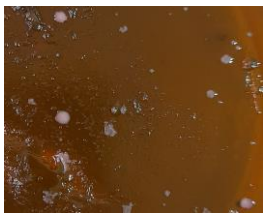
The purified strains, chosen for their similar macroscopic and microscopic criteria between multiple purifications, are organised following the sample label, the medium used, dilution, Aspect, the results of identification, and a representative picture, in the table below:

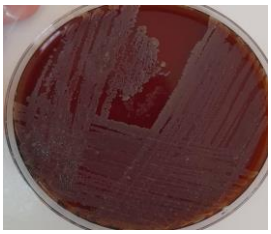
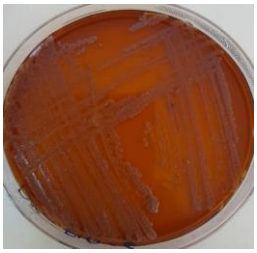
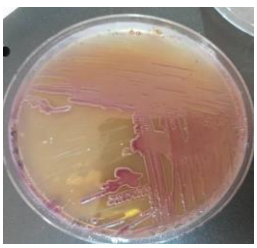
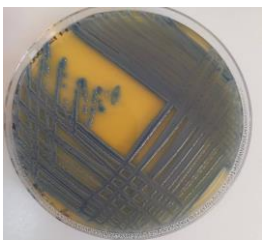
Table 12: Macroscopic Identification of healthy and disease cases with each sample strain.

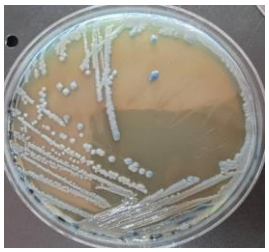
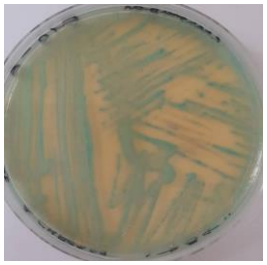
Medium	Macroscopic Identification				Figure	Strains
	Colour	Size	Form and Margin	Opacity and elevation		
Hektoen	Salmon	1-2mm	Circular, Smooth	Opaque, Flat		ED; E4; E8; E10; E11; EA; E2; E5; E7; E8.
		3-4mm	Circular, Smooth	Opaque, elevated		E2; E3; E5; E7; E9; E10; EA; EC; ED; E7.
	Salmon brownish black	4_5mm	Circular, smooth	Opaque, elevated		EA.

	Blueish green	1mm	Circular, Smooth	Transluent, Flat		EB; E10; ED; E11; EB;
	Blue_ Green with black centre	1mm	Circular, Smooth	Opaque, elevated		EC; E2; E3; E8; E11
Chapman	Yellow	5_6mm	Circular, smooth	Translucid, Flat		E10; E8; E6,
		3mm	Circular, Smooth	translucid, flat		ED; E7; E8; E10; E5.
		<1mm	Circular, Smooth	Translucid, elevated		EA; E7
	White	5mm	Circular, Smooth	Opaque, elevated		E11; E10,
		2mm	Circular, smooth	Opaque, elevated		E6; EA; E8,

		2_3mm	Circular, Smooth	Opaque, Flat		E8; EC
		1mm	Circular, Smooth	Opaque, Flat		ED; EC; E2,
Columbia	White	5_6mm	Circular, undulated, wrinkled	Opaque, elevated		E1
		5_6mm	Circular, undulated	Opaque, Flat		E4_3,
		1mm	Circular smooth	Opaque, flat		E7; E5;
	/	2mm	Circular, smooth	Translucid, flat		E5; E8; E7
		4mm	Circular, smooth	Translucid, flat		E11; E6;

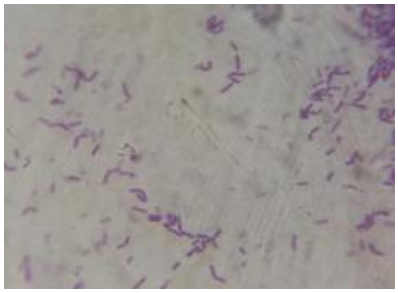
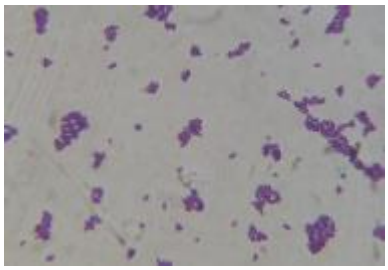
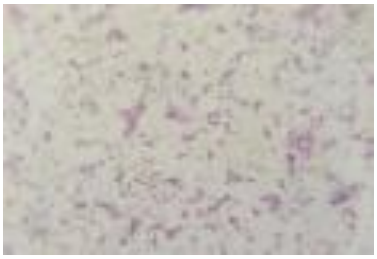
	White glistening	5mm	Circular smooth	Opaque, elevated		E7; E5;
	White greenish	5mm	Circular, smooth	Opaque, flat		EC_5,
MRS		2mm	Circular, smooth	Translucid , flat	-	E2; E3; E7
	white	1-2mm	Circular, smooth	Opaque, convex		EC
		1mm	Circular, smooth	Opaque, elevated		EA; E11; EC; E1; E2; E6; E10
EMB	white with pink centre	1_2mm	Circular, undulated	Opaque, flat		E6; E9
	Greyish purple	2_3mm	Circular, undulated	Opaque, flat		E6;

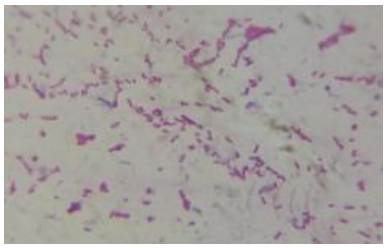
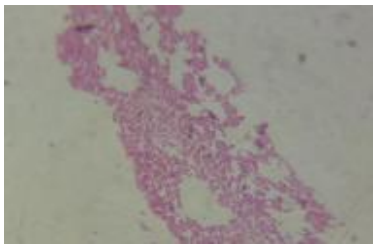
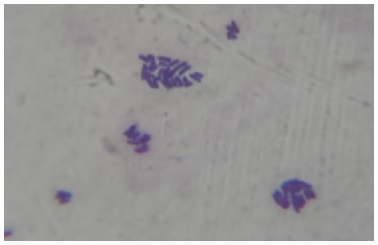
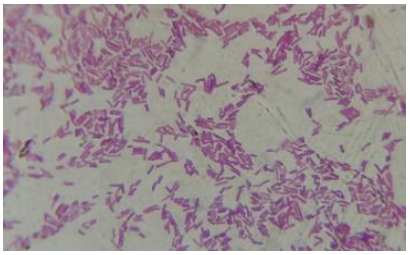
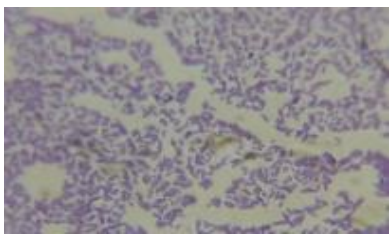
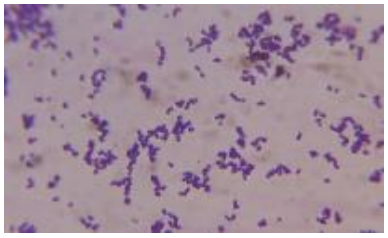
DX	Whitish brown	3mm	Circular, smooth	Opaque ,elevated		E10,
	glistenin greenish brown	1mm	Circular, smooth	translucid, elevated		E10; EC; E7;
Schaedler	brown	3mm	Circular, undulate d	opaque, flat	-	EC,
	green	4mm	Circular, undulate d	opaque, flat	-	EC,
Sabouraud	White glistenin g	5mm	Circular, smooth	Opaque, elevated		E6, E7; E10; E11; EA
		2mm	Circular, smooth	Opaque, flat		E10; EB; E7; E11
Chromagar	Purple red	<1mm_ 2mm	Circular, smooth	Opaque, flat		E11HKT; E2 ; E3; E7; E1
	Dark blue	mm	Circular, smooth	Opaque, convex		E5; E11;

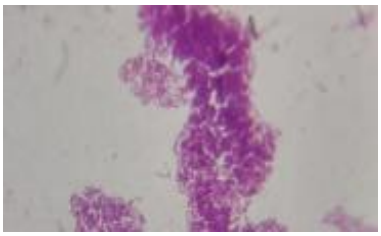
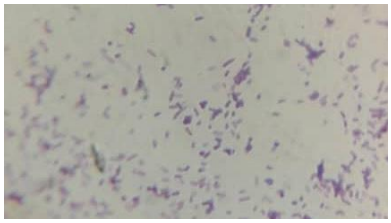
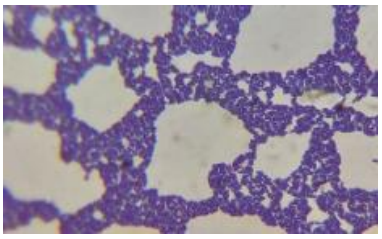
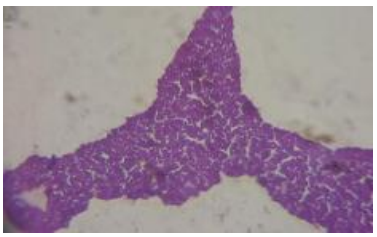
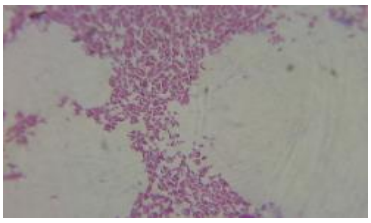
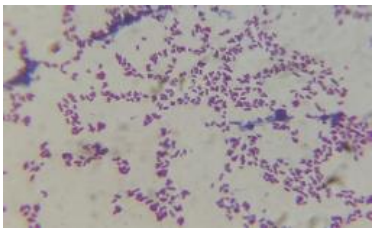
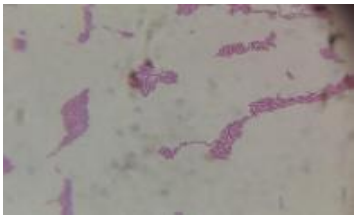
	Greyish blue	2mm	Circular, smooth	Opaque, convex		E8;
	Turquois blue	mm	Circular, smooth	Opaque, elevated		E1; E3; E5; E7; E10 chapman

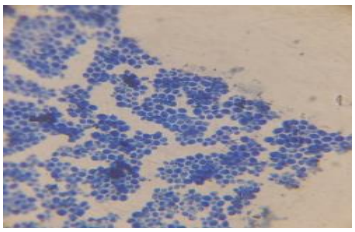
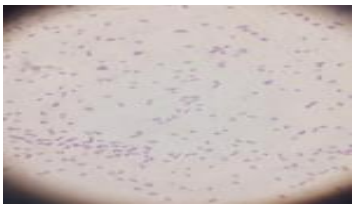
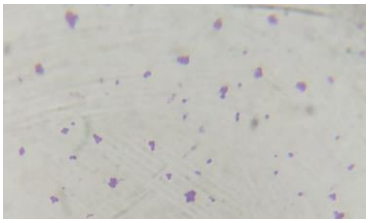
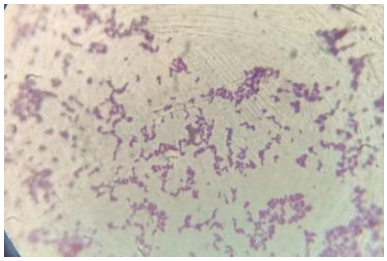
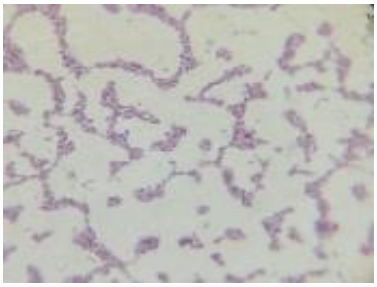
4.2. Microscopic identification

Table 13: Results from the microscopic identification.

Medium	Strain	Form	Gram	Figure
Hektoen	Salmon, green, salmon with black centre, green with black centre, salmon brown	Bacilli	–	
Chapman	Yellow	Diplococci, cocci clusters, cocci chains, cocci tetrads	+	
	White	Cocci amon, clusters	+	

	White	Bacilli	+	
			–	
Columbia	White	Bacilli	+	
	Translucid	Bacilli	–	
			+	
		Diplococci, chain	+	
	White glistening	Bacilli	–	

				
	Greenish white	Bacilli	–	
MRS	White	Bacilli	+	
		Diplococcus, cocci chain		
	Translucent	Cocci clusters	+	
EMB	Purple grey, pink	Bacilli	–	
DX	Green, Brownish white	Bacilli	–	
Schaedler	Brown, Green	Bacilli	–	

Sabouraud	white	Yeast	/	
Chromagar	Purple	Bacilli	_	
	Blue turquoise	Diplococci	+	
	Grey Blue	Cocci chain	+	
	Drak Blue	Bacilli	_	

The microscopic identification of 59 strains gave the following results:

Among group A: 35 (64.8%) strains were gram +, and 19 (35.2%) were gram –. 21 (38.9%) of them were bacilli, cocbacilli or bacilli, and 33 (61.1%) cocci.

Among group B: 12: (60%) were gram –, 8 (40%) were gram +, 16 (80%) were Bacilli (among them bacilli and cocobacilli) and 6 (20%) were cocci.

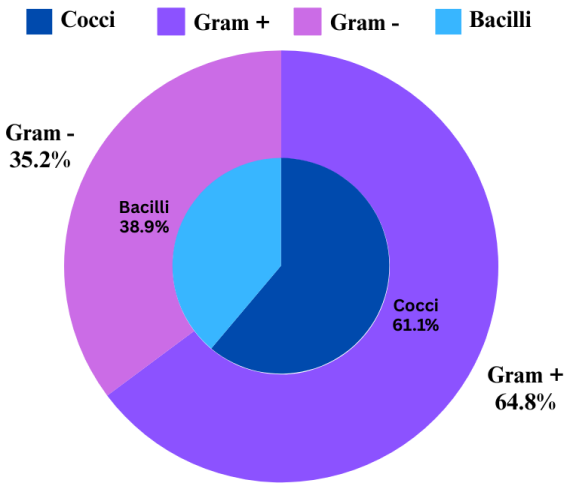


Figure 37: The percentage of gram +/_; Bacilli/Cocci found in Group A.

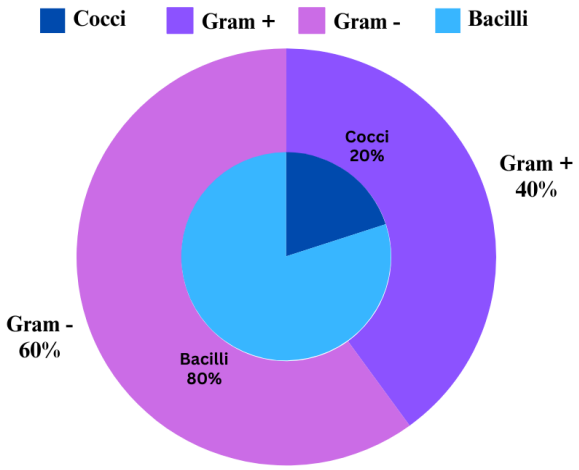


Figure 38: The percentage of gram +/_; Bacilli/Cocci found in Group B.

5. Biochemical identification

5.1. Identification of Gram+ Cocci

Table 14: Results of *Staphylococcus* and *Enterococcus* suspected strains identification.

Strain	Catalase	Mannitol	VF	Coagulase	Identification
E5	+	+	Aeroanaerob	+	***Staphylococcus aureus***

E6	+	+	Aeroanaerob	+	***S.aureus***
ED	+	+	Aeroanaerob	+	***S.aureus***
E5	+	_	Aeroanaerob	_	***S.xylosus***
E10	+	_	Aeroanaerob	_	***S.xylosus***
E3	+	/	Aeroanaerob microaerophile	/	***Pediococcus***
E8	+	_	Aerobic strict	/	***Micrococcus***
E7	_	+	aero anaerobic	/	***Enterococcus .sp***
E10	_	/	Aeroanaerob	/	***Enterococcus .sp***
E5 columbia	_	+	Aeroanarob	/	***Enterococcus .sp***
E5	_	+	Aeroanaerob	/	***Enterococcus. sp***
EB columbia	_	/	Aeroanaerob	/	***Enterococcus .sp***
EC	_	+	/	/	***Enterococcus .sp***
EC MRS	_	/	Aeroanaerob	/	***Enterococcus .sp***
E6MRS	_	/	Aeroanaerob	/	***Enterococcus .sp***

Followed by the standard identification, the strains suspected as *Enterococcus*, were purified on Chromagar medium and given a blue turquoise color, following the Chromagar orientation, confirming the results.

In another attempt to identify certain strains following the Chromagar orientation criteria, the strain E5 Chapman which was identified as an *Enterococcus sp*. was later purified on chromagar orientation and gave a blue turquoise coloration. Following these results, suspected *Enterococcus* strains were isolated on chromagar for identification. The same method followed E8 Hkt *E.coli* identification, which gave purple colouration, E11 HKT *Klebsiella* which gave Dark blue colouration, and *Streptococcus* identification, which gave dark blue-grey colouration.

The biochemical results of the staphylococcus identification are as follows:

Table 15: Identification results of *Staphylococcus* strains with API system.

Strain	X	GLU	FRU	MNE	MAL	LAC	TRE	MAN	XLT	MEL	NIT	PAL	VP	RAF	XYL	SAC	MDG	NAG	ADH	URE	Identification
E6 Chapman	–	+	+	-	+	-	+	+	+	-	+	-	+	+	+	+	-	+	+	-	***Staphylococcus xylosus***
E10 Chapman	–	–	–	–	–	–	+	+	+	+	+	–	–	+	–	–	–	–	+	–	***Staphylococcus .sp***

Table 16: The biochemical identification of *Enterococcus* presumed strains.

Strain	ADH	PAL	RAF	MAN	TRE	VP	LAC	Hemolysis	Identification
EC MRS	+	/	+	+	+	+	–	/	***Enterococcus .sp***
E5 chapman	+	/	+	+	+	+	–	γ	***Enterococcus gallinarum***

Table 17: The Biochemical identification of *Streptococcus* presumed strains.

Strain	VP	ADH	MAN	LAC	TRE	RAF	Hemolyse	Identification
ED	+	_	+	_	+	_	/	***Streptococcus agalactiae***
E5	+	+	+	+	+	+	β	***Streptococus aeruginosa***
EC	_	+	+	_	_	+	α	***Streptococcus***
E7	–	+	+	–	–	+	/	***Aerococcus sp***

Following the macroscopic, microscopic, biochemical identification test, and the chromagar results, among the 33 identified cocci, 5 were *Staphylococcus* (15.2%), 4 were *Streptococcus* (12.1%), 1 *Pediococcus* (3%), 1 *Micrococcus* (3%) , 1 *Aerococcus* (3%) , and 21 *Enterococcus* (63.6%).

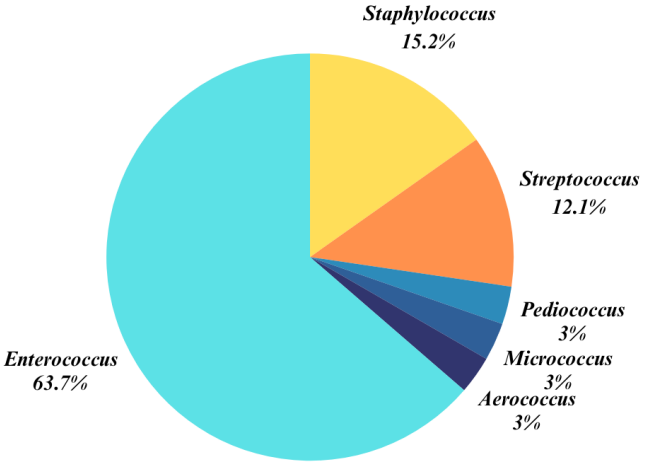


Figure 39: Pie chart with the identification results for Cocci G +.

5.2. Identification of Gram- Bacilli

Table 18: The standard identification tests for Gram- Bacilli.

Strain	Oxidase	Mannitol	LDC	ADH	Mobility	VF	Lactose	Glucose	Citrate	Nitrate	Identification
E7	-	+	-	-	-	Aeroanaerob	-	-	-	+	***Proteus vulgaris group***
EC	+	+	-	-	+	/	-	-	-	+	***Pseudomonas .sp***

Strain	Oxidase	Mannitol	Motility	VF	Nitrate	Identification
E4	–	+	/	Aeroanaerob	/	***E.coli***
E8	–	+	+	Aeroanaerob	+	***E.coli***
EB	–	–	+	Aeroanaerob	/	***E.coli***
E11S	–	+	+	/	+	***Enterobacter cloacae***
E10	–	–	–	Aeroanaerob	/	***Enterobacter cloacae***
E6	–	+	+	/	+/N2	***Enterobacter aerogenes***
E11B	–	+	+	/	+	***Serratia***
E11C	–	+	–	/	+	***Proteus***
E7	–	+	–	Aeroanaerob	+/N2	***Pantoea***
E9	–	+	–	Aeroanaerob	–	***Enterobacteriaceae***
E6	–	–	/	/	–	***Providencia rettgeri***
ED	_	+	/	/	+	***Klebsiella pneumoniae Sp pneumonia***
E11	_	+	_	Aeroanaerob	+	***Proteus vulgaris***
EC	_	+	/	Aeroanaerob, H2S, Gaz	/	***Salmonella .spp***
EA	–	+	+	Aeroanaerob	+/N2	***Enterobacteriaceae***
EA	–	+	–	/	-	***Enterobacteriaceae***
EC	–	+	+	Aeroanaerob	/	***Enterobacteriacea***
E8	–	+	+	/	+	***Enterobacteriaceae***
EB columbia	–	+	–	Aeroanaerob, H2S, Gaz	+/ N2	***Enterobacteriaceae***

The API identification results of Bacilli gram-, from both groups, were put in the biochemical identification database; they followed the highest probability, or the most probable. The results were as follows:

Table 19: Gram- Bacilli identification with API system.

Strain	ONPG	ADH	LDC	ODC	CIT	H2S	URE	TDA	IND	VP	GEL	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA	Identification
E4 Hkt, salmon	–	–	+	–	–	–	–	–	+	–	–	+	+	–	–	+	–	–	/	/	***E.coli 2***
E8 Hkt, Salmon	+	+	+	–	–	–	+	–	+	–	–	+	+	+	+	+	+	–	–	+	***E.coli 1***
EB Hkt	–	–	–	–	–	–	–	–	–	–	–	+	+	+	–	–	–	–	–	–	***E.coli 2***
E11 Hkt, salmon	–	+	+	+	+	–	–	–	–	+	–	+	+	+	+	+	+	+	+	+	***Enterobacter cloacae***
E10 DX	–	+	+	+	+	–	–	–	–	+	–	–	+	+	+	+	+	+	+	+	***Enterobacter cloacae***
E10DX	+	+	+	+	+	–	–	–	–	+	–	+	+	–	+	+	+	–	+	+	***Enterobacter cloacae***
EA Hkt	+	+	+	–	+	–	+	–	–	+	–	+	+	+	+	+	+	+	+	+	***Enterobacter cloacae***
E6 EMB pink	+	–	+	–	+	–	+	–	–	+	–	+	+	+	+	+	+	+	+	+	***Enterobacter aerogenes (Klebsiella)***
E9 Hkt	+	+	+	+	+	–	–	+	+	+	+	+	+	–	+	+	+	+	–	+	***Serratia***
E11 Hkt, Black	+	–	+	+	–	–	–	–	+	+	–	+	+	+	+	+	+	+	–	+	***Serratia oderifera 1***
E7 columbia	–	+	–	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	–	–	***Pantoea .sp***
E11 columbia	–	–	–	–	–	+	–	+	–	–	+	+	+	+	–	–	+	–	–	–	***Proteus vulgaris***

E6 EMB grey	–	–	–	–	+	–	–	+	–	–	–	–	–	+	+	+	+	–	–	+	***Providencia rettgeri***
EC Hkt	_	+	_	_	+	+	_	_	_	_	_	_	+	+	+	+	_	+	_	_	***Salmonella .spp***
ED Hkt	+	+	+	+	+	_	_	_	_	+	_	+	+	_	+	+	+	+	+	+	***Klebsiella pneumoniae Sp pneumonia***
E8 Chapman	_	_	_	_	_	_	_	_	_	+	+	_	+	+	+	_	+	_	_	–	***Pasteurella pneumotropica/ Mannheimia haemolytica***
E11 Chapman	+	+	+	–	+	–	+	–	–	+	–	+	+	+	+	+	+	+	+	+	***Klebsiella pneumonia Spp ozaenae***

The bacilli strains that grow on Chapman media may present with Halophil characteristics or were the result of strain specific adaptation. However, since the identified strains do not correlate with such characteristics, we are led to presume that the growth is the result of strain Growth conditions, preparation or medium issues, or contamination.

5.3. Identification of Bacilli Gram+

The biochemical identification results were put on an API Bacillus database in the site and followed the highest probability. Results with multiple low probabilities or improbable identification results were identified solely as *Bacillus*. A high probability was taken as a result, the identification was as follows:

Table 20: Bacilli G+ identification results.

Sample	ONPG	ADH	LDC	ODC	CIT	H2S	URE	TDA	IND	VP	GEL	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA	Identification
E1 columbia	+	+	–	–	–	–	–	–	–	–	+	+	+	–	–	–	+	–	+	–	***Bacillus lentis***
E4 columbia	+	+	+	–	–	–	+	+	–	–	+	+	–	+	+	–	+	–	+	–	***Virgibacillus pantohenticus***
E2 MRS	–	–	–	+	–	–	+	–	+	+	+	+	–	+	–	–	–	+	+	–	***Bacillus***
EA MRS	–	–	–	–	+	–	–	–	+	+	–	+	+	+	+	+	+	+	+	+	***Bacillus circulans***

5.4. Identification of Anaerobic Gram- Bacilli

The strains grown on the Schaedler medium were identified following an anaerobic identification database. The results were as follows:

Table 21: Anaerobic bacilli G- identification results.

Strain	IND	URE	GLU	MAN	SAC	ARA	GEL	SOR	RHA	CAT	Gram	Identification
EC	-	+	+	+	+	+	-	-	+	+	-	***Bacteroides ovatus/thetaiotaomicron***
EC	+	+	+	-	-	+	+	-	+	/	-	***Bacteroides ovatus/thetaiotaomicron***

Group A: Among the 33 identified cocci, 5 were *Staphylococcus* (9.3%), 4 were *Streptococcus* (7.4%), 1 *Pediococcus* (1.9%), 1 *Micrococcus* (1.9%), 1 *Aerococcus*(1.9%), and 21 *Enterococcus* (38.9%). Among 21 identified Bacilli, 15 were *Enterobacteriaceae* (27.8%), and 6 were *Bacillus* (11.1%).

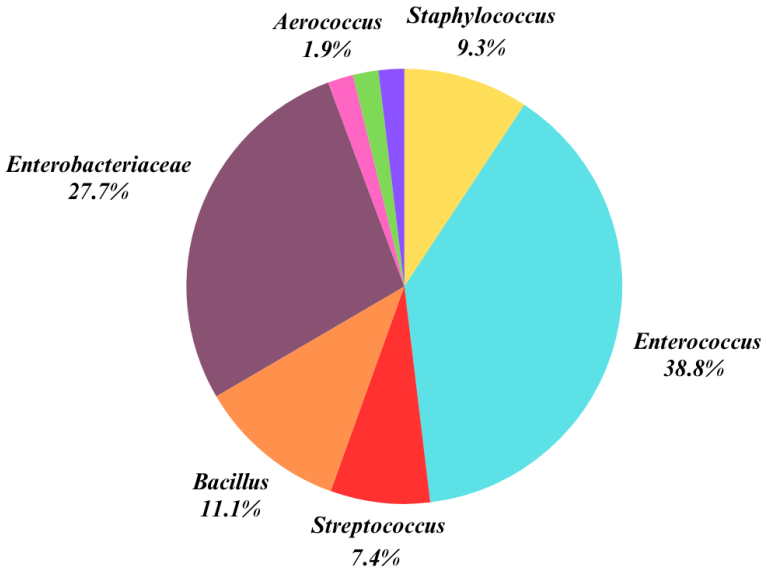


Figure 40: Pie chart with the identification results for Group A.

Group B: Among 6 cocci, 1 was *Staphylococcus* (4.5%), 4 were *Enterococcus* (18.2%), 2 *Streptococcus* (9.1%). In addition, among 16 bacilli, 1 *Pseudomonas* (4.5%), 2 *Bacteroides* (9.1%), 1 *Bacillus* (4.5%), 12 *Enterobacteriaceae* (54.5%).

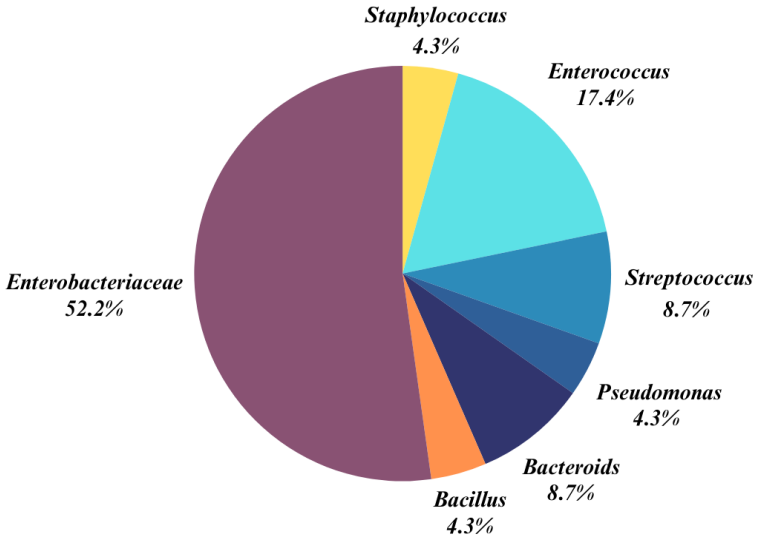


Figure 41: A pie chart with identification results for Group B (Healthy).

5.5. Yeast identification

Biochemical identification results of yeast are displayed in table 23. Yeast strain probability was given using an identification database.

Table 22: Yeast biochemical identification.

Strain	GLU	XYL	XLT	MDG	NAG	LAC	MAN	SAC	TRE	RAF	HYPH	Identification
E6	+	+	+	–	+	–	+	–	+	–	–	***Cryptococcus laurentii***

It is important to note that sample E6 was taken from a child presenting cutaneous infection.

5.6. Nitrate reduction

Among 26 verified and identified strains, 18 were nitrate reduction positive (68.23%), 3 were negative (11.54%), and 5 were inconclusive (19.23%).

Discussion

The microbiota is a component of the biological barrier, which has positive and negative effects on intestinal permeability. Anaerobes (Firmicutes and Bacteroidetes) are the main bacteria present in the gastrointestinal tract and represent about 90% of all microbiota. The host provides the gut microbiota with an anatomical niche for bacterial attachment and growth, nutrients and substrates, bacterial metabolism and biological processes, and immune tolerance that allows the microbiota to thrive

In respiratory disease cases (Group A), results showed a predominant *Enterococcus* flora, followed by *Enterobacteriaceae*, *Bacillus*, and a collection of *Staphylococcus*, *Streptococcus*, *Aerococcus*, *Pediococcus*, and *Micrococcus*. In the controls (Group B), the results showed a predominant *Enterobacteriaceae*, *Enterococcus*, *Streptococcus*, and a collection of *Bacillus*, *Staphylococcus*, *Bacteroides*, and *Pseudomonas*.

The Group A results showed higher diversity in Gram-positive cocci, such as *Staphylococcus* and *Enterococcus*, and fewer Gram-negatives. Group B had higher *Enterobacteriaceae* and lower *Enterococcus* in comparison with Group A. Both groups presented with *Staphylococcus* (A > B) and *Streptococcus* (A > B) strains. The abundance of Gram-positives in respiratory disease children correlates with the results of community-acquired pneumonia cases in 0-3-year-old infants (Ren *et al.*, 2020). The *Streptococcus* and

Enterococcus abundance results correspond with bronchiolitis and severe bronchiolitis in afflicted children as well (Raul *et al*., 2024).

The fermentative-type results showed similarities between the two Groups. The strains of Group B were aerobic facultative, but better growth was observed under aerobic conditions. In addition to identifying aerobic and microaerobic strains such as *Micrococcus* and *Aerococcus*, a deduction was made that the Group A flora may present with higher oxygen tolerance than Group B. This result is supported by findings in pneumonia infection cases, where a higher abundance of aerobic or microaerophilic bacteria was found in the gastrointestinal tract of young children (Ren *et al*., 2020).

Nitrate reduction by gut bacteria is affected by oxygen levels. High oxygen levels favour aerobic respiration over nitrate reduction, whereas low oxygen levels encourage nitrate reduction as an alternative pathway for energy production to sustain microbial growth and function. The predominance of nitrate-reducing microorganisms corresponds to the gut microbiota's overall ability to reduce nitrate (Tiso *et al*., 2015).

Given the researched link between oxygen availability and respiratory pathways, it is safe to assume that higher oxygen levels in the gastrointestinal system owing to lung inflammation may shift microbial metabolism toward aerobic respiration rather than nitrate reduction. The ramifications of nitrate reduction by gut bacteria are still being investigated. However, new research suggests that nitrate supplementation may benefit pulmonary inflammation, implying possible therapeutic implications that require further study (Ritz *et al*., 2021).

The high percentage of *Enterobacteriaceae* species present in Group A were identified as *Providencia*, *Proteus*, *Pantoea*, *Serratia*, and *Enterobacter*. These strains present a significant risk for children due to their potential pathogenicity and resistance mechanisms.

From several samples, *Enterobacter cloacae* were identified, famously known to cause nosocomial infections, even in post-natal cases at 2 months of age, with recorded cases of gastrointestinal tract lesions and, rarely, meningitis (Bonadio *et al*., 1991). It has also been associated with cases of pneumonia or respiratory exacerbation in pediatric units (Lasme-Guillao *et al*., 2011). Its presence in Group A is an indicator of nosocomial infections in samples E11 and E10, leading to pneumatic symptoms

Similarly, to *E. cloacae*, the identified *Pantoea* strain causes nosocomial infections, most commonly by *Pantoea agglomerans*. This infection can be caused by a previously mild non-*Pantoea* infection or contact with contaminated medical equipment and fluids (Shraddha *et al*., 2018). The identification was made from sample E7, an immune-deprived child presenting with respiratory exacerbations and undergoing oxygen treatment. The results indicate that the identified strain may have resulted from a nosocomial infection and highlight the risk to immune-deprived patients with respiratory diseases in clinical settings.

The identified bacterium *Serratia* causes urinary infections, respiratory infections, and various other diseases. In pediatric clinical settings, the strain may be found in both the gastrointestinal tract and the respiratory tract; colonisation begins in the respiratory tract and then spreads to the gastrointestinal tract. This leads to a higher abundance in the lungs, making them a reservoir for *Serratia* in respiratory infections (Albers *et al*., 2001). This suggests that the high abundance of *Serratia* in the lungs, subsequently spreading to the gut, may correlate with the clinical symptoms present in patient E11.

While the previously identified strains are common nosocomial infection agents, *K. pneumoniae* can be both a commensal human bacterium and a nosocomial infection agent, depending on various factors. However, the presence of this strain does not definitively indicate a cause of respiratory infections or exacerbations, although it is a possible causative agent. *K. Pneumoniae subsp*. Ozaenae on the other hand is a pathogenic bacterium that contributes to a chronic Atrophic rhinitis infection, also called ‘ozone’, and is commonly isolated from Bronchitis and meningitis infection cases (Delmas, 2018). The presence of the strain in faecal matter and the results of The case E11 survey revealed that the patient presented with Nasal obstruction, mucus production, and respiratory problems, all correlating to a *K.pneumoniae subsp. Ozaenae* infection.

While *Enterobacteriaceae* and *bacteroids* have a high presence in Pneumonia (Ren *et al*., 2020) and bronchiolitis (Raul et al., 2024), our study showed contrary results with few *Enterobacteriaceae*, mostly *E. coli*, a gut commensal bacterium.

The Group A strains were mostly Enterobacteriaceae and *Bacteroides* correlating with the results for healthy gut microbiota (Ren *et al*., 2020). The identification of *Salmonella*, A pathogen bacterium, is an indicator of the disease, but the age of the control patient who had the sample collected is to be considered. Since all control samples were from less than one-

week-old neonates, group B is not only that of healthy children but of the first colonizing bacterium in the gastrointestinal tract, since the faecal matter was that of meconium. The identification of *Pseudomonas*, *Enterobacteria*, *streptococcus* and *enterococcus* correlate with the results found from neonate gut microbiota test using a meconium sample (Yi-Sheng Chang *et al*., 2023).

The identification of the Yeast strain did not present with *Candida* identification, however, the identified *Cryptococcus laurentii*, is a rare pathogenic yeast that causes cryptococcosis, a pneumatic infection. Recent records revealed Gastric cryptococcosis in an immune-deprived patient who presented gastroesophageal reflux, abdominal pain, vomiting, and acute worsening of chronic watery diarrhoea (Walter de Araujo Eyer-Silva *et al*., 2019). While the patient had similar symptoms and presented with cutaneous infection, it is unlikely that this is a gastric Cryptococcosis infection but more likely that the sample was contaminated with the cutaneous infection during sampling.

6. Antibiogram results and interpretation

After 18-24h of incubation, the results of antibiotic susceptibility were revealed. Varied resistance patterns among the strains tested were observed. The diameter of the inhibition zones was measured using a ruler. The results are displayed in (Tab.24).

Table 23: Antibiotic susceptibility test results based on CASFM and PIA standards.

Strain	AMP	IPM	VA	CN	NA	CD	NTX	CZ	E	TE	COL
E. coli	R	-	-	S	S/S	-	R	R	-	-	-
E. cloacae 1	R	S	N/A	S	-	-	-	-	-	-	-
Salmonella ssp	R	S	N/A	R/I	-	-	-	-	-	-	-
Klebsiella pneumoniae	R	-	-	S	S	-	R	R	-	-	-
Proteus vulgaris	R	-	-	S	S	-	R	R	-	-	-
Bacteroides ovatus /thetaiotaomicron	R	S	R	N/A	-	-	-	-	-	-	-
Enterococcus sp. 1	R	-	-	-	-	N/A	-	-	R	R	-
Enterococcus sp. 2	R	-	-	-	-	N/A	-	-	R	R	-
E. aerogenes	R	-	-	S	S/I	-	R	R	-	-	-

Pasteurella pneumotropica/Manheim-ia haemolytica	R	N/A	N/A	N/A	-	-	-	-	-	-	-
K.pneumoniae ssp ozaenae 1	-	-	N/A	S	-	-	-	-	-	-	CMI
K.pneumoniae ssp ozaenae 2	R	S	N/A	R/S	-	-	-	-	-	-	-
Serratia oderifera	R	-	-	S	S	-	S	R	-	-	-
E.cloacae 2	R	-	-	R	S/I	-	-	R	-	-	-
E.cloacae 3	R	-	-	R	S/I	-	R	R	-	-	-
Pseudomonas spp	R	-	N/A	S	-	-	-	-	-	-	CMI
Bacteroides ovatus /thetaiotaomicron	R	S	R	N/A	-	-	-	-	-	-	-
E.gallinarum 1/2	N/A	-	-	-	-	N/A	-	-	N/A	N/A	-
Enterococcus	R	-	-	-	-	N/A	-	-	N/A	N/A	-
Bacillus spp1	R	-	-	S	N/A	-	-	N/A	-	-	-
Bacillus spp2	-	-	-	-	N/A	-	N/A	R	-	-	-

S: Sensitive **N/A: Not available**
R: Resistant **- : not tested**
I: Intermediate

Note: results are interpreted based on both CASFM and PIA standards. Results are written in the form of **CASFM/PIA** as in **R/S** in order

Antibiotic susceptibility test results for 16 strains from different samples were based on CASFM and PIA interpretation for their susceptibility towards antibiotics. The following figures are some of the interpreted results (Fig.42).

A) **B)**

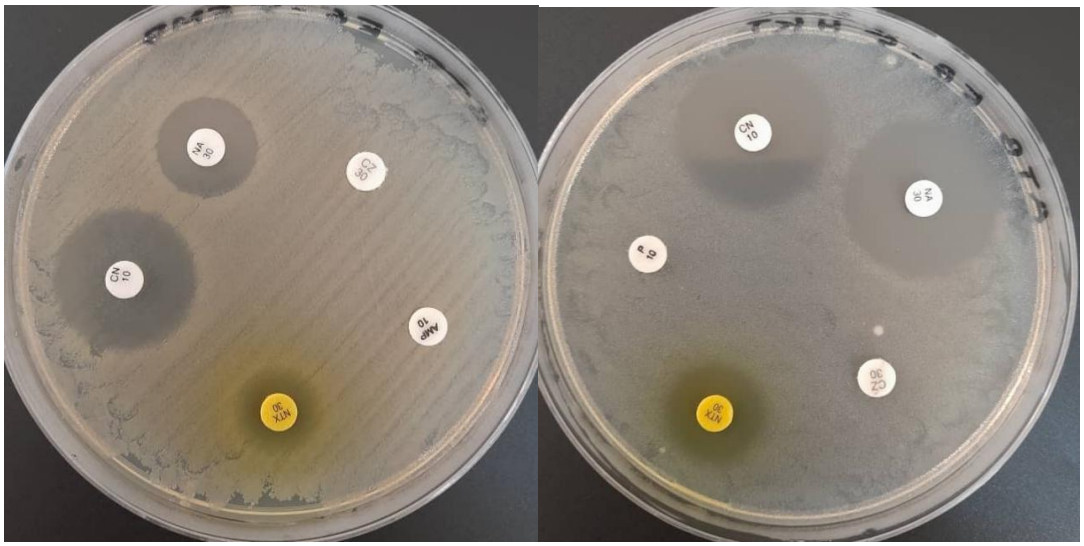


Figure 42: Antibiogram results for two strains that belong to the Enterobacteriaceae family A) *E. aerogenes*, B) *E. coli.*

While *E. coli* was completely resistant to Penicillins and slightly resistant to Nitroxolin, it showed high susceptibility towards NA and CN in which halo diameter was 27 mm and 25 mm respectively (See Appendix).

*E. cloacae***,** on the other hand, considering the three strains from the three different samples, showed high resistance towards Penicilines (100%). Strain number 1 was sensible towards CN (33.3%) unlike the strains numbered 2 and 3 which were resistant to it (66.6%), they were also both resistant to CZ according to CASFM. Strain number 1 was highly sensible towards IPM. Strains numbered two and three were both sensible towards NA according to CASFM (2023) still they were classified as intermediate according to PIA (2020).

Salmonella ssp showed high susceptibility towards IPM, still, it was not decided if the strain was susceptible or resistant towards VA according to CASFM and PIA. CN showed two different results, according to CASFM (2023) the strain is considered resistant while according to PIA (2020), it was considered intermediate.

K. pneumoniae showed resistance towards NTX and CZ in accordance with CASFM (2020) and PIA (2020) respectively. Still, the strain was susceptible towards both CN and NA (CASFM, 2023). *P. vulgaris* has identical results as *K. pneumoniae*. *S. oderifia* on the other hand differs from them both as being susceptible to NTX (CASFM, 2020).

E. aerogenes was resistant to both NTX and CZ according to CASFM (2020) and PIA (2020). It is considered sensitive against CN and NA as CASFM stated, still, it was considered intermediate according to PIA (2020).

K. pneumoniae ssp ozaenae was sensible towards CN according to both CASFM and PIA, its susceptibility towards COL was not decided as it needed CMI to be calculated. Other *K. pneumoniae ssp ozaenae* was resistant to CN according to CASFM terms but was considered susceptible according to PIA. It was also highly susceptible towards IPM.

Pseudomonas spp is susceptible to CN according to PIA while it was considered as EPI which mainly refers to ESBL according to CASFM, it showed also a slight resistance towards VA still no criteria were there to compare to; for COL a CMI test was needed to determine strain susceptibility or resistance.

Bacteroides ovatus/thetaiotaomicron showed a sensitivity towards IPM and resistance towards VA according to CASFM. For CN the result cannot' be decided completely as the CMI wasn't' decided.

Enterococcus strains showed an outstanding result as they look completely susceptible due to the cumulative effects of the 4 antibiotics with the appearance of several resistant colonies near them.

Discussion

Antibiotics susceptibility test results for the 16 strains from different samples revealed high resistance to AMP across all the tested strains. The high susceptibility towards IPM, CN, and NA respectively was effective against the family of Enterobacteriaceae. The different Mechanisms of resistance among bacteria became a very common thing in the modern world; between intrinsic and acquired resistance, the latter harbors more danger. Most bacteria possess at least one of the four antimicrobial resistance mechanisms, which makes most antibiotics ineffective.

E. cloacae and *E. coli*, both being part of the *Enterobacteriaceae* family, exhibit resistance to penicillin by nature due to extended-spectrum beta-lactamase (ESBL) production, which hydrolyzes the beta-lactam ring, causing resistance to beta-lactam antibiotics (Reygayert, 2018). Sensitivity to CN is also common in Enterobacteriaceae, but resistance is often due to acquired mechanisms, like reduced porin numbers. CN do part of Aminoglycosides which are particularly potent against Gram-negative and Gram-positive bacteria, that includes *E.coli, K.pneumoniae, E.cloacae, Proteus spp* and *Serratia spp* which have been proven in previous studies (Krause *et al*., 2016; Arafah, 2024)

Susceptibility to NA suggests that fluoroquinolones are effective at standard dosages. Several previous and recent studies have shown similar results, with bacteria belonging to the family Enterobacteriaceae exhibiting high resistance to penicillin and other classes of antibiotics. *Salmonella spp*. and *K. pneumoniae* also showed varying resistance and susceptibility patterns. These bacteria were affected by IPM, a carbapenem, due to its mechanism of inhibiting cell wall synthesis

Resistance to CN and NA in various strains indicates acquired resistance mechanisms limiting antibiotic uptake and effectiveness. *B.ovatus/thetaiotaomicron* and other gram-

negative bacteria often exhibit intrinsic resistance to VA due to their LPS layer (Reygayert, 2018). The observed multidrug-resistant (MDR) colonies in *Enterococcus* suggest a synergistic effect from the chosen antibiotic combinations, highlighting the complex interplay of resistance mechanisms across these bacteria.

Conclusion

Conclusion

The gut microbiota is the basis of human health, the present bacteria and its abundance can be the difference between wellness and disease development. Understanding the microbiota in diseased patients can help in disease prevention, making a diagnosis, and finding the most suitable treatment to diminish antibiotic resistance acquisition or side effects. A change in lifestyle or diet in adult patients may help resolve certain cases. However, infants and neonates require a more precise and direct approach for successful treatment due to their underdeveloped immune systems. Among the most prevalent cases are respiratory infections and chronic respiratory diseases.

In our study, 15 samples were taken from hospitalized children under 4 years old, 4 were healthy neonates and 11 were patients with respiratory infections. The patient's data were collected following a survey, and the identification followed conventional methods.

Lastly, the identified strains were tested for antibiotic susceptibility. The healthy and sick patients' results showed contradictory results, indicating the importance of commensal bacteria in maintaining homeostasis. The results in both groups presented nosocomial infection strains.

The antibiotic susceptibility results for 16 bacterial strains indicate critical insights for treating respiratory infections in children. The high resistance to ampicillin (AMP) across all strains suggests that this antibiotic may be ineffective in such cases. However, the observed high susceptibility to imipenem (IPM), gentamicin (CN), and nalidixic acid (NA) within the Enterobacteriaceae family highlights alternative effective treatments.

Given the presence of both intrinsic and acquired resistance mechanisms, and the greater threat posed by acquired resistance, it is crucial to monitor and tailor antibiotic use in pediatric care to prevent the spread of multidrug-resistant (MDR) strains.

The findings underscore the need for strategic use of potent antibiotics like carbapenems and fluoroquinolones to manage bacterial respiratory infections in children, ensuring better clinical outcomes and mitigating the risk of resistance development.

Conducting research, especially in a laboratory setting, often comes with a multitude of challenges that can hinder progress and affect the quality of outcomes. One of the primary hardships that we encountered is the lack of sufficient materials, which delayed our experiments. Time constraints add another layer of difficulty .limited access to laboratory space exacerbates this issue, as competing for time in a shared facility resulted in scheduling conflicts and reduced productivity. The pressure to produce results quickly lead to rushed experiments, which increased the risk of errors and reduced the reliability of findings. Contamination is another significant concern, particularly in a busy lab where many people come and go throughout the day. Each entry and exit increase the risk of introducing contaminants into the environment, which can compromise experiments and lead to false results.

Further studies are needed to strengthen the results we have obtained and to provide more relevant clinical information. The survey study should be done on large numbers of children.

The study of the gut microbiota can be strengthened by more advanced techniques: for example, the sequencing of the gene encoding for 16S RNA or MALDI-TOF mass spectrometry, because the intestinal microflora is difficult to study. Only a limited number of bacteria can be detected using conventional culture techniques used in our study.

Finally, our preliminary results could open new perspectives in gastroenterology and pediatric pneumonia.

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Appendices

Appendix 1: Survey

Respiratory Disease Questionnaire

This questionnaire is intended to gather information about your medical history and respiratory symptoms. Please answer all questions as accurately as possible.

Section 1: General information

Age:
Gender:

Section 2: Medical History

Have you ever been diagnosed with a respiratory disease?
If so, what disease?
How long has it been?
Have you been hospitalized for respiratory disease?
If so, how many times?
Are you taking medication for respiratory disease?
If so, what medications?

Section 3: Respiratory Symptoms

Do you have a cough?
How long has it been?
Is the cough dry or productive?
Do you have sputum?
If so, what is the color of sputum?
Do you have a fever?
If so, what is the temperature of your fever?
Do you have difficulty breathing?
Do you have a feeling of chest tightness?
Do you have wheezing?
Do you have chest pain?
Other symptoms:…..

Section 4: Aggravating factors

Do your symptoms worsen during exercise?
Do your symptoms worsen with exposure to certain irritants (smoke, dust, animals, pollen, etc.)?
Do your symptoms get worse at night?

Section 5: Quality of life

Do your breathing problems affect your quality of life?

Section 6: Medical follow-up

Do you regularly see a doctor for respiratory problems?
If yes, how often?

Section 7: Conclusion

Do you have any other symptoms to report?
Do you have any questions?
The mode of delivery
The mode of breastfeeding
Another characteristic to note:

Thank you for your participation.

Appendix 2: Material and instruments used

Material	Instruments
- Sterile specimen collection container	- Hot plate stirrer
- Spatula	- Vortex
- Recipients	- Bunsen Burner
- Measuring Flask	- PH metre
- Beaker	- Water bath
- Funnel	- Balance
- Glass rod	- Optic Microscope
- Tongs	- Refrigerator
- Media bottles	- Autoclave
- Test tubes	- Incubator
- Glass slides	
- Petri dishes	
- Tubes holder	
- Eppendorfs	
- Pro-pipette	
- Pipette Pasteur	
- Micropipette	
- Micropipette's tips	
- Syringes	

Appendix 3: Mediums Composition

PBS (Phosphate buffer saline)

Composition per liter:

Sodium phosphate dibasic
Na2HPO4…….……**1.42g**

Monopotassium phosphate
KH2PO4…….…….……**0.24g**

Sodium chloride
.NaCl…… **8.0g**

Potassium Chloride
KCl……..……..**0.2g**

Nutrient Agar

Composition per liter:

Sodium chloride NaCl……...**5.0g**

Peptone…….…….....**5.0g**

Meat extract ……..……...……..…**1.5g**

Yeast extract…….……..**1.5g**

pH 7.3 ± 0.2 at 25°C

Columbia C.N.A. Agar Base with Blood

Composition per liter:

Peptone, special ……..… **23.0g**

Agar …….……. **15.0g**

NaCl …… **5.0g**

Corn starch …… **1.0g**

Sheep blood, defibrinated ……. **50.0mL**

pH 7.3 ± 0.2 at 25°C

MRS Broth

Composition per liter:

Glucose ……..… **20.0g**

Peptone ……..… **10.0g**

Beef extract ……..… **5.0g**

Sodium acetate trihydrate …… **5.0g**

Yeast extract …… **5.0g**

Dipotassium hydrogen phosphate (K_2HPO_4) ……. **2.0g**

Ammonium hydrogen citrate …….. **2.0g**

Magnesium sulfate heptahydrate (MgSO_4·7H_2O) ……..……. **0.2g**

Manganese sulfate monohydrate (MnSO_4·H_2O) …… **38.0mg**

Tween 80 (Sorbitan monooleate) …… **1.0mL**

pH 6.2 ± 0.2 at 25°C

M17 Agar

Composition per liter:

Disodium β-glycerophosphate ……... **19.0g**

Agar ……... **11.0g**

Beef extract ……. **5.0g**

Pancreatic digest of soybean meal ……... **5.0g**

Yeast extract …… **2.5g**

Ascorbic acid ……... **0.5g**

MgSO_4·7H_2O ……... **0.25g**

Lactose solution …… **50.0mL**

pH 6.9 ± 0.2 at 25°C

Hektoen Enteric Agar

Composition per liter:

Agar ……... **13.5g**

Lactose …….. **12.0g**

Peptic digest of animal tissue …… **12.0g**

Sucrose …….. **12.0g**

Bile salts …….. **9.0g**

NaCl (Sodium Chloride) ……. **5.0g**

$Na_2S_2O_3$ (Sodium Thiosulfate) …… **5.0g**

Yeast extract …… **3.0g**

Salicin …… **2.0g**

Ferric ammonium citrate …… **1.5g**

Acid Fuchsin ……... **0.1g**

Bromthymol Blue …… **0.064g**

pH 7.6 ± 0.2 at 25°C

MRS Agar (DeMan, Rogosa, Sharpe Agar)

Composition per liter:

Glucose …….. **20.0g**

Peptone …….. **10.0g**

Agar ……... **10.0g**

Beef extract …… **8.0g**

Sodium acetate·3H2O …… **5.0g**

Yeast extract …… **4.0g**

K2HPO4 …….. **2.0g**

Triammonium citrate ……... **2.0g**

MgSO4·7H2O ……. **0.2g**

MnSO4·4H2O ……... **0.05g**

Sorbitan monooleate (Tween80) ……... **1.00mL**

pH 6.2 ± 0.2 at 25°C

EMB Agar (Eosin Methylene Blue Agar)

Composition per liter:

Agar ……... **13.5g**

Pancreatic digest of casein …… **10.0g**

Lactose …… **5.0g**

Sucrose …… **5.0g**

K2HPO4 …….. **2.0g**

Eosin Y …… **0.4g**

Methylene Blue ……... **0.065g**

pH 7.2 ± 0.2 at 25°C

Schaedler Agar (Schaedler Anaerobic Agar)

Composition per liter:

Agar ……... **13.5g**

Glucose …….. **5.83g**

Pancreatic digest of casein …… **5.7g**

Proteose peptone No. 3 …… **5.0g**

Yeast extract …… **5.0g**

Tris(hydroxymethyl)aminomethane buffer ……. **3.0g**

NaCl …….. **1.65g**

Papainic digest of soybean meal ……. **1.0g**

K_2HPO_4 …… **0.83g**

L-Cystine ……. **0.4g**

Hemin …… **0.01g**

pH 7.6 ± 0.2 at 25°C

Columbia C.N.A. Agar Base with Blood

Composition per liter:

Peptone, special ……. **23.0g**

Agar ……... **15.0g**

NaCl …… **5.0g**

Corn starch …….. **1.0g**

Sheep blood, defibrinated …….. **50.0mL**

pH 7.3 ± 0.2 at 25°C

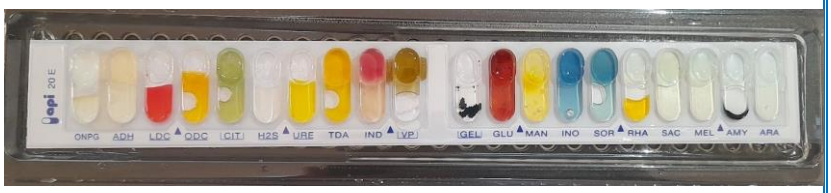
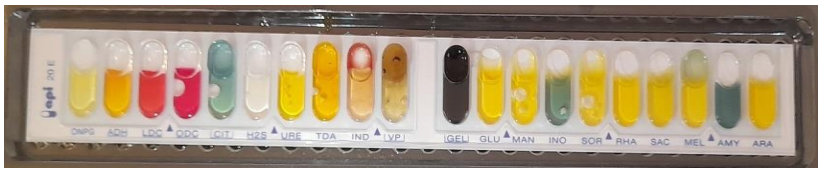
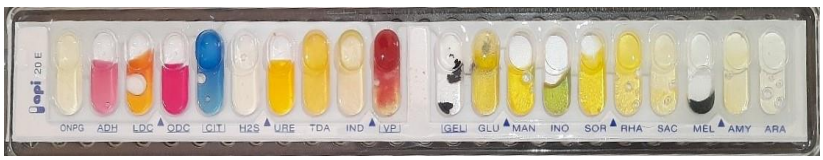
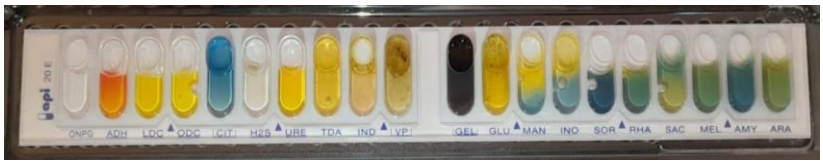
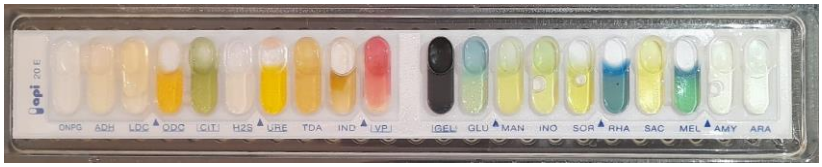
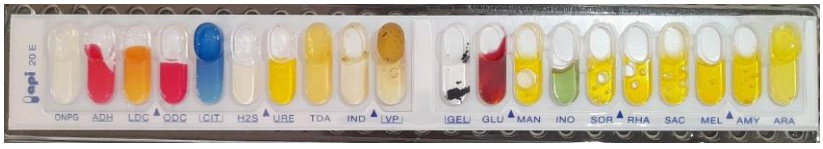
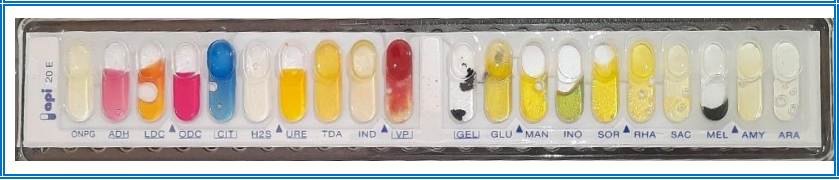
Columbia CNA Agar (Columbia Colistin Nalidixic Acid Agar)

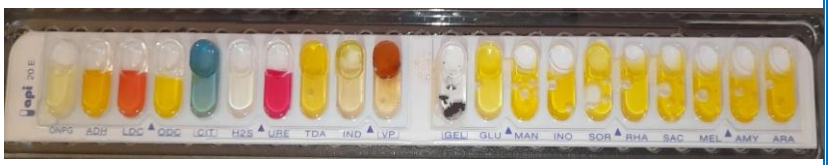
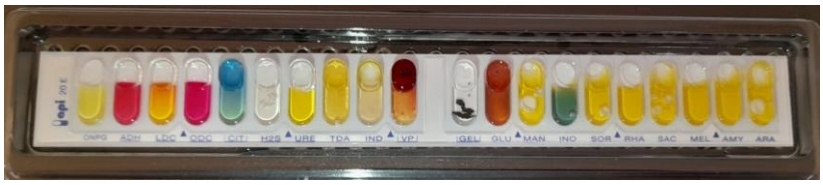
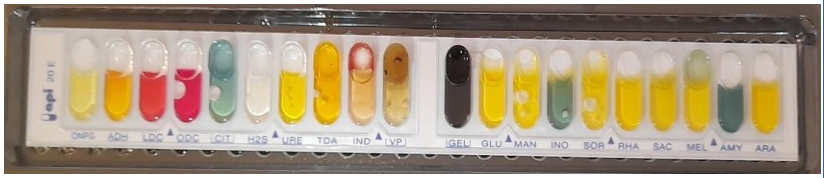
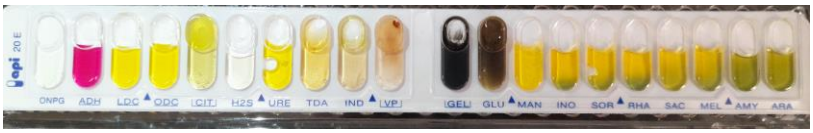
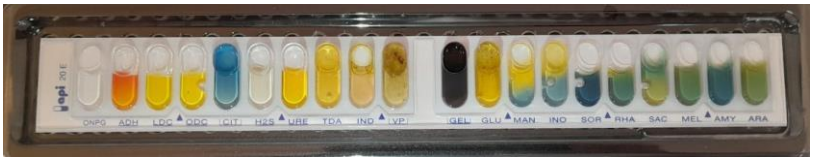
Composition per liter:

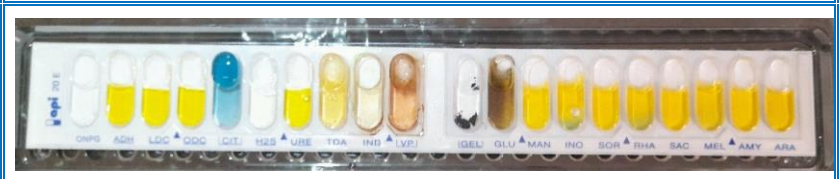
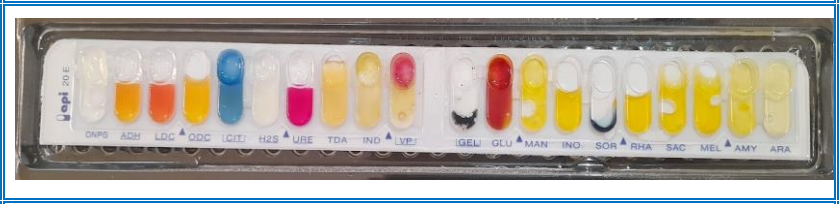
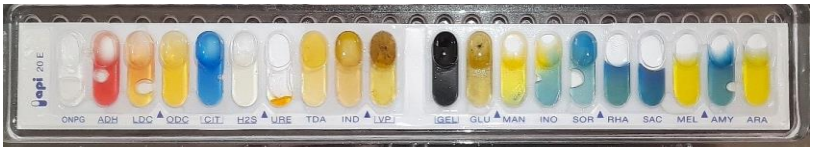
Columbia blood agar base …….. **950.0L**
Sheep blood ……... **50.0mL**

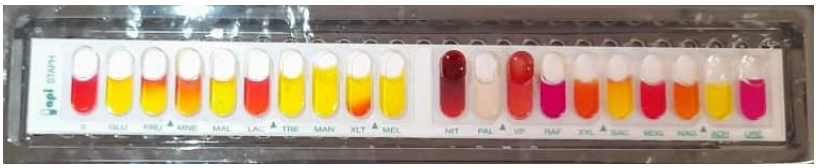
pH 7.3 ± 0.2 at 25°C

Appendix 4: API results

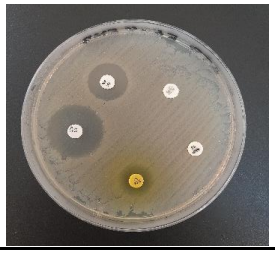
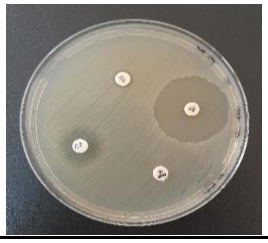
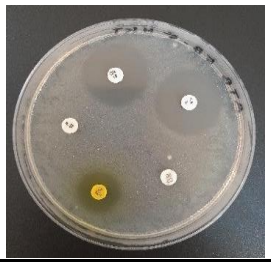
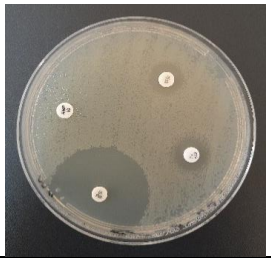
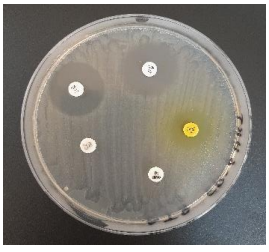
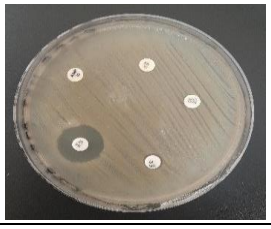
Code	Figure	Identification
404411		*Escherichia coli*
52157735		*Enterobacter aerogenes*
70547325		*Escherichia coli 1*
73675720		Enterobacteriaceae
7305533		*Enterobacter cloacae*
22063200		*Pantoea Spp* 1
0003724		*Pasteurella pneumotropica/ Mannheimia haemolytica*
63017735		*Enterobacter cloacae*
7305533		*Enterobacter cloacae*

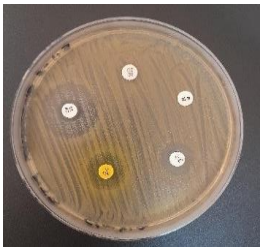
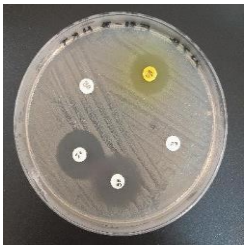
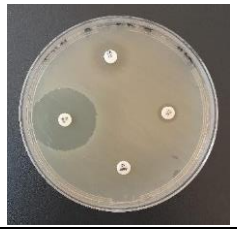
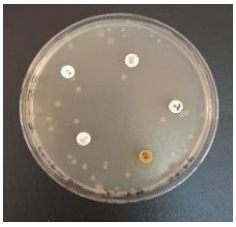
Code	Figure	Identification
6305773		*K.pneumoniae Ssp ozaenae*
72157730		*Klebsiella pneumonia Spp ozaenae*
04263205		*Proteus vulgaris*
7305573		*Klebsiella pneumoniae Sp Pneumonia*
00043000		*Escherichia coli*
73675720		*Serratia*
20477701		*Pantoea spp*
220632		***Providencia rettgeri***
0227503		No official identification was made

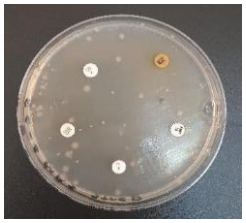
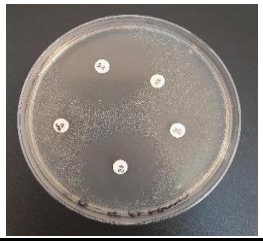
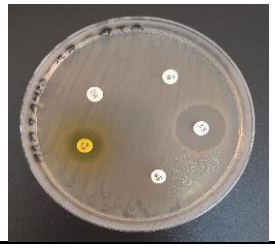
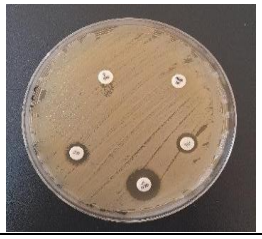
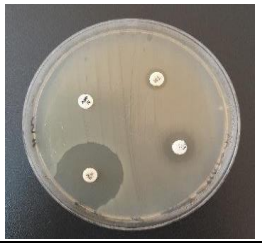
Code	Figure	Identification
7164552		*Virgibacillus pantohenticus*
04027776		*Bacillus circulans*
6704751		*Salmonella*
5145772		*Serratia oderifera*
IND-, URE+, GLU+, MAN+, SAC+, ARA+, GEL-, SOR-, RHA+, CAT +, GRAM		***Bacteroides ovatus/thetaiotaomicron***
IND+, URE+, GLU+, MAN-, SAC-, ARA+, GEL+, SOR-, RHA+, GRAM- , COCC -		***Bacteroides ovatus/thetaiotaomicron***

Code	Figure	Identification
0073201		***Staphylococcus***
VP-, ADH+, MAN+, LAC-, TRE-, RAF+		***Aerococcus***
Hemolysis ***B***		***Staphylococcus aureus***
VP +, ADH -, RAF -, TRE +, LAC -, PAL		***Streptococcus agalactia***
		***Enterococcus***
		***Cryptococcus laurentii***

Appendix 5: Antibiogram

Strain	Genus/ Specie	ANTB	inhibition diameter (mm)	Antibiogram	Cd (CASFM) (mm)		Cd (PIA)(mm)		
					R	S	R	I	S
E6	*Enterobacter aerogenus*	NA 30	17		<14	≥14	<13	14-18	≥19
		CN 10	24		<17	≥17	<12	13-14	≥15
		NTX 30	13		<15	≥15	**N/A**	**N/A**	**N/A**
		CZ 30	0		**N/A**	**N/A**	<19	20-22	≥23
		AMP 10	0		<14	≥14	<13	14-16	≥17
E10	*Enterobacter cloacae*	IMP 10	30		<19	≥22	<19	20-22	≥23
		CN 10	15		<17	≥17	<12	13-14	≥15
		AMP 10	0		<14	≥14	<13	14-16	≥17
EB	*E.coli*	NA 30	27		<14	≥14	<13	14-18	≥19
		CN 10	25		<17	≥17	<12	13-14	≥15
		NTX 30	15		<16	≥16	**N/A**	**N/A**	**N/A**
		CZ 30	0		**N/A**	**N/A**	<19	20-22	≥23
		P 10	0		<14	≥14	<13	14-16	≥17
EC	*Salmonella ssp*	IMP 10	40		<19	≥22	<19	20-22	≥23
		CN 10	13		<17	≥17	<12	13-14	≥15
		VA 10	14		**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
		AMP 10	0		<14	≥14	<13	14-16	≥17
ED	*Klebsiella pnemoniae*	NA 30	23		<14	≥14	<13	14-18	≥19
		CN 10	25		<17	≥17	<12	13-14	≥15
		NTX 30	15		<16	≥16	**N/A**	**N/A**	**N/A**
		CZ 30	0		**N/A**	**N/A**	<19	20-22	≥23
		AMP 10	0		<14	≥14	<13	14-16	≥17
EA	*E. cloacae*	CN 10	7		<17	≥17	<12	13-14	≥15
		NA 30	18		<14	≥14	<13	14-18	≥19
		CZ 30	0		**N/A**	**N/A**	<19	20-22	≥23
		AMP 10	0		<14	≥14	<13	14-16	≥17

E11	***Proteus vulgaris***	**CN 10**	23		<17	≥17	<12	13-14	≥15
		NTX 30	15		<16	≥16	**N/A**	**N/A**	**N/A**
		NA 30	14		<14	≥14	<13	14-18	≥19
		P 10	0		<14	≥14	<13	14-16	≥17
		CZ 30	0		**N/A**	**N/A**	<19	20-22	≥23
E11	***E.cloacae***	**NA 30**	18		<14	≥14	<13	14-18	≥19
		CN 10	8		<17	≥17	<12	13-14	≥15
		NTX 30	15		<16	≥16	**N/A**	**N/A**	**N/A**
		CZ 30	0		**N/A**	**N/A**	<19	20-22	≥23
		P 10	0		<14	≥14	<13	14-16	≥17
E11	***Serratia oderifera***	**NA 30**	20		<14	≥14	<13	14-18	≥19
		CN 10	23		<17	≥17	<12	13-14	≥15
		NTX 30	18		<16	≥16	**N/A**	**N/A**	**N/A**
		CZ 30	0		**N/A**	**N/A**	<19	20-22	≥23
		P 10	0		<14	≥14	<13	14-16	≥17
E10	***K. pnemoniae ssp ozaenae***	**CN 10**	19		<17	≥17	<12	13-14	≥15
		COL 10	10		**CMI**	**CMI**	**CMI**	**CMI**	**CMI**
		VA 10	9		**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
E10	***K. pnemoniae ssp ozaenae***	**IMP 10**	30		<19	≥22	<19	20-22	≥23
		CN 10	15		<17	≥17	<12	13-14	≥15
		AMP 10	0		<14	≥14	<13	14-16	≥17
EC	***Bacteroides ovatus/the-taiotaomicro n***	**IMP 10**	30		<50	≥17	**N/A**	**N/A**	**N/A**
		CN 10	8		**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
		VA 10	8		<17	≥17	**N/A**	**N/A**	**N/A**
		AMP 10	0		<14	≥14	<13	14-16	≥17
E5	***Enterococcus sp***	**AMP 10**	0		<8	≥10	<16	**N/A**	≥17
		E 15	0		<23	>23	<13	14-22	≥23
		CD 2	0		**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
		TE 30	0		**N/A**	**N/A**	<14	15-18	≥19

EC	***Enterococcus sp***	**AMP 10**	0		<8	≥10	<16	**N/A**	≥17
		E 15	0		<23	>23	<13	14-22	≥23
		CD 2	0		**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
		TE 30	0		**N/A**	**N/A**	<14	15-18	≥19
ED	***Bacillus spp 1***	**CN 10**	35		<17	≥17	**N/A**	**N/A**	**N/A**
		NA 30	40		**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
		CZ 30	11		**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
		AMP 10	0		**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
EA	***Bacillus spp 2***	**NA 30**	20		**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
		NTX 30	15		**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
		CZ 30	0		**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
EC	***Pseudomonas spp***	**CN 10**	15		**EPI**	**EPI**	<12	13-14	≥15
		COL	9		**CMI**	**CMI**	**N/A**	**N/A**	**N/A**
		VA 10	10		**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
		AMP 10	0		**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
E8	***Pasteurella pneumotropica/ Mannheimia***	**IPM 10**	28		**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
		CN 10	14		**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
		VA 10	10		**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
		AMP 10	0		<17	≥17	**N/A**	**N/A**	≥27

ANTB: Antibiotics **Cd: critic diameter** **N/A: Not available**

CASFM: Comité de l'Antibiogramme de la Société Française de Microbiologie
PIA: Pasteur Institution of Algeria
CMI: Some strains suggest the need to apply other tests in order to decide resistance or sensibility of strains such as the determination of critic concentration (CMI) using microdilution method in a liquid medium
EPI: typically refers to Extended Spectrum Beta-Lactamase (ESBL) Producing Isolates. These are bacteria that produce enzymes capable of breaking down a wide range of beta-lactam antibiotics, including penicillin and cephalosporins, thereby conferring resistance to these drugs.