Characterization of typical and atypical bacteria in pediatric upper respiratory tract infections at Kisumu County Hospital

Loice Mahila Machanja[1]
Godfrey Gitonga Mutuma[2]
Nathan Shaviya[3]
Eliphalet Ochieng Otunga[4]

[1]mahilamachanja1@gmail.com (+254702505256)
[2]mutumagitonga1@gmail.com
[3]shavianathan@gmail.com
[4]otungaely@gmail.com

[1]https://orcid.org/0000-0001-9483-1486
[2]https://orcid.org/0000-0003-1891-1817
[3]https://orcid.org/0000-0001-7347-3130
[4]https://orcid.org/0009-0009-9561-3638

[1,2,3,4] Masinde Muliro University of Science and Technology, Kenya

https://doi.org/10.51867/scimundi.6.1.40

ABSTRACT

Upper respiratory tract infections (URTIs) are among the leading causes of illness and mortality among children under five, particularly in low and middle-income countries. Management becomes complicated due to bacterial pathogens and antimicrobial resistance, particularly in areas with limited diagnostic capacity. The objective of this study was to isolate and characterize the bacteriological pathogens associated with upper respiratory tract infections among children at Kisumu County Hospital. A cross-sectional descriptive study was conducted involving 100 purposively recruited children under five years of age presenting with URTI symptoms. Throat swabs were collected between June 2025 and July 2025 and subjected to culture on blood and MacConkey agar, then incubated at 37°C for 24 hours. Bacteria were identified using morphological, Gram staining, and biochemical tests. Antimicrobial susceptibility was determined using the Kirby-Bauer disk diffusion method following CLSI guidelines. Molecular characterization using the PCR technique was used to detect resistance genes. Descriptive and inferential statistics were used for analysis. The most frequently isolated bacteria were Staphylococcus aureus (40%), Streptococcus pyogenes (31%), and Enterococcus faecalis (19%). Antimicrobial sensitivity was highest in S. pyogenes (52%) and lowest in Klebsiella species (30%), which also showed the highest resistance (45%) and multidrug resistance (60%). PCR analysis confirmed high prevalence of the 16S rRNA mutation gene and MecA in all five samples tested isolates. Other AMR genes identified were TEM, TYLA, VANA/B, EF-Tu mutants, and APH(3) IIIa. Although there is no statistically significant association between bacterial species and the overall sensitivity patterns with the antibiotics ($p = 0.135$), there was a statistically significant association between the type of bacterial isolate and MDR ($p = 0.045$). These results indicate no statistically significant association between bacterial isolates and specific AMR gene profiles ($p > 0.05$). Bacterial pathogens, particularly Gram-positive cocci, are major contributors to URTIs among children in Kisumu. A high prevalence of antimicrobial and multidrug resistance, especially in urban areas, threatens treatment outcomes. There is a need for the ministry of health to incorporate molecular diagnostics, including PCR and metagenomics, into routine hospital laboratory workflows for early detection of resistance genes and more accurate pathogen identification in all county-level 5 hospitals for better diagnosis and management of patients. Clinicians should also consider isolating both typical and atypical bacteria present in patients with respiratory tract infections.

Key Words: Atypical Bacteria, Characterization, Infections, Typical, Upper Respiratory Tract

I. INTRODUCTION

Respiratory tract infections (RTIs) refer to infections that affect the respiratory system, which includes the organs involved in breathing: the nose, throat (pharynx), voice box (larynx), windpipe (trachea), bronchi, and lungs (Reed, 2015). RTIs can be caused by various microorganisms including viruses, bacteria, fungi, and even parasites. These infections can range from mild, self-limiting illnesses to severe and life-threatening conditions. These infections can occur in both upper and lower respiratory tract and are a major cause for the high morbidity and mortality rates around the world especially to young children.

Globally, there were 488.9 million incident cases of upper respiratory tract infections (URTIs) in 2019, with an age-standardized incidence of 6,295.0 per 100,000 population. URTIs are also a main cause of deaths among children

under 5 years of age and approximately 0.67 million children under 5 years died from URIs globally in 2019. Upper Respiratory Tract Infections (URTIs) account for 5.8 million deaths globally and 50% of these deaths occur in sub-Saharan Africa. The prevalence of URI for all the African countries was 25.3%. Congo (39.8%), Gabon (38.1%), Lesotho (35.2%), and Tanzania (35.2%) these were also the countries with the highest prevalence of ALRIs (Acute lower respiratory infections) (Safiri *et al*., 2023).

In Africa, URTIs are a leading cause of pediatric morbidity and mortality, especially in children under five years old. Epidemiological studies across countries like Nigeria, Ghana, and South Africa indicate that S. pneumoniae, H. influenzae, and M. catarrhalis dominate the bacterial spectrum of URTIs. However, improved access to molecular diagnostics has also led to increasing reports of atypical bacteria such as M. pneumoniae and C. pneumoniae, especially in cases of persistent cough and community-acquired pneumonia. Contributing factors to the high burden of URTIs in Africa include poor sanitation, overcrowding, indoor air pollution, and limited access to vaccines and healthcare services. Although immunization programs targeting H. influenzae type b and S. pneumoniae have been rolled out in many countries, gaps in coverage and vaccine hesitancy continue to undermine their impact. Furthermore, irrational antibiotic use, limited diagnostic capabilities, and weak regulatory frameworks exacerbate antimicrobial resistance in both typical and atypical pathogens (Ciccacci *et al*., 2025).

In Kenya, among children aged below 5 years, the estimated annual incidence of diseases associated with AURTIs (Acute upper respiratory tract infections) was 206 per 1000 children. The estimated annual rates of hospitalized and non-hospitalized URIs were 1077 per 100,000 children (Sarfo *et al*., 2023). The estimated annual number of in- and out-of-hospital deaths associated with URIs infection in Kenya were 1921 per 100,000 children. In Kenya, these infections accounted for 25% of all reported illness cases in public health facilities surpassing other types of infections in children younger than 5 years old (Achoki *et al*., 2018). The infections although commonly caused by viruses can also be caused by bacterial pathogens which remain a major cause of RTIs in children, particularly in developing countries. The most commonly known respiratory bacteria pathogens found globally and especially in Kenya include but are not restricted to the following bacteria: Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes, Pseudomonas species, Klebsiella species, Haemophilus influenza. Most of these pathogenic bacteria have developed a resistance to the commonly used antibiotics especially in Kenya (Miriti *et al*., 2023).

Bacteria may develop resistance to antibiotics due to some mechanisms which may include: decreased permeability of the cell membrane, modification of drug target and gene mutations. This can be attributed to many factors including but not limited to improper use of antibiotics and transmission of bacterial pathogens. Treatment for antimicrobial resistant infections is not advanced in developing countries like Kenya; this may be because laboratory services are costly and results take time. Resistance to the commonly used antibiotics has led to failure in the treatment and management of most diseases. The commonly used antibiotics used in the treatment include the following: Amoxicillin, Penicillin, Cefadroxil, Erythromycin, Amoxicillin and clavulanate, Ceftriaxine, Azithromycin.

The increase in the number of microorganisms that are resistant to the commonly used antibiotics has led to the increase widespread epidemics and endemic dissemination of multidrug-resistant pathogens globally. In some cases, phenotypic results are not conclusive that is why molecular methods are useful in identifying the presence of a given gene or a mutation and give conclusive results. Some of the molecular methods used to analyze samples include: PCR is an in vitro method that allows the exponential amplification of specific sequences of DNA and RNA, a technique applied widely due to its high specificity. Metagenomics in which assemblies formed from small sequencing reads are called contigs, which can be annotated to search for resistance genes. The search for resistance genes is mostly done by methods that consider the similarity of the contigs to the genes contained in reference databases.

1.2 Statement of the Problem

Bacterial respiratory tract infections are among the leading causes of child mortality and morbidity worldwide and especially in third world countries like Kenya. In Kisumu County especially in Kisumu Central sub county due to a large number of slums, overcrowding, smoke emissions and immunization status the number of children suffering from acute respiratory infections is high. The infections are easily spread and due to their mild clinical manifestations and during diagnosis most clinicians do not consider this a serious health issue. Misdiagnosis, over the counter medicine and misuse of antibiotics have contributed to increase in the number of cases of infection. Proper treatment, however, depends on correct identification of the pathogen involved so as to give the right drug for treatment. Most of the bacteria have developed resistance to commonly used antimicrobial agents. Multidrug-resistant bacteria remain more common despite extensive attempts to combat antimicrobial resistance; this presents an immediate threat to successful infection control. Currently in Kenya there are not many studies which focus on the molecular characterization of resistance genes in pathogenic bacteria that causes upper respiratory infections despite the issue being a major concern in public health. Therefore, this study will determine the respiratory tract pathogenic bacterial that are resistant to commonly used antibiotics and characterize their genomic structure to determine the bacteria with mutated genes that causes antimicrobial resistance.

1.2 Research Objective

i. To establish the bacterial profiles of typical and atypical bacteria isolated from the upper respiratory tract infections cases among children attending Kisumu County Hospital.
ii. To examine antimicrobial sensitivity pattern of typical and atypical isolated from the upper respiratory tract infections cases in children attending Kisumu County Hospital.
iii. To determine Molecular characterization of bacterial isolated from throat swab of children suffering from upper respiratory tract infections attending Kisumu County Hospital.

II. LITERATURE REVIEW

2.1 Respiratory tract infection overview

The primary function of the respiratory tract (RT) is gaseous exchange; therefore, it is continuously exposed to various environmental materials which can include microorganisms such as bacteria, viruses and spores. The respiratory tract is divided into two parts; the upper and the lower respiratory tract. The upper respiratory tract consists of the airways from the nostrils to the vocal cords in the larynx, including the paranasal sinuses and the middle ear. The lower respiratory tract covers the continuation of the airways from the trachea and bronchi to the bronchioles and the alveoli. Respiratory infections (RIs) are classified as upper respiratory tract infections (URIs) or lower respiratory tract infections (LRIs). It is estimated that 10.8 million children die each year due to RTIs. It's estimated that in 2000, 1.9 million of them died because of ARIs, 70 percent of them in Africa and Southeast Asia. The World Health Organization (WHO) estimates that 2 million children under five dies of pneumonia each year (Safiri *et al*., 2023).

2.2 Typical and Atypical Bacteria Causing URTIs

Upper respiratory tract infections (URTIs) are caused by a wide range of bacteria and viruses, and promptly identifying the specific pathogens in each case is essential for effective clinical management (Guibas & Papadopoulos, 2017). Bacterial causes of respiratory can be divided into typical and atypical bacteria. Typical bacteria have a conventional cell wall structure, can be seen easily with a Gram stain, grow well on standard laboratory media. They often cause more acute symptoms and usually respond to beta-lactam antibiotics like penicillin and cephalosporins (Rohde, 2019). Examples of typical bacteria commonly found in upper respiratory tract infections include: Streptococcus pyogenes, Streptococcus pneumoniae, Enterococcus faecalis, Klebsiella species, Pseudomonas aeruginosa, Haemophilus influenzae, Moraxella catarrhalis. Atypical bacteria lack a typical cell wall e.g., Mycoplasma and Chlamydophila. They do not show up well on Gram stain and may require special culture techniques or molecular tests for detection. These types of bacteria tend to cause milder or more prolonged symptoms and may often require macrolides, tetracyclines, or fluoroquinolones for treatment, as beta-lactams are ineffective in their management.

Some of the most common atypical bacterial pathogens found on the upper respiratory tract include the following Mycoplasma pneumoniae a well-known childhood pathogen and is highly transmissible. Most infections caused by this organism include pharyngitis, tracheobronchitis, bronchiolitis, and croup. C pneumoniae identified in acute lower respiratory infection with mild dyspnea and wheezing and asthma in children. C trachomatis is transmitted by infected women to their infants at birth via contact with infected cervicovaginal secretion and should therefore not be ruled out in infants less than 6 months of age with clinical symptoms of respiratory tract disease for which no other pathogen can be found.

2.3 Upper Respiratory Tract Infections

The Upper respiratory tract infections are the most common infectious diseases and they include rhinitis (common cold), sinusitis, ear infections, acute pharyngitis or tonsils, epiglottitis, laryngitis and pharyngitis. A huge cause of URIs is viruses e.g. Rhinoviruses that account for 30% of URIs however, bacterial infections can occur, especially in cases where there's bacterial colonization or secondary bacterial infection following a viral infection. Bacterial upper respiratory tract infections (URIs) among children below 5 years old can be caused by various bacteria. Some of the common bacterial pathogens associated with URIs in this age group include: Streptococcus pneumoniae: This bacterium is a leading cause of bacterial pneumonia, otitis media (middle ear infection), sinusitis, and other upper respiratory tract infections in children.

Haemophilus influenzae type b: Prior to widespread vaccination, Hib was a major cause of bacterial meningitis, pneumonia, and epiglottitis in young children. The Hib vaccine has significantly reduced the incidence of these infections (Slack *et al*., 2021). Staphylococcus aureus: can cause various upper respiratory tract infections, including sinusitis and tonsillitis. It's also a common cause of skin and soft tissue infections. Group A Streptococcus (Streptococcus pyogenes): can cause streptococcal pharyngitis (strep throat) and, less commonly, other upper respiratory tract infections like sinusitis and tonsillitis. Moraxella catarrhalis is a common cause of otitis media and sinusitis in children. Other less common bacteria can also cause upper respiratory tract infections in children, including Mycoplasma pneumoniae, Chlamydophila pneumoniae, and Bordetella pertussis (which causes whooping cough) (Reed, 2015).

2.4 Isolation of bacteria

Isolation, purification and identification of bacteria are essential steps in bacteriological studies. Clinical specimens are collected, transported appropriately, cultured on selective and non-selective media, and purified for further characterization.

2.4.1 Specimen Collection

Many different specimens are sent for microbiological examination from patients with suspected bacterial infection. Common specimens include urine, faeces, wound swabs, throat swabs, vaginal swabs, sputum, and blood. Less common specimens include cerebrospinal fluid, pleural fluid, joint aspirates, tissue, bone and prosthetic material. Some types of specimens are normally sterile e.g. blood (Lagier *et al*., 2015). These samples are usually obtained via a percutaneous route with needle and syringe, using appropriate skin disinfection and an aseptic technique. The culture of bacteria from such specimens is usually indicative of definite infection except if they are skin contaminants. It is preferred to obtain the samples for bacteriological culture before antibiotic therapy is started. This maximizes the sensitivity of the investigations and reduces false-negative results. Similarly, samples of tissue or pus are preferred to maximize the recovery of bacteria in the laboratory. Specimens must be accurately labelled and accompanied by a properly completed requisition form, indicating the nature of the specimen, the date of sample collection, relevant clinical information, the investigations required, and details of antibiotic therapy (Safiri *et al*., 2023). This helps in the interpretation of results and reporting of the results.

2.8 Antimicrobial Resistance

Antimicrobial resistance results from intrinsic or acquired mechanisms including gene mutation, horizontal gene transfer, target modification, enzymatic drug inactivation and efflux pumps.

2.8.1 Mechanisms of antibiotic resistance

A variety of mechanisms are responsible for acquired bacterial resistance to various antibiotics. Bacteria utilize these resistance mechanisms for protection against antibiotics; inactivation or destruction of the antimicrobial agent; alteration or protection of the target site; blocking the active transport mechanism, decreasing the cell surface permeability or (efflux) removal from the cell and the creation of alternative metabolic pathway instead of that was inhibited by antimicrobial agent.

Beta symbol-lactam agents such, as penicillin, cephalosporins and carbapenems are molecules of choice to treat a variety of infections. Their introductions into therapy were rapidly followed with development of resistances being increasingly being reported from different geographical regions. The acquisition and accumulation of resistant determinants have given rise to multidrug ESBL producers, further limiting therapeutic options since the 35 identifications of ESBL microorganisms in the early 1980s and shortly after the introduction of oxyimino-beta-lactam. In many European countries a rapid dissemination of E. coli and others entrobacteria producing ESBLs have been reported (Munita & Arias, 2016).

In Latin America significant studies on resistance in E. coli isolates have been reported showing an increase in trends among the children. In Africa the E. coli isolates, the resistance is seen in both pathogenic and nonpathogenic isolates, in Morocco Madagascar, Central Africa, Nigeria. In Kenya several studies have shown similar trends being observed as in other regions pointed out in the above studies of an increase in E. coli strains showing resistance. Recognizing the potential for emergence of resistance and an upward trend of ESBLs should garner newfound respect for the discovery of new agents so urgently needed to cure infectious diseases which currently on the decline before the world get to the prebiotic era.

2.12 Polymerase Chain Reaction (PCR)

PCR enables amplification of specific DNA targets and is widely used for pathogen identification and detection of antimicrobial resistance genes.

2.13 Prevalence of antimicrobial Resistance Genes in Respiratory Tract Infections

Antimicrobial resistance (AMR) has become a major global public health threat, with respiratory tract infections (RTIs) acting as one of the main sources for resistant pathogens. Children below five years are the most affected with respiratory tract infections (RTIs) in Sub-Saharan Africa, with increasing evidence linking these infections to multidrug-resistant bacterial strains. Sub-Saharan Africa reported some of the highest AMR-attributable mortality rates globally, especially among children under five years old suffering from lower respiratory tract infections (Mehrotra *et al*., 2023).

Over the years several genes identified with drug resistance in respiratory pathogens. Among Gram-positive bacteria, the mecA gene, which confers resistance to methicillin and other β-lactam antibiotics, remains one of the most frequently detected. A large surveillance study in the Vietnam reported mecA in 34.9% of Staphylococcus aureus isolates from respiratory specimens, highlighting the continued burden of methicillin-resistant S. aureus (MRSA) (An

et al., 2024). Similarly, the vanA and vanB genes, which confer resistance to vancomycin in Enterococcus species, were present in 15.9% of respiratory isolates in the same cohort. In sub-Saharan Africa, available data shows a high prevalence of resistance genes. A study done in Nigeria reported that mecA was detected in approximately 51% of S. aureus isolates, while genes conferring resistance to tetracyclines (tet), sulfonamides (sul), and macrolides (erm) were each found in 19–27% of isolates from both human and environmental samples (Ugbede *et al*., 2025).

In Kenya, several hospital-based studies have reported a high prevalence of resistance genes in bacterial respiratory pathogens. For instance, mecA and bla_TEM have been frequently detected in S. aureus and E. coli isolates, respectively, in both pediatric and adult respiratory samples (Ong'era *et al*., 2023). A study conducted at Kenyatta National Hospital found a high frequency of multidrug-resistant Klebsiella pneumoniae and Pseudomonas aeruginosa, with resistance genes such as OXA-48 and CTX-M contributing to the observed resistance patterns (Kyany'a *et al*., 2019). In respiratory Gram-negative organisms, extended-spectrum β-lactamases (ESBLs), such as TEM, CTX-M, and SHV, contribute significantly to AMR. ESBL-producing strains of Klebsiella pneumoniae and Escherichia coli were found in 41.9–70.5% of respiratory isolates in a multi-year study conducted in Fujian Province, China (Chen *et al*., 2013). These strains also exhibited resistance to carbapenems, with rates as high as 19.6% in Acinetobacter baumannii and 8.9% in K. pneumoniae. The presence of carbapenemase genes, although less prevalent, is of growing concern, especially in ICU settings and among ventilated patients (Li *et al*., 2024)

In sub-Saharan Africa, AMR surveillance remains underdeveloped, yet available data indicate a high prevalence of resistance genes. A meta-analysis from Nigeria reported that mecA was detected in approximately 51% of S. aureus isolates, while genes conferring resistance to tetracyclines (tet), sulfonamides (sul), and macrolides (erm) were each found in 19–27% of isolates from both human and environmental samples. These findings align with those from local studies in Kenya, which have shown high levels of multi-drug resistance and frequent detection of resistance genes in both community and hospital settings. In Kenya, Staphylococcus aureus, Streptococcus pneumoniae, and Escherichia coli are the most frequently isolated bacteria in children with RTIs, many of which show resistance to first-line antibiotics (Miriti *et al*., 2023). Molecular studies have identified the mecA gene in over 50% of S. aureus isolates and β-lactamase genes such as bla_TEM and OXA-48 in E. coli and Klebsiella pneumoniae from pediatric respiratory specimens. Resistance genes conferring resistance to aminoglycosides (Aph(3) IIIa), macrolides (ermB, 16S rRNA mutations), and fluoroquinolones (qnr) have also been detected. The lack of targeted pediatric antimicrobial resistance surveillance has limited the development of age-specific treatment guidelines, often leading to poor clinical outcomes.

The use of metagenomic and next-generation sequencing tools has enhanced the detection of resistance genes in respiratory samples. In a recent study using DNA/RNA metagenomics in critically ill patients, AMR genes such as mecA, blaZ, msrA, and others were detected in hospital-onset infections within minutes of sequencing, enabling real-time resistance surveillance. These technologies are especially useful for detecting difficult-to-culture or polymicrobial infections. These studies show the growing prevalence of AMR genes in respiratory pathogens globally. The consistent detection of genes such as 16srRNA mutations,mecA, vanA/B, tem, and CTX-M in respiratory isolates reinforces the need for molecular surveillance, especially in pediatric populations. This may be essential in informing treatment guidelines and reducing AMR-driven morbidity and mortality especially in younger populations.

III. METHODOLOGY

3.1 Study Design

This study employed a cross-sectional descriptive methodology to evaluate the prevalence and clinical characteristics of upper respiratory tract infections in children under five at Kisumu County Hospital. Data were gathered at a singular moment, offering a picture of illness burden, risk factors, and demographic attributes to guide public health initiatives and further research.

3.2 Study site and Population

The study was conducted in Kisumu County Hospital, which is located in Kisumu County, in Kenya. The total area of Kisumu County is approximately 2,496.1 km². This county lies between latitude 0° 26' to 0° 18' north and longitude 33° 58' east and 34° 33' west. According to the latest national housing and population census, the population of the county is about 842,304 with 20.4% under 5 years of age. The majority of residents are ethnically Luo tribe and earns a living through agriculture or fishing.

Kisumu County was selected as the study area because Kisumu is one of Kenya's largest urban centers and serves as a major referral hub for the Lake Victoria Basin region. The county has a high population density, with over 1.2 million residents, a significant proportion of whom are children under 14 years of age a group particularly vulnerable to upper respiratory tract infections. Kisumu experiences a high burden of communicable diseases, including acute respiratory infections (ARIs), which are among the leading causes of pediatric outpatient visits and hospital admissions in the region (Ministry of Health. According to the Kenya Health Information System (KHIS), Kisumu County

consistently reports a high number of respiratory infection cases among children, particularly during the rainy seasons when environmental conditions favor the spread of airborne pathogens.

3.3 Study Population

The target population were children under 5 years of age who presented with clinical symptoms suggestive of upper respiratory tract infections at Kisumu County hospital. A total of 100 study participants were purposively recruited. Children under five years are biologically more vulnerable to respiratory infections due to their developing immune systems and narrower airways, which predispose them to more frequent and severe respiratory illnesses. According to the World Health Organization (WHO, 2022), respiratory tract infections are a leading cause of morbidity and mortality in this age group, especially in low- and middle-income countries like Kenya. Therefore, focusing on this demographic allowed the study to capture the highest disease burden, which is crucial for public health planning and intervention.

3.4 Sample size determination

The sample size (N) was calculated using the Cochran method as indicated in the formula below (Cochran, 1977).

$$n = \frac{Z^2.p.(1-p)}{e^2}$$

Where:-

N= minimum sample size required

Z= confidence level at 95% (standard value of 1.96)

P= prevalence of respiratory tract infections in children in rural Kenya 7 % (Tornheim *et al.,* 2007)

E = margin of error. Desired level of precision

$$\frac{1.96^2 \times 0.07 \times 0.93 =}{0.05^2} \quad 100$$

3.5 Inclusion and exclusion criteria

3.5.1 Inclusion criteria

All patients were children below the age of 5 years given consent by parents /guardians. Residents of Kisumu County. All patients with signs and symptoms of respiratory tract infection were to participate in the study

3.5.2 Exclusion criteria

Patients above the age of 5 years, Patients who were immunocompromised or had chronic diseases. The parents or guardians of patients refused to consent to participate in the study

3.6 Sampling Design

A purposive sampling design was adopted in this study. Children attending the Kisumu County hospital who have clinical symptoms of respiratory infections were approached through their parents/guardians for enrolment into the study.

3.7 Laboratory procedures

3.7.1 Sample collection

Clinical samples of throat swabs were collected using sterile swabs and transported to the hospital's bacteriology laboratory using Amies transport medium for bacteriological analysis. A loopful of each sample was then inoculated onto sterile Blood agar, Chocolate agar, sabouroud's agar and MacConkey agar immediately on arrival and then incubated aerobically at 37^{o}C for 24hours except for Chocolate agar which was incubated under anaerobic conditions. The isolates were identified using standard biochemical test based on their colony morphology, colour, haemolysis on blood agar and Gram staining characteristics (10).

3.7.2 Culture and Isolation of Bacteria

In the laboratory, each swab was inoculated onto two types of media: Blood Agar (BA) and MacConkey Agar (MAC). The plates were incubated at 37°C for 24 to 48 hours under aerobic conditions. Blood Agar was used to support the growth of both Gram-positive and fastidious organisms and to observe hemolytic patterns. MacConkey Agar was used for the selective isolation of Gram-negative enteric bacteria. Colonies were examined for morphological characteristics such as color, shape, and hemolysis. Pure colonies from primary culture were subcultured on fresh Blood Agar and MacConkey Agar to obtain isolated colonies for further testing.

3.7.3 Preliminary Identification and Gram Staining

Gram staining was performed on the isolated colonies. A loop full of bacterial growth was emulsified on a clean glass slide and air-dried. The smears were heat-fixed and subjected to sequential staining using crystal violet, iodine solution, decolourization with alcohol, and counterstaining with safranin. Gram-positive organisms appeared purple while Gram-negative organisms appeared pink under oil immersion microscopy.

3.7.4 Biochemical Characterization

Several biochemical tests were performed for bacterial identification, including: Catalase Test: A small amount of bacterial growth was mixed with 3% hydrogen peroxide on a slide. Immediate bubbling indicated a positive result. Coagulase Test: A bacterial suspension was mixed with human plasma. Clot formation within 10 seconds indicated the presence of *Staphylococcus aureus*. Citrate Utilization Test: Organisms were streaked on Simmon's Citrate Agar slants and incubated. A change in color from green to blue indicated citrate utilization. Bacitracin and Optochin Sensitivity Tests: Blood agar plates were inoculated with bacterial suspensions and antibiotic discs were placed on the surface. The diameter of inhibition zones was measured after incubation. The results of morphological, Gram stain, and biochemical tests were interpreted together to identify bacterial species.

3.7.5 Antimicrobial Susceptibility Testing

Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton Agar, following Clinical and Laboratory Standards Institute (CLSI, 2021) guidelines. Standardized bacterial suspensions equivalent to 0.5 McFarland turbidity were prepared. A sterile swab was used to spread the inoculum evenly over the surface of the Mueller-Hinton Agar plate. Commercial antibiotic discs were placed at least 24 mm apart. The antibiotics tested included: Amoxicillin, Cloxacillin, Tetracycline, Erythromycin and Ceftriaxone. Plates were incubated at 37°C for 18 to 24 hours. After incubation, inhibition zones were measured in millimeters and interpreted as "sensitive," "intermediate," or "resistant" based on CLSI breakpoints.

3.7.6 Molecular Characterization

Genomic DNA Extraction: Genomic DNA was extracted from overnight bacterial cultures using the QIAamp DNA Mini Kit following the manufacturer's protocol. Bacterial pellets were obtained by centrifugation at 8000 rpm for 5 minutes. The pellet was re-suspended in enzymatic lysis buffer and incubated with lysozyme and proteinase K. DNA was bound to silica columns and washed sequentially with buffers AW1 and AW2. Elution was done with 50 µL of Buffer AE, and DNA was stored at −20°C until analysis.

Polymerase Chain Reaction (PCR): PCR was used to amplify regions of the 16S rRNA gene to identify resistance-associated mutations. The reaction mix (25 µL) included: 12.5 µL OneTaq Quick-Load 2X Master Mix, 1 µL forward primer, 1 µL reverse primer, 2 µL DNA template and 8.5 µL nuclease-free water. After the desired number of cycles. The amplification PCR products were stored at −20°C for downstream applications. *Gel Electrophoresis:* Amplified DNA was separated on a 1% agarose gel stained with ethidium bromide. A 5 µL aliquot of each PCR product was loaded into wells along with a DNA ladder. Electrophoresis was performed at 100 volts for approximately 45 minutes. DNA bands were visualized using a UV transilluminator and documented photographically.

Genomic Sequencing: Selected DNA samples were subjected to genomic sequencing using nanopore technology. The MinION platform and MinKnow software were used for sequencing and data analysis. Reads were assembled into contigs and aligned to reference databases to detect resistance genes, including mutations in 16S rRNA, MecA genes and other commonly implicated genes.

3.8 Data Management and Analysis

Data from laboratory results were entered into Microsoft Excel and exported to SPSS version 25.0 for statistical analysis. Descriptive statistics such as frequencies, means, and percentages were computed. Associations between bacterial isolates, resistance profiles, and demographic variables were analyzed using chi-square tests or Fisher's exact test where appropriate. A p-value of less than 0.05 was considered statistically significant.

3.9 Quality Assurance

Standard operating procedure (SOPs) were followed when caring out all the laboratory investigations. Control for all the equipment were run prior to testing and calibration performed regularly to ensure accuracy and precision of results. All the reagents in use were inspected regularly for their expiry and prepared in accordance to the manufacturer's instruction. All the laboratory investigations were performed by qualified lab personnel licensed by the Kenya Medical Laboratory Technologists and Technicians Board (KMLTTB).

3.10 Ethical Consideration

Written informed consent was obtained from the parents or legal guardians of all participating children prior to data and specimen collection. For minors aged between 0–5 years, both parental/guardian consent and written assent

from the child were obtained in accordance with ethical standards. The study protocol, consent forms, and specimen collection procedures were reviewed and approved by the Institutional Review Board (IRB) of Masinde Muliro University Ethics Review Committee (MUERC: ref :ISE approval NO: MMU/COR: 403012V6) and the Kisumu County Department of Health Research and Ethics Committee (KCHREC:ref 970330). Ethical guidelines adhered to the principles outlined in the Declaration of Helsinki (2013 revision) and the Kenyan National Guidelines for Research Involving Human Participants. Participation in the study was voluntary, and confidentiality was assured through the use of unique study identifiers and secure data handling. No invasive procedures beyond standard clinical practice were carried out. Participants were free to withdraw from the study at any time without affecting their access to healthcare.

IV. FINDINGS & DISCUSSION

4.1 Socio-demographic Characteristics of the Participants

This study enrolled a total of 100 pediatric participants presenting with symptoms of upper respiratory tract infections (URTIs) at Kisumu County Hospital. The age distribution of the children revealed that the highest burden of URTIs occurred among infants aged 0 to 1 year, who accounted for 38% of the study population. This finding aligns with global evidence indicating that infants are more susceptible to respiratory tract infections due to immature immune systems and high exposure to environmental pathogens.

The second most affected group was children aged 4 to 5 years, representing 21% of the participants. The relatively high prevalence in this group may be attributed to increased exposure in school and daycare environments, as reported by O'Brien et al. (2019). Children aged 1 to 2 years comprised 18%, those aged 2 to 3 years were 12%, and those aged 3 to 4 years accounted for 11%. These age-based differences highlight a trend where vulnerability to URTIs is highest in early infancy and gradually declines as children grow older and develop immunological resilience (Khan *et al.*, 2020). In terms of sex distribution, 64% of the participants were female, while 36% were male. Although the reasons for this disparity were not explored in depth, previous studies have shown varying susceptibility and care-seeking behaviors across genders, with some indicating a slightly higher incidence of respiratory infections among male infants (Tregoning & Schwarze, 2010), though cultural and caregiver bias in seeking care may also contribute.

Geographically, the majority of the children (58%) were from Kisumu Central, suggesting a concentration of healthcare access or disease burden in urban regions. Participants from Kisumu East accounted for 19%, Kisumu West for 14%, and Kisumu South for 9%. The higher proportion from Kisumu Central could reflect better access to Kisumu County Hospital due to proximity or a higher population density in the urban center. Regarding the severity of URTI symptoms, 82% of the children were diagnosed with mild cases, which were primarily characterized by symptoms such as fever and cough. Severe cases, constituting 15%, included more alarming signs such as chest in-drawing, while very severe cases (3%) involved critical symptoms like convulsions or inability to feed. These findings are consistent with WHO classifications for pediatric respiratory infections, which guide clinical decisions in resource-constrained settings (Gill *et al.*, 2025). These results are presented in table 1 as shown below.

Table 1

Socio-demographic Characteristics of the Participants

Variable	Category	Frequency (n)	Percentage (%)
Age (in years)	$0 \leq 1$	38	38%
	$1 \leq 2$	18	18%
	$2 \leq 3$	12	12%
	$3 \leq 4$	11	11%
	$4 \leq 5$	21	21%
	Totals	**100**	**100.00%**
Sex	Female	64	64%
	Male	36	36%
	Totals	**100**	**100**
Residence (Sub- County)	Kisumu Central	58	58%
	Kisumu East	19	19%
	Kisumu West	14	14%
	Kisumu South	9	9%
	Totals	**100**	**100**
URTI Severity Classification	Mild (fever, cough)	82	82%
	Severe (chest drawing)	15	15%
	Very severe (convulsions/inability to feed)	3	3%
	Totals	**100**	**100**

4.1.1 Isolation of Bacterial Pathogens and antimicrobial susceptibility patterns

In this study, throat swabs collected from 100 children under the age of five presenting with symptoms of upper respiratory tract infections (URTIs) were cultured and analyzed to identify the causative bacterial pathogens. The results revealed five distinct bacterial species. The most frequently isolated organism was Staphylococcus aureus, accounting for 40% of all isolates. This aligns with previous studies which have shown that S. aureus is commonly associated with pediatric respiratory tract infections due to its colonization of the nasopharynx and ability to cause opportunistic infections (Kalu *et al*., 2022). Following this, Streptococcus pyogenes was identified in 31% of the samples, confirming its known role as a major etiological agent in bacterial pharyngitis among children (Pato *et al*., 2018). Enterococcus faecalis was isolated in 19% of the cases, while Streptococcus pneumoniae and Klebsiella species were identified in 6% and 4% of the samples respectively. Although Klebsiella spp. was the least frequently isolated pathogen, its presence is still clinically significant, as this organism is increasingly associated with nosocomial and community-acquired respiratory infections in children (Rahmat Ullah *et al*., 2024).

Antimicrobial susceptibility testing revealed varied sensitivity patterns across the isolated organisms. Notably, Streptococcus pyogenes exhibited the highest antibiotic sensitivity rate at 52%. This high sensitivity is consistent with global findings that suggest S. pyogenes remains susceptible to beta-lactam antibiotics, particularly penicillin, which continues to be the treatment of choice (Olsen *et al*., 2022). In contrast, Klebsiella species demonstrated the highest resistance rate at 45%. This is particularly concerning as Klebsiella is a Gram-negative bacterium with multiple intrinsic and acquired resistance mechanisms, making it difficult to treat, especially in pediatric populations where antibiotic options are limited (Li *et al*., 2023). Furthermore, an assessment of multi-drug resistance (MDR), defined as resistance to three or more antibiotic classes revealed the highest MDR rate in Klebsiella spp. at 60%, followed closely by Staphylococcus aureus (55%) and Enterococcus faecalis (50%). These figures highlight the growing threat of antimicrobial resistance, which complicates the clinical management of URTIs and increases the risk of treatment failure (Salam *et al*., 2023). Figure 1, 2 and Table 2 presents the results.

Table 2

Prevalence of bacteria isolates in relation degree of sensitivity to antibiotics

Isolated bacteria	Antibiotic	Degree of resistance		x^2	df	sig
		Sensitive	Resistant			
Staphylococcus aureus *n= 40*	Erythromycin	29	11	66.67	3	0.001
	Tetracycline	25	15			
	Ampicillin/cloxacillin	5	35			
	Amoxicillin	0	40			
Streptococcus pyogenes *n= 31*	Erythromycin	20	11	42.11	3	0.001
	Tetracycline	9	22			
	Ampicillin/cloxacillin	2	29			
	Amoxicillin	0	31			
Enterococcus faecalis *n= 19*	Erythromycin	7	12	2.86	3	0.413
	Tetracycline	5	14			
	Ampicillin/cloxacillin	10	9			
	Amoxicillin	8	11			
Klebsiella species *n=4*	Erythromycin	4	0	8.89	3	0.031
	Tetracycline	2	2			
	Ampicillin/cloxacillin	1	3			
	Amoxicillin	0	4			
Streptococcus pneumoniae *n=6*	Erythromycin	5	1	13.51	3	0.004
	Tetracycline	2	4			
	Ampicillin/cloxacillin	0	6			
	Amoxicillin	0	6			

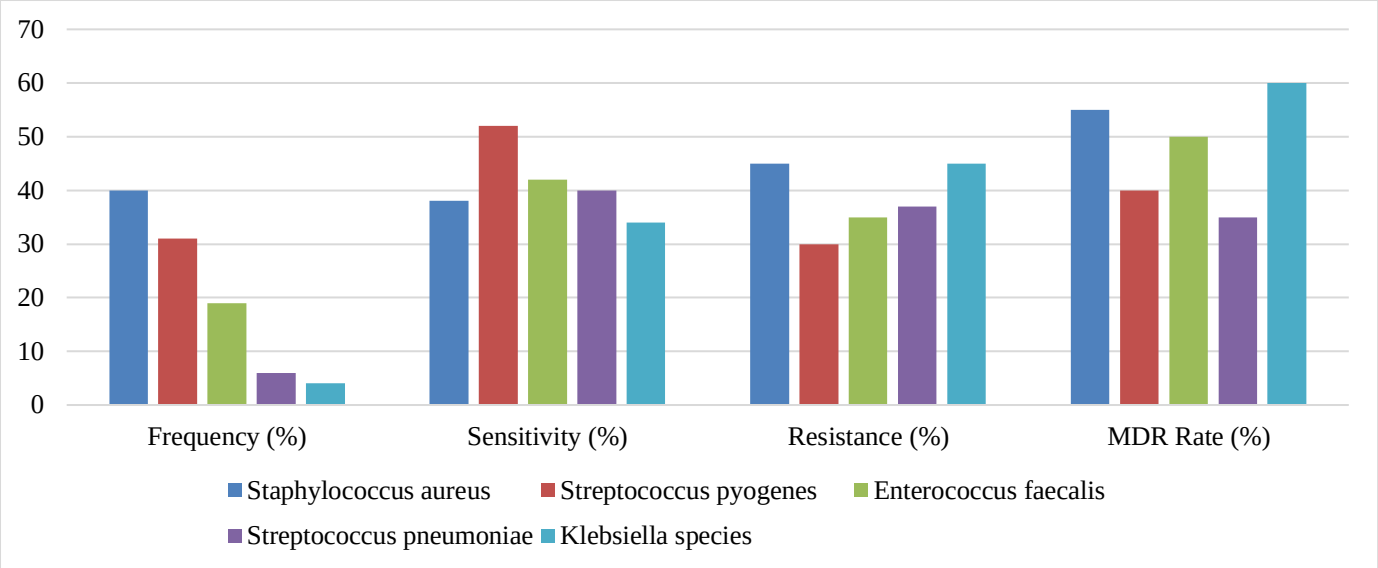

Figure 1
Isolation of Bacterial Pathogens

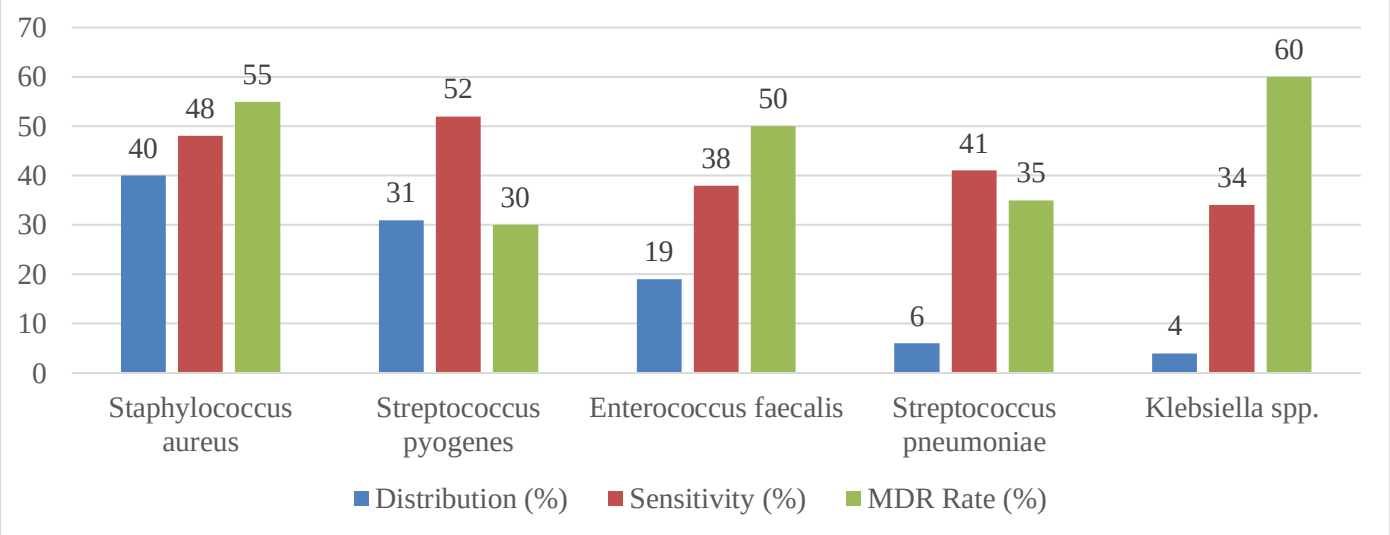

Figure 2
Distribution of Bacteria

4.1.2 Gene Resistance Patterns in the Isolated Bacterial Pathogens

The molecular analysis of bacterial isolates associated with pediatric upper respiratory tract infections (URTIs) in Kisumu County Hospital revealed the presence of key antimicrobial resistance (AMR) genes that contribute significantly to bacterial resistance mechanisms. Notably, 100% of the bacterial isolates examined exhibited the presence of the 16S rRNA gene mutation and the MecA gene. The 16S rRNA gene mutation is commonly associated with resistance to macrolide antibiotics, such as erythromycin and azithromycin. This resistance arises because the mutation alters the ribosomal RNA, impairing the binding of macrolides to the bacterial ribosome, thereby reducing their effectiveness (Cocozaki *et al.*, 2016).

The MecA gene, on the other hand, is a well-known genetic determinant of methicillin resistance in Staphylococcus aureus (MRSA). This gene encodes an alternative penicillin-binding protein (PBP2a), which has a low affinity for β-lactam antibiotics, rendering these drugs ineffective (Lade & Kim, 2023). The presence of MecA in all samples indicates a widespread occurrence of MRSA, which presents significant treatment challenges, especially in pediatric populations with limited antibiotic options as indicated in the Table 3.

Table 3

Gene Determination

Resistance Gene	Function / Associated Antibiotic Class	Frequency (n)	Prevalence (%)
16S rRNA Mutation	Ribosomal modification – Macrolides	5	100%
mecA	Methicillin resistance – β-lactams	5	100%
TEM	β-lactamase production – Penicillins/Cephalosporins	3	60%
TylA	Target modification – Macrolides/MLSB group	3	60%
vanA/vanB	Vancomycin resistance – Glycopeptides	3	60%
EF-Tu Mutants	Elongation factor – General protein synthesis interference	3	60%
aph(3')-IIIa	Aminoglycoside-modifying enzyme – Kanamycin, Amikacin	2	40%

4.2 Discussion

Although age and gender showed no significant association with the type of bacterial isolate or resistance pattern, a notable link was found between place of residence and the presence of multidrug-resistant organisms. Children from urban areas were more likely to harbor MDR pathogens. This is consistent with findings by Miriti et al in 2023, who observed that urban populations had higher antibiotic misuse rates, likely due to over-the-counter access, self-medication, and limited diagnostic facilities. However, another study done in Ethiopia suggested that males have a higher frequency of bacterial culture isolates while age remaining an insignificant factor (Ali & Kebede, 2008).

The study results also revealed that Staphylococcus aureus (40%) and Streptococcus pyogenes (31%) were the most common bacterial pathogens isolated from throat swabs of children under five years presenting with upper respiratory tract infections (URTIs) at Kisumu County Hospital. These findings are consistent with several studies conducted both locally and internationally. For instance, a study done in Bulgaria reported that S. aureus and S. pyogenes were among the predominant pathogens in paediatric URTIs (Cheung et al., n.d.). Similarly, a study in Meru Kenya identified S. aureus and S. pyogenes as key etiological agents in childhood respiratory infections (Miriti *et al*., 2023). The predominance of these organisms may be attributed to their colonization ability, virulence factors such as exotoxins, and ease of transmission in crowded settings typical of urban areas like Kisumu.

The presence of Enterococcus faecalis (19%), although not traditionally considered a primary respiratory pathogen, aligns with emerging literature recognizing its role in nosocomial and opportunistic infections in children with weakened immunity (Gaeta *et al*., 2023). Streptococcus pneumoniae and Klebsiella species were less frequently isolated in this study (6% and 4% respectively), which contrasts with previous findings where S. pneumoniae was the leading cause of respiratory infections in children (Thadchanamoorthy & Dayasiri, 2021). This difference may be due to increased pneumococcal vaccination coverage in recent years or regional differences in pathogen prevalence.

The study found that S. pyogenes exhibited the highest sensitivity (52%), followed by S. pneumoniae (50%) and S. aureus (45%). In contrast, Klebsiella species showed the highest resistance rate (45%). These findings are broadly consistent with regional and global trends in antimicrobial resistance (AMR). A study conducted by Ventola in 2015 reported rising resistance among Gram-negative pathogens, especially Klebsiella species, due to the production of extended-spectrum beta-lactamases (ESBLs) and carbapenemases. In a Kenyan study by Kimang'a in 2012, Klebsiella and E. coli were found to be highly resistant to ampicillin and cephalosporins, supporting the current findings. On the other hand, the relatively higher sensitivity among Gram-positive bacteria may reflect continued effectiveness of certain antibiotics like erythromycin and penicillin in this demographic, although resistance is gradually increasing.

It is also notable that E. faecalis showed moderate antimicrobial sensitivity (40%) and intermediate resistance (35%) to the antimicrobial drugs selected. These patterns reflect the adaptive mechanisms of Enterococci, such as intrinsic resistance to cephalosporin and the ability to acquire vancomycin resistance genes (Miller *et al*., 2014). The significant intermediate resistance suggests the potential for treatment failure with suboptimal dosing, underscoring the need for guided therapy based on laboratory data. Multidrug resistance (MDR) was observed in all five bacterial species, with the highest rate found in Klebsiella species (60%), followed by S. aureus (55%) and E. faecalis (50%). These findings are in line with global MDR surveillance reports that identify Klebsiella pneumoniae and S. aureus as leading multidrug-resistant organisms. The higher MDR rate in Klebsiella may be due to its ability to acquire plasmid-borne resistance genes, especially in hospital environments. Meanwhile, the high MDR rate in S. aureus may reflect the emergence of methicillin-resistant S. aureus (MRSA) strains in both community and healthcare settings.

This study employed polymerase chain reaction (PCR) techniques to detect antibiotic resistance genes in in the isolated bacterial pathogen in 10 out of 100 samples of patients with URTIs. 5 samples out of the 10 contained antimicrobial resistant genes (IDs 30, 65, 80, 83, and 54) and each showed multiple bacterial infections and the detection of specific AMR genes associated with resistance to key antibiotics used in the treatment and management of URTIs. The 16S rRNA gene mutation and MecA genes caused resistance in most of the isolated bacteria samples (100%). These findings are in line with two studies done where the aminoglycoside resistance associated with 16S rRNA and MecA gene mutations was high (90–96%) (24, 25) . Tem, TylA, vanA/B and EF-Tu Mutants AMR genes were each identified

in 3 out of the 5 samples (60%). The least prevelant gene identified was Aph(3)IIIa was identified in 2 out of 5 samples (40%), this gene encodes an enzyme that inactivates aminoglycoside antibiotics like kanamycin and amikacin (Zeng & Jin, 2003). The lack of statistical significance ($p > .05$) in the association tests which may be attributed to the small sample size ($n = 5$), the recurring detection of specific resistance genes across multiple samples suggests a biological trend that warrants further investigation with a larger dataset.

The molecular analysis showed that all the bacterial samples (100%) carried the 16S rRNA gene mutation and the MecA gene. The 16S rRNA gene mutation contributes to macrolide resistance by altering the ribosomal target site, while the MecA gene is typically associated with methicillin-resistant Staphylococcus aureus (MRSA), as it encodes a modified penicillin-binding protein that renders β-lactam antibiotics ineffective. Additionally, other resistance genes such as TEM, VanA/B, TylA, and EF-Tu mutants were each detected in 60% of the isolates, and the Aph(3')-IIIa gene which inactivates aminoglycosides was found in 40% of the samples.

V. CONCLUSION & RECOMMENDATIONS

5.1 Conclusion

These results point to an alarming presence of multidrug-resistant organisms among pediatric URTI cases. Such resistance undermines the effectiveness of commonly used antibiotics, complicates clinical management, and increases the risk of treatment failure, prolonged illness, and healthcare costs. The study highlights the urgent need for improved diagnostic methods, surveillance of antimicrobial resistance, and rational use of antibiotics to safeguard child health in the region

5.2 Recommendations

Based on the findings of this study, there should be policy development and implementation that focuses on Strengthening Laboratory Capacity for Culture and Sensitivity Testing; Health facilities, particularly in urban counties like Kisumu, should be equipped with reliable microbiological laboratories to guide evidence-based antibiotic prescriptions. Additionally, Routine molecular screening for resistance genes (such as 16S rRNA) should be adopted to improve early detection and monitoring of antimicrobial resistance.

Declaration of Interest

The authors declare that they do not have any known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding Declaration

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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