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Bacterial Resistance Test to Antibiotics Through Throat Swab Specimens of Children with Acute Respiratory Tract Infections (ARIs)

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ABSTRACT

Acute Respiratory Infections (ARIs) are contagious diseases that affect the respiratory tract and remain a leading cause of morbidity and mortality, particularly among children. The use of antibiotics without proper sensitivity testing can lead to bacterial resistance; therefore, research is needed to determine the effectiveness of commonly prescribed antibiotics. ARIs is one of the primary causes of morbidity and mortality, especially in children in developing countries. The irrational use of antibiotics or the absence of adequate sensitivity testing is one of the main factors contributing to the rise in bacterial resistance, which may reduce the effectiveness of treatment and increase the public health burden. Objective to determine the sensitivity levels of several types of antibiotics against bacteria that cause ARIs, obtained from throat swab specimens of children with ARIs in several community health centers (Puskesmas) under the Ternate City Health Office. This study is a descriptive research using a laboratory approach, applying the Kirby-Bauer method for antibiotic sensitivity testing against bacteria using Mueller Hinton Agar (MHA) media. Bacterial identification showed a predominance of *Staphylococcus epidermidis* (45%), followed by Streptococcus pneumoniae (35%), and *Streptococcus pyogenes* (20%). Sensitivity testing revealed that *Streptococcus pneumoniae* was most sensitive to gentamicin (100%), while *Staphylococcus epidermidis* showed high sensitivity to ciprofloxacin (100%). On the other hand, cefixime exhibited the highest level of resistance. Sensitivity testing is crucial before administering antibiotics to prevent resistance and ensure appropriate treatment for children with ARIs.

INTRODUCTION

Acute Respiratory Infections (ARI) remain one of the major global health concerns, particularly in developing countries. ARI refers to an infection that affects one or more parts of the respiratory tract, either the upper respiratory tract (such as the nose, pharynx, and larynx) or the lower respiratory tract (such as the trachea, bronchi, and lungs). It is classified as acute because it typically lasts less than 14 days. ARI often presents with symptoms such as fever, cough, runny nose, sore throat, and shortness of breath. In some cases, it may lead to severe complications and even death, especially among vulnerable groups such as infants and young children (Wang et al., 2024).

According to data from the World Health Organization (WHO) in 2020, Acute Respiratory Infections (ARI) account for up to 4.25 million deaths globally each year. The prevalence of ARI is particularly high among children aged 1–5 years, with an incidence

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rate reaching 42.91%. In Indonesia, ARI is among the top 10 most common illnesses that lead to visits to healthcare facilities, with high prevalence reported in several provinces, including Jakarta, East Java, Banten, and West Papua. In Ternate City, the local Health Office recorded a total of 159,978 ARI cases from 2017 to 2020, indicating that this disease remains a major health burden in the region (Purnama et al., 2025).

The high incidence of Acute Respiratory Infections (ARI) is directly proportional to the increased use of antibiotics in the community, especially among children. This becomes a concern when antibiotics are administered without proper justification, such as without laboratory testing or bacterial sensitivity analysis. In fact, the majority of ARI cases are caused by viruses, which do not require antibiotic therapy. This irrational use of antibiotics significantly contributes to the rise in antimicrobial resistance where antibiotics are no longer effective in inhibiting or killing the microorganisms responsible for infections (Darod et al., 2023).

Antibiotic resistance is a global issue that affects the effectiveness of bacterial infection treatments. Numerous studies have indicated that resistance arises from various factors, such as improper antibiotic use, incorrect dosage and duration, and administration without strong medical indications. One crucial step in addressing this problem is conducting antibiotic sensitivity testing a microbiological method used to determine the effectiveness of antibiotics against specific bacterial isolates from patients. By identifying bacterial sensitivity to antibiotics, physicians can prescribe the most appropriate and effective treatment, while also reducing the risk of resistance development (Tirago et al., 2025).

At the local level, particularly in Ternate City, ARI ranks highest among communicable diseases. According to data from the Ternate City Health Office, a total of 159,978 ARI cases were recorded between 2017 and 2020. A significant surge occurred in 2019, with the number of cases reaching 95,542. This situation indicates that ARI remains a very serious public health issue in the region, especially among children, who are the most vulnerable age group. In addition to its health impacts, ARI also carries considerable social and economic consequences, such as school absenteeism among children and reduced productivity of parents who must care for their sick children (WHO, 2022).

ARI can be caused by various pathogenic agents, including both viruses and bacteria. In clinical practice, viral infections are the predominant cause; however, bacterial infections may also be the primary cause or arise as complications following a viral infection. This presents a major issue in healthcare services widespread and inappropriate use of antibiotics. Although antibiotics have no effect on viruses, many ARI patients are still prescribed antibiotics without bacteriological diagnosis. This common practice has contributed to the alarming rise in antibiotic resistance cases (Petat et al., 2025).

Antibiotic sensitivity testing is a microbiological method aimed at determining the effectiveness of a particular antibiotic in inhibiting bacterial growth. This method is essential to ensure that the prescribed antibiotic is truly effective against the bacteria causing the infection. One commonly used technique is the disk diffusion method, also known as the Kirby-Bauer method. In this method, bacterial isolates obtained from patient specimens (such as throat swabs) are cultured on Mueller Hinton Agar (MHA). Antibiotic-impregnated disks are then placed on the agar surface to observe the formation of inhibition zones. The larger the inhibition zone, the more sensitive the bacteria are to the antibiotic (Bayot ML, 2024).

Antibiotic resistance occurs when bacteria undergo genetic changes or acquire resistance genes from other microorganisms, enabling them to survive despite antibiotic treatment. The World Health Organization has stated that antibiotic resistance is a serious threat to global public health and requires comprehensive cross-sectoral efforts to control it.

The use of antibiotics without sensitivity testing accelerates the emergence of resistant bacteria and leads to increasingly limited treatment options. As a result, infections that should be easily treatable can become more severe and potentially life-threatening (Sur & Plesa, 2022; Tirago et al., 2025).

The high prevalence of upper respiratory tract infections (URTIs) and their associated impacts have led to increased consumption of over-the-counter medications (such as anti-influenza, cough medicines, and multivitamins) as well as antibiotics. In practice, antibiotics are frequently prescribed to treat these infections. Excessive antibiotic prescribing is particularly common in respiratory tract infections, especially acute upper respiratory tract infections, even though the majority of cases are caused by viruses. One contributing factor is the overuse of antibiotics by clinicians, often as a preventive measure against secondary bacterial infections which, in reality, cannot always be prevented. The consequence of this practice is a growing problem of bacterial resistance (Kementerian Kesehatan RI, 2021).

MATERIALS/METHOD

This study is a descriptive research. The purpose of this descriptive study is to provide an overview of the antibiotic sensitivity of bacteria causing acute respiratory infections.

The population of this study consists of patients with Acute Respiratory Infections (ARI) at community health centers (Puskesmas) within the working area of the Ternate City Health Office, including Puskesmas Siko, Puskesmas Kota, Puskesmas Kalumpang, and Puskesmas Kalumata. The sputum specimen examinations will be conducted at the Integrated Laboratory of the Poltekkes Kemenkes Ternate. This study has received ethical approval from the Health Research Ethics Committee under the approval number UM.2.03/6/526/2025.

The procedures carried out in this study consist of the following steps:

1. Sterilization of Equipment
   Glassware is washed, wrapped in paper or aluminum foil, and then sterilized in an oven at 180°C for 1–2 hours.
2. Preparation of Culture Media
   a. Preparation of Mannitol Salt Agar (MSA) begins by weighing 111.5 grams of the medium and dissolving it in distilled water in an Erlenmeyer flask to a final volume of 1 liter, then homogenizing the solution. Sterilize the medium in an autoclave at 121°C for 15 minutes. Pour approximately 25 mL of the medium into sterile petri dishes and allow it to solidify.
   b. Preparation of MacConkey Agar (MCA) begins by weighing 18.03 grams of MacConkey agar using an analytical balance. The medium is placed into an Erlenmeyer flask and dissolved in 350 mL of distilled water, then heated and stirred until boiling and homogeneous. Sterilize the medium in an autoclave at 121°C for 15 minutes, then pour it into sterile petri dishes and allow it to solidify.
   c. Preparation of Mueller Hinton Agar (MHA) begins by weighing 35.7 grams of the medium and dissolving it in distilled water in an Erlenmeyer flask to a final volume of 1 liter, then homogenizing the solution. Sterilize the medium in an autoclave at 121°C for 15 minutes. Pour approximately 25 mL of the medium into sterile petri dishes and allow it to solidify.
3. Collection of Throat Swab Samples
   The procedure for collecting throat swab samples is carried out using a sterile swab. The container for the throat swab sample must also be sterile to prevent contamination by other bacteria. The patient is asked to open their mouth, ensuring that the tongue does not obstruct the throat. The throat area is then swabbed using the sterile cotton swab.

After the swabbing is completed, the specimen is transported to the laboratory for immediate examination.

4. Culturing of Throat Swab Samples
   Prepare a suspension by placing sterile physiological NaCl solution into a test tube, then immerse the sterile cotton swab into the solution. Inoculate the bacterial culture by dipping a sterile loop into the suspension and streaking it onto the culture medium. Close the petri dish aseptically near a spirit lamp flame. Then, re-sterilize the inoculating loop to eliminate any remaining bacteria. Seal the edge of the petri dish that has been inoculated, and incubate it at 37°C for 24 hours in an incubator. This step is followed by bacterial identification through biochemical testing.
5. Biochemical Testing
   Biochemical testing is performed to identify the bacteria in the samples, using the following procedures: Using a straight inoculating needle, take a single colony and stab it aseptically into TSIA and SIM media. Then, using a loop, take another colony and streak it aseptically onto citrate and urea media. Next, take another colony and suspend it aseptically in MR and VP media. Incubate all media at 37°C for 24 hours. After 24 hours, observe color changes and bacterial growth in TSIA, SIM, urea, and citrate media. Then, add 3 drops of methyl red indicator to the MR medium, and 3 drops of 40% KOH solution along with 3 drops of alpha-naphthol solution to the VP medium. Observe any resulting color changes.
6. Preparation of 0.5 McFarland Standard Solution
   A total of 0.05 mL of 1% Barium Chloride ($BaCl_2$) in distilled water is added to 9.95 mL of 1% Sulfuric Acid (H_2SO_4). The mixture is then homogenized and stored in a place protected from direct sunlight.
7. Preparation of Bacterial Test Suspension
   Bacterial colonies that have grown on MCA, MSA, and Blood Agar media are collected using a sterile inoculating loop and suspended in a test tube containing 5 mL of sterile 0.9% NaCl solution until the turbidity reaches the equivalent of a 0.5 McFarland standard. The same procedure is applied to each type of test bacteria. Inoculation of the bacterial suspension onto MSA media and bacterial susceptibility testing using the Kirby-Bauer method: After preparing the pure bacterial suspension adjusted to the 0.5 McFarland standard, the suspension is inoculated onto MHA (Mueller Hinton Agar) by aseptically taking one loopful of the bacterial suspension. The suspension is then spread evenly across the entire surface of the medium. The plate is divided into four sections, and one antibiotic paper disc is placed in each section. The plates are then incubated in an incubator at 37°C for 24 hours.

RESULTS AND DISCUSSION

The study was conducted at Puskesmas Siko, Puskesmas Kota, Puskesmas Kalumata, Puskesmas Kalumpang, and the Integrated Laboratory of the Health Polytechnic (Poltekkes) Kemenkes Ternate. The research focused on the bacterial sensitivity to antibiotics using throat swab specimens from children diagnosed with Acute Respiratory Infections (ARI). A total of 30 throat swab samples were collected over a 7-day period, from May 9 to June 15, 2025. The research findings are presented in the following tables.

Table 1. Respondent characteristics

Respondent characteristics	Frequency	Percentage
gender		
male	19	63
female	11	37

age		
children aged 5-11	16	53
children aged 12-15	14	47
symptoms suffered		
cough	21	70
itchy throat	6	20
dispneu	3	10

As presented in Table 1, the characteristics of the respondents show that the majority of the children were male and aged between 5 and 15 years, with the main clinical symptom being cough (70%), accompanied by sore throat and shortness of breath. This finding is consistent with pediatric studies reported in China, where cough, sore throat, fever, and dyspnea were identified as the predominant symptoms of acute respiratory tract infections (ARTIs) in children (Wang et al., 2024).

Table 2. Percentage of identification of ARIs bacteria

No	Bacteria	Frequency	Percentage
1	*S. epidermidis*	14	45
2	*S. pneumoniae*	11	35
3	*S. pynogenes*	6	20
Number of pure culture isolates		31	100

Based on Table 2, Bacteriological analysis revealed a predominance of Gram-positive pathogens, with *S. epidermidis* identified in 45% of isolates, followed by S. pneumoniae (35%) and *S. pyogenes* (20%). This distribution is consistent with other studies in pediatric tonsillitis. For instance, Tirago et al. found *S. pyogenes* to be the most frequently isolated pathogen (25.4%), followed by *S. aureus*, *S. epidermidis*, and *S. pneumoniae*. Although S. epidermidis is a common component of normal oropharyngeal flora, it is frequently isolated in ARI cases in children (Tirago et al., 2025).

Table 3. Percentage of Bacterial Sensitivity to Gentamicin, Ciprofloxacin, Amoxicillin and Cefixime

Antibiotics/ Bacteria	Sensitive	%	Resistant	%	Intermediate	%
Gentamicin						
S. epidermidis	10	71	3	21	1	8
S. pneumoniae	11	100	-	-	-	-
S. pynogenes	4	67	1	17	1	16
Ciprofloxacin						
S. epidermidis	14	100	-	-	-	-
S. pneumoniae	9	82	-	-	2	18
S. pynogenes	3	50	-	-	3	50
Amoxicillin						
S. epidermidis	5	36	2	14	7	50
S. pneumoniae	2	18	6	55	3	27
S. pynogenes	3	50	1	17	2	33
Cefixime						
S. epidermidis	-	-	14	100	-	-

S. pneumoniae	-	-	11	100	-	-
S. pynogenes	-	-	6	100	-	-

Gentamicin is a narrow-spectrum antibiotic belonging to the aminoglycoside class and is effective against both Gram-positive and Gram-negative bacteria. Its primary mechanism of action involves the inhibition of protein synthesis through disruption of the translation processes of RNA and DNA, resulting in the production of defective proteins and ultimately exerting a bactericidal effect. To reach the ribosomes and interfere with protein biosynthesis, gentamicin, a positively charged cation, passively binds to the negatively charged outer membrane of Gram-negative bacteria. This electrostatic interaction facilitates the formation of pores or membrane disruptions via the transmembrane electrical potential. These disruptions not only lead to the leakage of intracellular contents but also enhance antibiotic penetration, enabling gentamicin to cross the cytoplasmic membrane. Ciprofloxacin, a broad-spectrum antibiotic of the fluoroquinolone class, acts by inhibiting nucleic acid synthesis, specifically DNA and RNA, thereby interfering with bacterial replication and transcription and preventing normal cellular development. Amoxicillin, a member of the penicillin group specifically the broad-spectrum aminopenicillins inhibits the synthesis of mucopeptides essential for bacterial cell wall formation. This inhibition compromises the structural integrity of the bacterial cell wall, resulting in cell lysis and death. Cefixime, a third-generation cephalosporin, is used to treat a range of bacterial infections, including bronchitis, gonorrhea, and infections of the ears, throat, tonsils, and urinary tract (Nurdin et al., 2023; Wang et al., 2024).

Based on the sensitivity test data presented in Tables 3 through it is evident that each antibiotic exhibited varying degrees of effectiveness against the different bacterial species tested. In the sensitivity test using gentamicin, *S. pneumoniae* demonstrated the highest sensitivity, with 100% (11 colonies) showing susceptibility. Conversely, the highest resistance was observed in *S. epidermidis* at 23%, while the highest intermediate response was noted in *S. pyogenes* at 16%. Meanwhile, in the ciprofloxacin test, *S. epidermidis* exhibited maximum sensitivity at 100%. *S. pyogenes* showed the highest intermediate response at 10%, and no resistance was detected, indicating the high effectiveness of ciprofloxacin against the tested bacterial strains. Figure 1 also illustrates the results of the antibiotic susceptibility tests for the following bacterial strains: A1 : *S. epidermidis*, A2 : *S. pneumoniae*, and A3 : *S. pyogenes*.

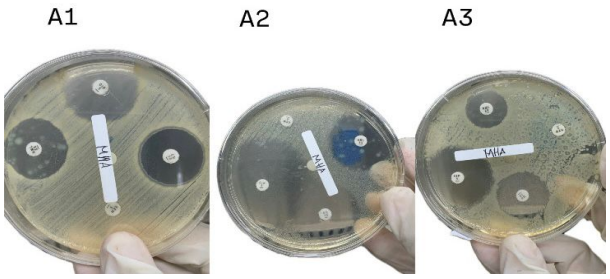

Figure 1. Results of Bacterial Sensitivity Test to Antibiotics (A1: *S. epidermidis*, A2: *S. pneumoniae*, A3: *S. pyogenes*)

Antimicrobial susceptibility testing demonstrated limited efficacy of both amoxicillin and cefixime against the bacterial strains evaluated. *S. epidermidis* exhibited the greatest sensitivity to amoxicillin; however, this was restricted to only 36% of isolates, reflecting suboptimal overall effectiveness. A pronounced resistance pattern was observed in *S. pneumoniae*, with 55% of isolates displaying resistance to amoxicillin. Notably, *S. epidermidis* presented the highest rate of intermediate susceptibility at 50%, further

emphasizing the limited therapeutic potential of amoxicillin against the majority of tested strains. In stark contrast, cefixime exhibited no detectable antimicrobial activity against any of the bacterial isolates assessed. All strains of *S. epidermidis*, *S. pyogenes*, and *S. pneumoniae* demonstrated complete resistance (100%) to cefixime, with no intermediate responses recorded. These results collectively indicate that cefixime was the least effective agent among those tested, underscoring the urgency of exploring alternative antimicrobial strategies for the treatment of infections caused by these pathogens.

These results are consistent with previous findings by Darod *et al*., who reported that *S. pneumoniae* isolates were completely resistant to ampicillin, and with Tirago *et al*., who observed resistance levels of approximately 45% to cephalosporins and penicillin among *S. pyogenes* isolates (Darod et al., 2023; Tirago et al., 2025; Wang et al., 2024).

Overall, ciprofloxacin demonstrated the highest effectiveness among the four antibiotics tested, whereas cefixime showed the lowest effectiveness, with complete resistance observed in all bacterial strains. These findings provide a crucial basis for selecting appropriate antibiotics based on the specific bacterial pathogens causing the infection (Tirago et al., 2025; Wang et al., 2024).

The observed variation in antibiotic efficacy underscores the essential role of antimicrobial susceptibility testing in guiding appropriate therapeutic interventions. Consistent with this, Tirago et al. emphasized the necessity of culture and sensitivity testing to inform clinical decision-making in the management of pediatric tonsillitis. Similarly, Darod et al. advocated for laboratory-based diagnostic approaches and targeted antibiotic selection to enhance treatment efficacy and minimize both recurrence and the development of antimicrobial resistance. The present findings align with these recommendations, reinforcing the importance of evidence-based antibiotic prescribing practices, particularly in the management of pediatric acute respiratory infections (ARIs) (Darod et al., 2023).

This study cannot serve as a definitive reference for the overall use of antibiotics in the treatment of acute respiratory infections (ARIs). However, it may provide a useful reference or supplementary information regarding the antibiotic sensitivity patterns of bacteria that cause ARIs.

CONCLUSIONS

Based on the results of the sensitivity test of the antibiotics gentamicin, ciprofloxacin, amoxicillin, and cefixime against bacteria isolated from throat swabs of children with acute respiratory infections (ARIs), ciprofloxacin was found to be the most effective antibiotic against *S. epidermidis*, exhibiting a sensitivity rate of 100%. In contrast, cefixime showed no antibacterial activity against any of the tested isolates, with a resistance rate of 100% across all three bacterial species. Meanwhile, *S. epidermidis* exhibited the highest intermediate response, at 50%.

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