Frontiers in Health Informatics
ISSN-Online: 2676-7104
www.healthinformaticsjournal.com
2024; Vol 13: Issue 3
Open Access

Phenotypic Analysis of Antibiotic Resistance Mechanisms in Bacteria Causing Tonsillitis: Focus on ESBL, MBL, and MRSA

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Cite this paper: Vaishnavi B. Shevale1, Dr. Ravindra V. Shinde2, Dr. Satish R. Patil3 (2024) Phenotypic Analysis of Antibiotic Resistance Mechanisms in Bacteria Causing Tonsillitis: Focus on ESBL, MBL, and MRSA. *Frontiers in Health Informatics, 13* (3), 6268-6281

Abstract

Introduction*: Tonsillitis, a common inflammatory condition primarily affecting children, is caused by various bacterial and viral pathogens. The condition poses a significant public health challenge due to the rise in antimicrobial resistance, especially among methicillin-resistant Staphylococcus aureus (MRSA), Extended Spectrum Beta-Lactamase (ESBL)-producing, and Metallo Beta-Lactamase (MBL)-producing bacteria. This study investigates the prevalence of these resistant bacterial strains in tonsillitis cases to inform effective treatment strategies and improve clinical outcomes.*

Methods and Materials: This cross-sectional observational study was conducted at the Krishna Institute of Medical Sciences, India, from November 2021 to November 2022. Throat swab samples from tonsillitis patients were collected and cultured. Confirmed bacterial isolates were tested for antibiotic resistance using phenotypic methods: MRSA was detected using cefoxitin disc diffusion, ESBL production was confirmed with ceftazidime and ceftazidime-clavulanic acid discs, and MBL production was assessed via imipenem-EDTA disc testing. Data were statistically analyzed, and results were expressed as percentages.

Results: Out of 96 bacterial isolates, 58.97% of Staphylococcus aureus isolates were MRSA, 36.53% of gram-negative isolates were ESBL producers, and 46.15% were MBL producers. MRSA isolates were highly susceptible to linezolid and chloramphenicol, while doxycycline and azithromycin showed moderate effectiveness against ESBL and MBL producers. The high prevalence of resistant strains highlights the need for careful antibiotic selection and emphasizes the importance of local resistance data in guiding treatment protocols.

Conclusion: *The study reveals a significant prevalence of MRSA, ESBL, and MBL-producing bacteria among tonsillitis-causing pathogens, underscoring the critical need for routine susceptibility testing and antimicrobial stewardship. Effective infection control strategies and continuous surveillance are essential to mitigate the impact of antibiotic resistance, ensuring optimal patient outcomes and public health protection.*

Keywords: *Tonsillitis, MRSA, ESBL, MBL, antibiotic resistance, infection control, susceptibility testing, antimicrobial stewardship, Staphylococcus aureus, gram-negative bacteria*

I. Introduction

Tonsillitis, an inflammation of the palatine tonsils, is a common condition primarily affecting children and young adults. This inflammation, generally triggered by viral or bacterial infections, manifests in various forms: acute, chronic, and recurrent. The condition not only affects the individual's quality of life due to the associated pain, fever, and difficulty in swallowing but also poses a considerable public health challenge due to the increasing incidence of antimicrobial resistance among causative agents [1]. The most common bacterial pathogens associated with tonsillitis include *Streptococcus pyogenes* and *Staphylococcus aureus*, which are known for their ability to adapt and survive in the human host, often resulting in recurrent infections and complications.

The tonsils, as part of the Waldeyer's ring, play a critical role in immune defense. They act as a primary line of defense against inhaled or ingested pathogens by presenting antigens and stimulating both humoral and cell-mediated immune responses. Tonsillar tissue contains T-lymphocytes, B-lymphocytes, and macrophages that aid in immune response, especially in children whose immune systems are still developing. With the advent of antimicrobial resistance, particularly methicillin-resistant *Staphylococcus aureus* (MRSA), Extended Spectrum Beta-Lactamase (ESBL) producers, and Metallo Beta-Lactamase (MBL) producers, the treatment landscape for bacterial tonsillitis is becoming more complex and challenging. This study investigates these specific resistance mechanisms to better understand the challenges they pose in clinical treatment and explore potential mitigation strategies [2].

Figure 1(a). Methicillin resistance Staphylococcus aureus

Figure 1(b). Methicillin Sensitive Staphylococcus aureus

Tonsillitis affects children more frequently than adults due to the higher immunological activity of the tonsils in early childhood. Studies estimate that children experience a high incidence of tonsillitis-related doctor visits, especially in the 5-10 age group, during which tonsils are most immunologically active. While tonsillitis can be caused by a variety of pathogens, the infection is often the result of exposure to common respiratory pathogens that easily spread within school and daycare environments.

The primary bacterial agents responsible for tonsillitis include *Streptococcus pyogenes* (Group A Streptococcus) and *Staphylococcus aureus*. These bacteria can invade the tonsillar tissue, causing an immune response characterized by inflammation, redness, and swelling. Streptococcus pyogenes is known for causing not only

tonsillitis but also complications such as rheumatic fever and glomerulonephritis. *Staphylococcus aureus*, on the other hand, can colonize the tonsils and lead to both acute and recurrent infections. In recent years, other gram-negative bacteria like *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* have also emerged as significant pathogens, particularly in chronic or hospital-acquired tonsillitis cases [3].

Viral infections, including adenoviruses and Epstein-Barr virus, also play a role in the development of tonsillitis, typically resulting in self-limiting conditions. However, bacterial infections often require medical intervention, with antibiotics as the primary line of treatment. Despite antibiotic therapy, recurrent tonsillitis is a common outcome, suggesting a need to address underlying issues such as microbial resistance and host immune factors.

Role of Tonsils in Immune Response

The tonsils serve as part of the mucosa-associated lymphoid tissue (MALT) system, playing a crucial role in immune defense against airborne and ingested pathogens. Tonsillar tissue contains crypts, or deep folds, that capture pathogens, presenting them to immune cells such as macrophages, T-lymphocytes, and B-lymphocytes [4]. The tonsils are particularly significant in early childhood when the immune system is still developing. They act as a primary site for antigen presentation, allowing the immune system to recognize and respond to foreign agents, leading to the production of immunoglobulins. This immunological function, however, also makes the tonsils a reservoir for pathogens that can occasionally evade immune detection and establish persistent infections.

The process of antigen presentation in the tonsils begins with pathogens being captured by antigen-presenting cells (APCs), which process the antigens and present them to T-cells, thereby initiating an adaptive immune response [5]. The presence of specialized epithelial cells in the tonsils aids in antigen transport and the development of memory T-cells, which contribute to long-term immunity. Nevertheless, this immune activity also renders the tonsils susceptible to infections, as they become a target for various bacterial pathogens that have developed mechanisms to evade immune detection and resist immune-mediated destruction.

Antibiotic Resistance in Tonsillitis

Antibiotic resistance is one of the most pressing issues in modern healthcare, and tonsillitis is no exception to this challenge. The overuse and misuse of antibiotics in treating tonsillitis have contributed to the emergence of resistant strains of bacteria, complicating treatment outcomes. Among the most problematic resistant pathogens are MRSA, ESBL-producing gram-negative bacteria, and MBL-producing bacteria, all of which exhibit resistance to commonly used antibiotics.

1. **Methicillin-Resistant Staphylococcus aureus (MRSA):** MRSA is a significant contributor to antibiotic resistance in tonsillitis, especially in recurrent cases. It is resistant to beta-lactam antibiotics, including methicillin, penicillin, and amoxicillin, which are commonly prescribed for tonsillitis [6]. The resistance in MRSA strains is due to the acquisition of the mecA gene, which encodes a penicillin-binding protein (PBP2a) that has low affinity for beta-lactams, thereby rendering these antibiotics ineffective. MRSA not only complicates the treatment process but also poses a higher risk of spreading to other parts of the respiratory tract, leading to more severe infections.
2. **Extended Spectrum Beta-Lactamase (ESBL) Producers:** ESBLs are enzymes produced by certain gram-negative bacteria, including *Escherichia coli* and *Klebsiella pneumoniae*, which confer resistance to a broad range of beta-lactam antibiotics, including penicillins, cephalosporins, and aztreonam. ESBL-producing bacteria pose a challenge in the treatment of tonsillitis as they limit the efficacy of first-line antibiotic treatments [7]. In healthcare settings, ESBLs are associated with higher rates of morbidity

and mortality, primarily because these infections often require treatment with more toxic and expensive antibiotics like carbapenems.

3. **Metallo Beta-Lactamase (MBL) Producers:** MBL-producing bacteria represent a newer and particularly concerning resistance mechanism. MBLs can hydrolyze a wide range of beta-lactam antibiotics, including carbapenems, which are often considered a last line of defense against resistant infections. MBL-producing organisms, commonly found among *Pseudomonas aeruginosa* and other gram-negative bacteria, are associated with higher treatment failure rates, as they render many antibiotic options ineffective. MBL production among tonsillitis-causing bacteria is a growing concern, particularly in regions with high antibiotic usage.

This study focuses on evaluating the prevalence of ESBL, MBL, and MRSA in tonsillitis-causing bacteria. These resistance mechanisms are among the most challenging for clinicians, as they significantly limit the choice of effective antibiotics. Understanding the prevalence and distribution of these resistant bacteria in tonsillitis cases is essential for guiding antibiotic selection and improving patient outcomes [8]. The presence of resistant bacteria not only complicates treatment but also increases the risk of complications, prolonged hospital stays, and the spread of resistant pathogens within the community. The need for empirical antibiotic selection, informed by local resistance patterns, is critical for optimizing treatment strategies. Moreover, identifying the phenotypic characteristics of these resistant strains can aid in developing more effective infection control measures and surveillance programs [9][10]. In conclusion, tonsillitis represents a significant burden on healthcare systems, and the rise of antibiotic-resistant strains among causative bacteria exacerbates this issue. This study aims to provide a comprehensive understanding of the prevalence of MRSA, ESBL, and MBL producers in tonsillitis cases, thereby contributing valuable insights for clinicians to manage and control resistant infections effectively. Ongoing research and surveillance efforts are essential to combat the growing threat of antimicrobial resistance in tonsillitis and to ensure that effective treatment options remain available for affected patients.

II. Materials and Methods

a. Study Design

This research was conducted as a cross-sectional observational study at the Department of Microbiology, Krishna Institute of Medical Sciences, Krishna Hospital and Medical Research Centre, Karad, Maharashtra, India. The study spanned from November 2021 to November 2022 and aimed to evaluate the prevalence of Extended Spectrum Beta-Lactamase (ESBL), Metallo Beta-Lactamase (MBL), and Methicillin-Resistant *Staphylococcus aureus* (MRSA) among bacterial isolates causing tonsillitis. The findings from this study were intended to guide antibiotic therapy and enhance clinical decision-making to address antibiotic resistance challenges.

b. Sample Collection and Study Population

The study population comprised patients across all age groups who visited Krishna Hospital and Medical Research Centre with clinical symptoms suggestive of tonsillitis, such as sore throat, fever, and difficulty swallowing. Patients were selected based on inclusion and exclusion criteria to ensure relevant data collection:

- **Inclusion Criteria:**
  - All patients presenting with symptoms of tonsillitis, including children and adults.
  - Patients who consented to participate in the study after understanding its purpose.
- **Exclusion Criteria:**
  - Patients who did not consent to the study.

- Patients unavailable for sample collection during the study period.

After obtaining informed consent, clinical samples were collected from each patient. Throat swabs were taken using sterile cotton swabs, and each sample was immediately transported to the microbiology laboratory in a sterile medium to ensure sample integrity. To standardize the data collection, a single investigator handled each patient's sample collection, documentation, and transfer to the lab, minimizing variations in sampling and data handling.

c. Microbiological Processing of Samples

Upon arrival at the microbiology laboratory, each sample was cultured to isolate and identify bacterial pathogens. The samples were initially plated on various selective and differential media, including blood agar and MacConkey agar, to promote the growth of pathogenic bacteria and distinguish them based on colony morphology and hemolysis patterns [11]. The plates were incubated at 37°C for 24-48 hours to allow for optimal bacterial growth. Gram staining was then performed on representative colonies to determine the presence of gram-positive or gram-negative bacteria, as this would guide further testing procedures.

The primary bacterial pathogens isolated included *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. These bacteria were then subjected to a battery of tests for identification and confirmation, such as catalase and coagulase tests for *Staphylococcus aureus* and biochemical tests like triple sugar iron (TSI) agar, citrate, and urease for gram-negative isolates. Only confirmed bacterial isolates were selected for further antibiotic resistance testing.

d. Phenotypic Testing for Antibiotic Resistance

The phenotypic detection of antibiotic resistance, particularly for ESBL, MBL, and MRSA, was performed using the disc diffusion method, based on the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI). This method allowed for a standardized, reproducible approach to assess antibiotic susceptibility across multiple isolates.

1. **Methicillin-Resistant *Staphylococcus aureus* (MRSA) Detection:**
   To detect MRSA, the confirmed *Staphylococcus aureus* isolates were subjected to cefoxitin disc diffusion testing. Cefoxitin (30 µg) was selected due to its reliability in predicting mecA gene presence, a marker for methicillin resistance. The testing procedure was as follows:
   - The inoculum was prepared by suspending bacterial colonies in sterile saline and adjusting the suspension to match a 0.5 McFarland turbidity standard.
   - A sterile swab was used to evenly spread the bacterial suspension over the surface of a Mueller-Hinton agar plate.
   - A cefoxitin disc (30 µg) was placed on the agar surface, and the plate was incubated at 37°C for 24 hours.
   - Following incubation, the zone of inhibition around the cefoxitin disc was measured. An inhibition zone of ≤ 21 mm indicated resistance, confirming the presence of MRSA.

   The control strain used for MRSA testing was *Staphylococcus aureus* ATCC 25923, a well-characterized strain known for its antibiotic susceptibility pattern.
2. **Extended Spectrum Beta-Lactamase (ESBL) Detection:**
   ESBL production among gram-negative isolates was detected using a combination disc method involving ceftazidime and ceftazidime-clavulanic acid discs. This method leverages the ability of clavulanic acid to inhibit beta-lactamases, allowing for differentiation between ESBL-producing and non-ESBL-producing bacteria. The steps for ESBL detection were as follows:

- A 0.5 McFarland standard suspension of each gram-negative isolate was prepared.
- The bacterial suspension was spread on Mueller-Hinton agar plates to achieve semi-confluent growth.
- A ceftazidime (30 µg) disc and a ceftazidime-clavulanic acid (30 µg + 10 µg) disc were placed 20 mm apart on the agar surface.
- The plates were incubated at 37°C for 18-24 hours.
- After incubation, the inhibition zones were measured. An increase of ≥ 5 mm in the zone of inhibition with the ceftazidime-clavulanic acid disc compared to the ceftazidime disc alone indicated ESBL production.

Positive and negative controls were included to validate the test. *Escherichia coli* ATCC 25922 served as the control strain for ESBL testing.

3. **Metallo Beta-Lactamase (MBL) Detection:**
   MBL production was detected using the imipenem-EDTA combination disc method, which allows for the differentiation between MBL producers and non-producers. The procedure for MBL detection included the following steps:
   - A 0.5 McFarland standard suspension of the test organism was prepared.
   - The suspension was spread on Mueller-Hinton agar plates.
   - An imipenem (10 µg) disc and an imipenem-EDTA (10 µg + 750 µg) disc were placed 20 mm apart on the agar surface.
   - The plates were incubated at 37°C for 18-24 hours.
   - After incubation, an increase of ≥ 7 mm in the inhibition zone around the imipenem-EDTA disc compared to the imipenem disc alone was interpreted as a positive result for MBL production.

   For quality control, positive and negative controls were used to ensure the reliability of results. *Pseudomonas aeruginosa* ATCC 27853 served as the control strain for MBL testing.

e. Statistical Analysis

Data from the study were recorded and analyzed using Microsoft Excel software. Descriptive statistics were employed to summarize the prevalence rates of MRSA, ESBL, and MBL-producing organisms. Data were expressed as percentages, and statistical tests, such as the chi-square test, were used to compare resistance rates among different bacterial isolates. A p-value of <0.05 was considered statistically significant.

f. Ethical Considerations

The study was conducted following ethical guidelines to ensure the protection of participants. Ethical approval was obtained from the Ethics Committee at Krishna Institute of Medical Sciences, and informed consent was obtained from all patients or their guardians before sample collection. Participation in the study was voluntary, and patients were informed about their right to withdraw at any time without affecting their medical care. All personal information was anonymized to protect patient confidentiality, and data were used solely for research purposes.

g. Quality Control Measures

Throughout the study, stringent quality control measures were employed to ensure the reliability and accuracy of results:

- **Control Strains:** Control strains were included in each batch of tests to validate the accuracy of the results. For MRSA detection, *Staphylococcus aureus* ATCC 25923 was used. For ESBL detection,

Escherichia coli ATCC 25922 was used, and for MBL detection, *Pseudomonas aeruginosa* ATCC 27853 was used.

- **Standardization of Procedures:** The 0.5 McFarland turbidity standard was consistently used to standardize bacterial suspensions across all phenotypic tests, ensuring uniformity in bacterial concentrations.
- **Documentation and Data Handling:** A single investigator was responsible for sample collection, processing, and recording of data, minimizing the risk of errors due to multiple handlers.
- **Environmental Controls:** The microbiology laboratory adhered to standard aseptic procedures to prevent contamination during sample handling and processing.

h. Data Management and Record Keeping

All data collected during the study were meticulously recorded and stored in a secure database. Access to patient information was restricted to authorized personnel to maintain confidentiality. Data were backed up periodically to prevent loss, and all study-related documents were maintained following institutional guidelines for record retention.

i. Limitations of the Study

While this study provides valuable insights into the prevalence of antibiotic resistance mechanisms among tonsillitis-causing bacteria, it is essential to acknowledge its limitations:

1. **Single-Center Study:** Being conducted at a single medical center, the findings may not fully represent the broader population.
2. **Sample Size:** Although 96 isolates were included, a larger sample size could improve the reliability and generalizability of the results.
3. **Phenotypic Methods Only:** This study relied solely on phenotypic detection methods, which, while reliable, may lack the sensitivity and specificity of molecular techniques.

This cross-sectional observational study at Krishna Institute of Medical Sciences involved the phenotypic detection of MRSA, ESBL, and MBL among tonsillitis-causing bacterial isolates. By following standardized testing protocols and incorporating rigorous quality control measures, this study aimed to provide accurate prevalence data on antibiotic-resistant organisms in tonsillitis, which could inform treatment protocols and contribute to ongoing efforts in combating antimicrobial resistance. The results of this study underscore the importance of targeted antimicrobial therapy and routine surveillance to manage the increasing challenge of antibiotic resistance in clinical settings.

III. Results

The study analyzed 96 bacterial isolates obtained from patients with tonsillitis to determine the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA), Extended Spectrum Beta-Lactamase (ESBL)-producing, and Metallo Beta-Lactamase (MBL)-producing bacteria. The results are presented in tables and figures, showing the distribution and resistance profiles of these strains.

1. Prevalence of MRSA among Staphylococcus aureus Isolates

Out of the 39 *Staphylococcus aureus* isolates analyzed, 23 (58.97%) were identified as MRSA based on the cefoxitin disc diffusion test. The remaining 16 isolates (41.03%) were methicillin-sensitive *Staphylococcus aureus* (MSSA).

Table 1: Distribution of MRSA and MSSA among *Staphylococcus aureus* Isolates

Bacterial Type	Total Isolates	MRSA (Resistant)	MSSA (Sensitive)
Staphylococcus aureus	39	23 (58.97%)	16 (41.03%)

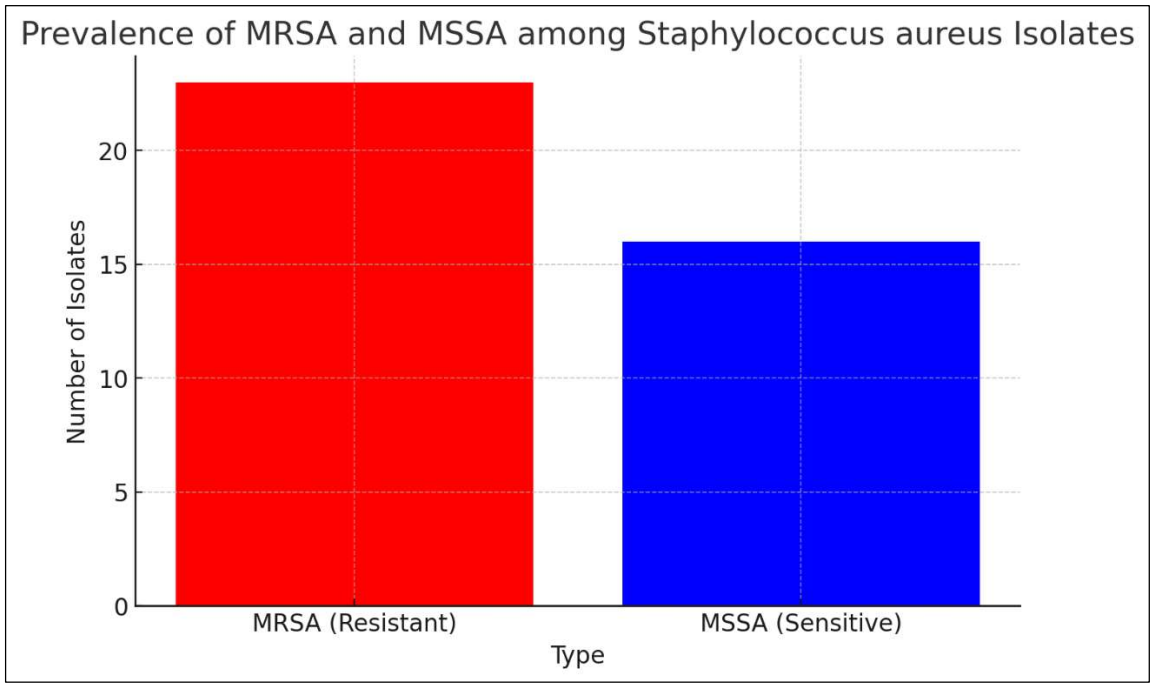

Figure 2: Prevalence of MRSA and MSSA among *Staphylococcus aureus* Isolates

The bar chart in Figure 2 displays the prevalence of MRSA and MSSA among the *Staphylococcus aureus* isolates. Over half of the isolates exhibited methicillin resistance, suggesting a high prevalence of MRSA in the study population. This finding underscores the need for caution when prescribing beta-lactam antibiotics in the treatment of tonsillitis, as a significant portion of *Staphylococcus aureus* isolates were resistant.

2. Prevalence of ESBL-Producing Gram-Negative Isolates

Among the 52 gram-negative isolates, 19 (36.53%) were found to be ESBL producers, indicating resistance to multiple beta-lactam antibiotics. The majority of ESBL-producing isolates included *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii*.

Table 2: Distribution of ESBL-Producing Gram-Negative Bacteria

Bacterial Species	Total Isolates	ESBL Producers (Resistant)	Non-ESBL Producers (Sensitive)
Pseudomonas aeruginosa	14	5 (35.71%)	9 (64.29%)
Escherichia coli	12	5 (41.67%)	7 (58.33%)
Klebsiella pneumoniae	10	3 (30%)	7 (70%)
Acinetobacter baumannii	8	1 (12.5%)	7 (87.5%)
Total	**52**	**19 (36.53%)**	**33 (63.47%)**

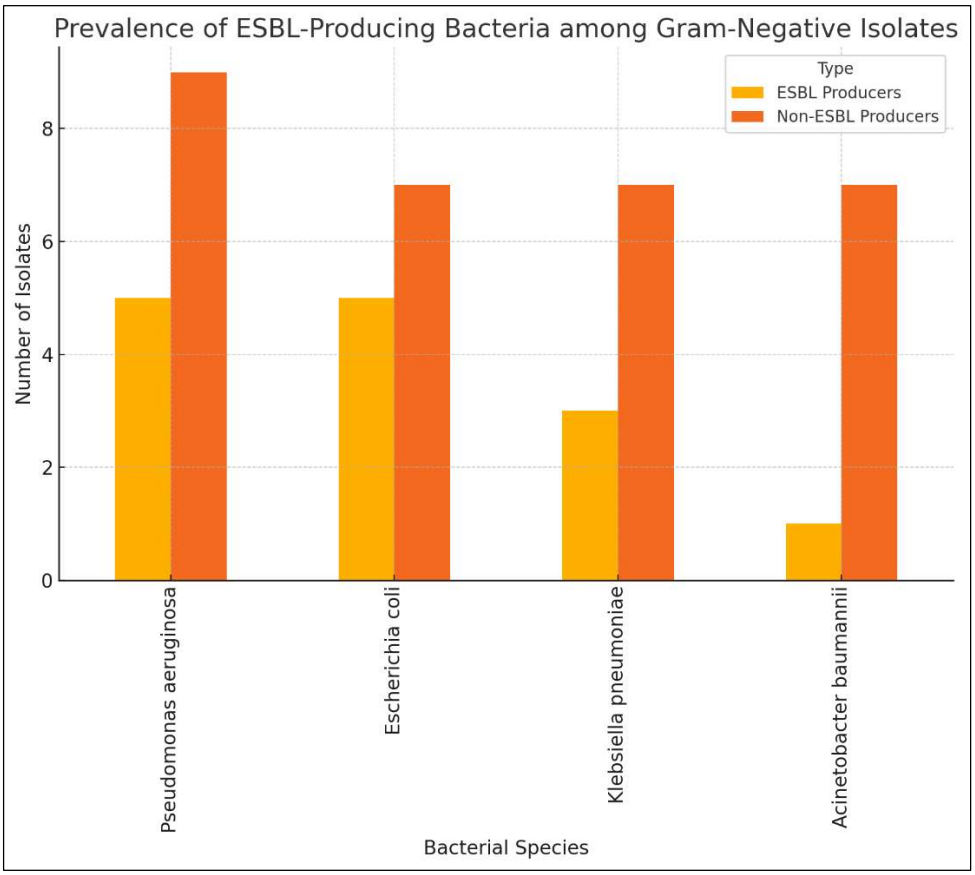

Figure 3: Prevalence of ESBL-Producing Bacteria among Gram-Negative Isolates

Figure 3 illustrates the distribution of ESBL-producing bacteria among gram-negative isolates. *Escherichia coli* showed the highest proportion of ESBL production (41.67%), followed by *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. These findings highlight the significant resistance seen among gram-negative bacteria causing tonsillitis, which can complicate treatment by limiting the effectiveness of common beta-lactam antibiotics.

3. Prevalence of MBL-Producing Gram-Negative Isolates

Out of the 52 gram-negative isolates, 24 (46.15%) were MBL producers, indicating resistance to carbapenem antibiotics. MBL production was most common among *Pseudomonas aeruginosa*, followed by *Escherichia coli* and *Klebsiella pneumoniae*.

Table 3: Distribution of MBL-Producing Gram-Negative Bacteria

Bacterial Species	Total Isolates	MBL Producers (Resistant)	Non-MBL Producers (Sensitive)
Pseudomonas aeruginosa	14	10 (71.43%)	4 (28.57%)
Escherichia coli	12	5 (41.67%)	7 (58.33%)
Klebsiella pneumoniae	10	4 (40%)	6 (60%)
Acinetobacter baumannii	8	5 (62.5%)	3 (37.5%)
Total	**52**	**24 (46.15%)**	**28 (53.85%)**

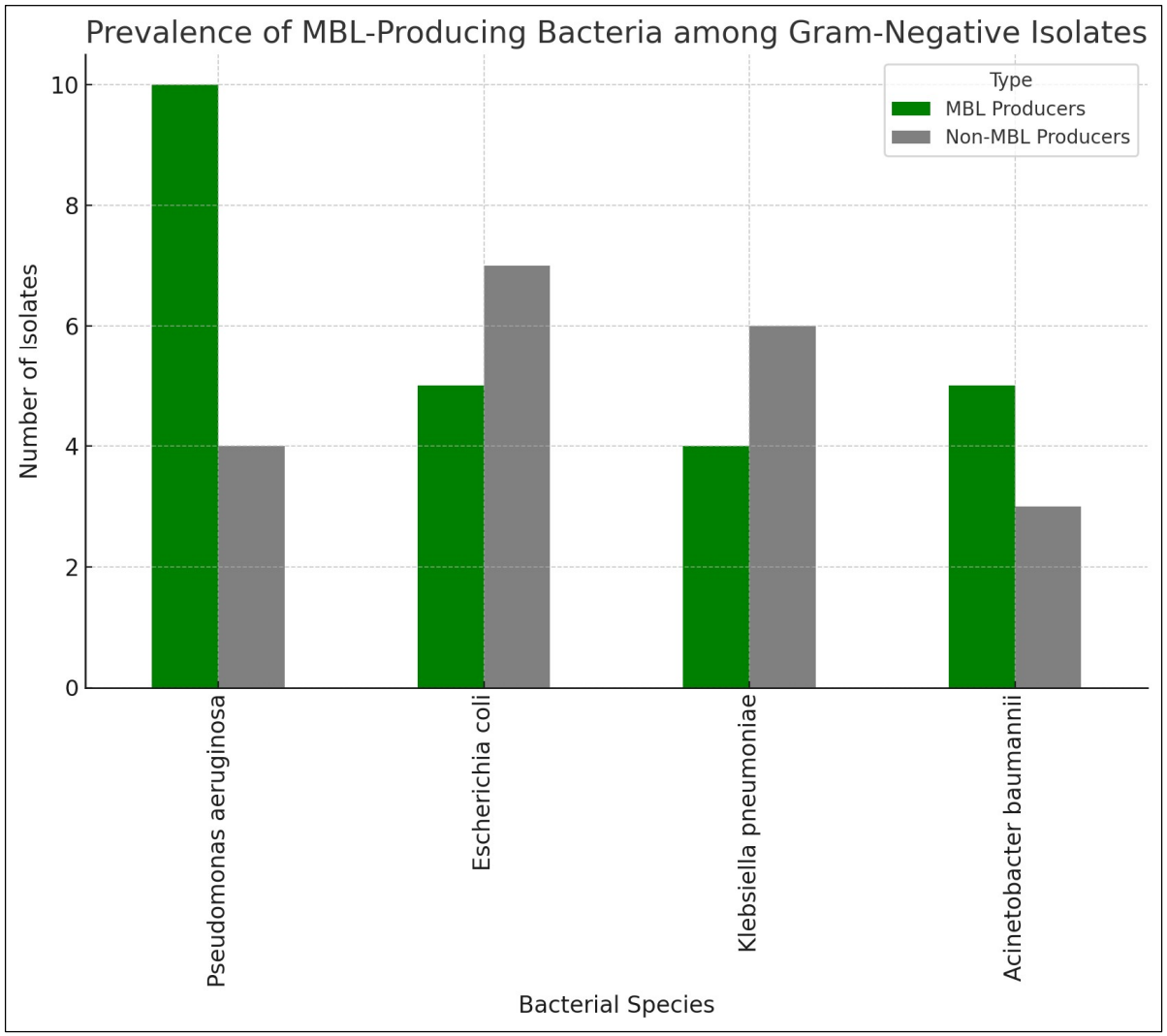

Figure 4: Prevalence of MBL-Producing Bacteria among Gram-Negative Isolates

In Figure 4, the high prevalence of MBL-producing bacteria, particularly among *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, is evident. The ability of MBL producers to hydrolyze carbapenem antibiotics poses a significant clinical challenge, as carbapenems are often used as a last line of defense against multidrug-resistant bacterial infections.

4. Antibiotic Susceptibility Profiles

The antibiotic susceptibility of MRSA, ESBL, and MBL-producing isolates was also evaluated. The susceptibility profiles indicate which antibiotics remain effective against these resistant strains and highlight the options available for treatment.

Table 4: Antibiotic Susceptibility Profiles of Resistant Isolates

Bacterial Type	Antibiotic	MRSA (n=23)	ESBL Producers (n=19)	MBL Producers (n=24)
Staphylococcus aureus	Linezolid	100%	-	-
	Chloramphenicol	82.6%	-	-

Gram-Negative (General)	Azithromycin	-	68.4%	45.8%
	Gentamicin	-	74.5%	50%
	Doxycycline	-	89.5%	63.7%
	Imipenem	-	79.6%	0%

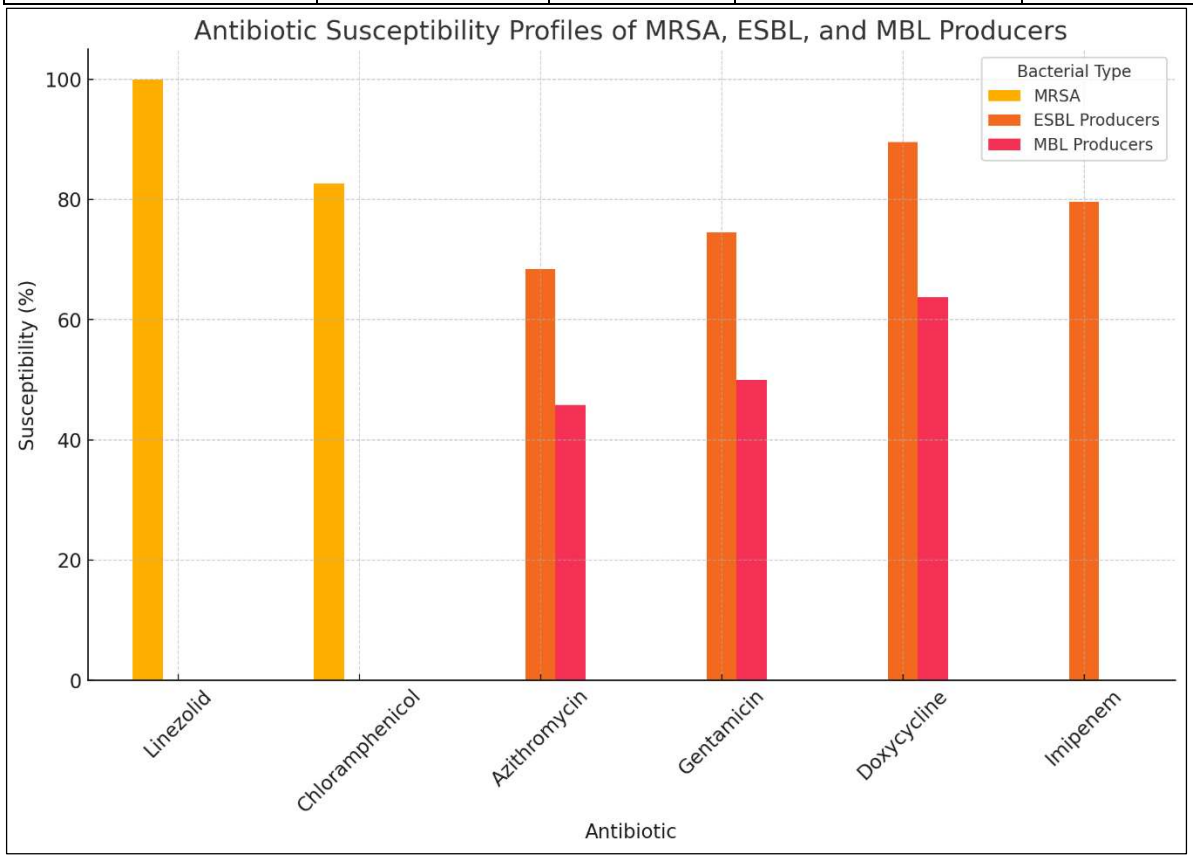

Figure 5: Antibiotic Susceptibility Profiles of MRSA, ESBL, and MBL Producers

Table 4 and Figure 5 display the antibiotic susceptibility patterns of the MRSA, ESBL, and MBL-producing isolates. Linezolid and chloramphenicol were effective against MRSA, with linezolid showing 100% susceptibility. Among gram-negative ESBL and MBL producers, azithromycin, gentamicin, and doxycycline were moderately effective, though the resistance levels were considerably high. Notably, imipenem was ineffective against MBL producers, emphasizing the limitations in treatment options for these resistant strains.

IV. Discussion

The findings of this study shed light on the alarming rates of antibiotic resistance among bacteria causing tonsillitis, with a particular focus on methicillin-resistant *Staphylococcus aureus* (MRSA), Extended Spectrum Beta-Lactamase (ESBL)-producing, and Metallo Beta-Lactamase (MBL)-producing gram-negative bacteria. These resistant pathogens pose a significant threat to public health, as they limit treatment options and increase the complexity of managing tonsillitis, particularly in children and young adults who are frequently affected by this condition. The discussion below explores the implications of these findings, compares them with existing research, and highlights the importance of strategic interventions to mitigate antibiotic resistance.

a. Prevalence of MRSA in Tonsillitis

The study identified that 58.97% of *Staphylococcus aureus* isolates were methicillin-resistant (MRSA), indicating a high prevalence of MRSA among tonsillitis patients. This prevalence rate is consistent with similar studies that have reported an increasing incidence of MRSA in respiratory infections. The high rate of methicillin resistance in *Staphylococcus aureus* isolates suggests that commonly prescribed beta-lactam antibiotics may be ineffective for a substantial proportion of tonsillitis cases, necessitating the use of alternative antibiotics such as linezolid and chloramphenicol, which showed effectiveness against MRSA in this study.

Comparing these findings with previous studies reveals a variable MRSA prevalence globally, with some studies showing even higher rates, particularly in hospital settings where MRSA is a known cause of nosocomial infections. The variation in MRSA prevalence can be attributed to differences in local antibiotic use practices, infection control measures, and patient demographics. This study's results emphasize the importance of implementing routine MRSA screening in tonsillitis cases to guide antibiotic therapy and prevent inappropriate prescribing.

b. Prevalence of ESBL-Producing Gram-Negative Bacteria

The study revealed that 36.53% of gram-negative isolates were ESBL producers, underscoring a substantial level of resistance among gram-negative pathogens associated with tonsillitis. *Escherichia coli* and *Pseudomonas aeruginosa* were among the most common ESBL producers in this study, consistent with findings from other research in clinical microbiology. The production of ESBLs by these pathogens confers resistance to multiple beta-lactam antibiotics, including penicillins and cephalosporins, significantly limiting treatment options.

ESBL-producing bacteria are a major concern in both community and hospital settings, as they often require treatment with carbapenems, a class of antibiotics generally reserved for severe infections. However, the overuse of carbapenems can promote the selection of carbapenem-resistant organisms, including MBL-producing bacteria. In this study, the high rate of ESBL production among gram-negative isolates highlights the need for antimicrobial stewardship programs that promote the rational use of antibiotics to curb the spread of resistant strains.

c. Prevalence of MBL-Producing Gram-Negative Bacteria

Among the 52 gram-negative isolates, 46.15% were identified as MBL producers, with *Pseudomonas aeruginosa* and *Acinetobacter baumannii* being the most prevalent. The production of MBLs confers resistance to carbapenems, which are often considered last-resort antibiotics for multidrug-resistant infections. The high prevalence of MBL-producing organisms in this study population presents a serious therapeutic challenge, as it renders many commonly used antibiotics ineffective.

The growing prevalence of MBL production among gram-negative pathogens reflects a global trend, particularly in regions with high antibiotic use and limited infection control measures. Studies from various parts of the world have reported similar or even higher rates of MBL production, indicating the need for enhanced surveillance and strict antibiotic stewardship policies. In clinical practice, the detection of MBL producers is crucial, as these bacteria may not respond to standard antibiotic regimens, requiring alternative approaches or combination therapies.

d. Antibiotic Susceptibility Profiles and Clinical Implications

The antibiotic susceptibility profiles obtained in this study provide important insights into the effectiveness of various antibiotics against MRSA, ESBL, and MBL producers. Linezolid and chloramphenicol demonstrated high effectiveness against MRSA, while azithromycin, gentamicin, and doxycycline were moderately effective

against ESBL and MBL-producing gram-negative bacteria. Imipenem, which is generally effective against gram-negative infections, showed no efficacy against MBL producers, highlighting the limitations in treatment options for these highly resistant organisms.

These susceptibility patterns underscore the need for tailored antibiotic therapy based on local resistance profiles. In the absence of susceptibility testing, empirical treatment of tonsillitis can result in treatment failures and contribute to the selection of resistant strains. Therefore, clinicians must consider local resistance data when choosing antibiotics for tonsillitis, especially in cases of recurrent infections or where initial treatments have failed.

e. Comparison with Other Studies

The prevalence rates of MRSA, ESBL, and MBL producers in this study are comparable to those reported in similar studies across different regions. For instance, research from other healthcare settings has shown that MRSA rates among tonsillitis patients can range from 40% to 70%, depending on the local prevalence of resistant strains and infection control practices. Similarly, the prevalence of ESBL-producing bacteria in respiratory infections has been reported to vary widely, with rates ranging from 20% to 60% in different countries. This variability highlights the importance of region-specific studies to inform local healthcare practices.

Studies focusing on MBL-producing bacteria have also reported increasing prevalence rates, particularly in hospital-associated infections. The findings of this study align with the global trend of rising MBL production, emphasizing the need for enhanced diagnostic and preventive measures. By comparing this study's results with those of other research, it becomes evident that antibiotic resistance is a widespread issue that requires coordinated efforts across regions to control its impact.

f. Limitations of the Study

While this study provides valuable insights into antibiotic resistance in tonsillitis-causing bacteria, several limitations should be noted:

1. **Single-Center Study:** The research was conducted at a single medical center, which may limit the generalizability of the findings to other populations. Multi-center studies would provide a broader perspective on resistance trends.
2. **Sample Size:** Although 96 isolates were included, a larger sample size could improve the reliability and generalizability of the results.
3. **Reliance on Phenotypic Methods:** The study used phenotypic methods for detecting resistance, which, while reliable, may not capture all resistance mechanisms. Molecular methods could provide additional insights into the genetic basis of resistance.

Despite these limitations, the study offers a comprehensive overview of antibiotic resistance patterns among tonsillitis-causing bacteria in the study population, contributing to the existing body of knowledge on this critical issue.

V. Conclusion

Infection control strategies are critical for preventing the spread of pathogens and safeguarding public health, particularly in healthcare settings. This study underscores the importance of various preventive measures, including hand hygiene, personal protective equipment, environmental disinfection, and the use of isolation protocols tailored to the modes of transmission. Moreover, antimicrobial stewardship, vaccination programs, and the careful handling and disposal of medical waste serve as essential tools in minimizing infection risk. Through vigilant screening, surveillance, and ongoing education of healthcare workers, patients, and the public,

the effectiveness of infection control measures can be amplified. Adopting a multi-faceted approach not only improves patient outcomes but also curtails the development of antibiotic-resistant strains, which pose a growing threat worldwide. As we continue to encounter new infectious challenges, maintaining and advancing infection control practices is fundamental to sustaining the safety and resilience of healthcare systems and communities alike.

References

[1] Abraham, Z. S., Bazilio, J., Ntunaguzi, D., Massawe, E. R. (2019). Prevalence and bacteriology of tonsillitis among patients attending otorhinolaryngology department at Muhimbili National Hospital, Dar Es Salaam-Tanzania. Medical Journal of Zambia, 46(1), 33-40.

[2] Al Ahmary, M. S., Al Mastour, A. S., Ghnnam, W. M. (2012). The microbiology of tonsils in Khamis Civil Hospital, Saudi Arabia. International Scholarly Research Notices, 2012, Article ID 813581.

[3] Stelter, K. (2014). Tonsillitis and sore throat in children. GMS Current Topics in Otorhinolaryngology, Head and Neck Surgery, 13. Available at: https://pubmed.ncbi.nlm.nih.gov/25587367/

[4] Ughasoro, M. D., Akpeh, J. O., Echendu, N., Mgbachi, N. G., Okpala, S., Amah, L., & Okolo, O. H. (2021). Profile of microorganisms associated with acute tonsillitis in children and their antibiotics sensitivity pattern in Nigeria. Scientific Reports, 11(1), 1-9. https://doi.org/10.1038/s41598-021-99570-9

[5] Buname, G., Kiwale, G. A., Mushi, M. F., Silago, V., Rambapu, P., & Mshana, S. E. (2021). Bacterial pattern on tonsillar surface and tonsillar core tissue among patients scheduled for tonsillectomy at Bugando Medical Centre, Mwanza, Tanzania. Pathogens, 10(12), 1560. https://pubmed.ncbi.nlm.nih.gov/34959515/

[6] Abd El Gail, S. Y., Abd El Gawad, S. C., El Ateeq, E. A. (2021). Isolation and identification of microorganisms causing tonsillitis among children in the Hail region. Journal of Infection and Public Health.

[7] Clinical and Laboratory Standards Institute (CLSI). (2022). Performance Standards for Antimicrobial Susceptibility Testing. 32nd Edition, CLSI Supplement M100. Clinical and Laboratory Standards Institute, USA.

[8] Mackie, M. T., & McCartney, J. G. (2006). Practical Medical Microbiology. 14th Edition. Churchill Livingstone.

[9] Al-Tameemi, K. A., Hraishawi, R. M., Aaiz, F. L. (2020). Isolation, identification, and evaluation of resistance of bacterial isolates from patients with tonsillitis. Annals of Tropical Medicine and Public Health, 23(10).

[10] Darod, H. H., Melese, A., Kibret, M., Mulu, W. (2023). Throat swab culture positivity and antibiotic resistance profiles in children 2-5 years of age suspected of bacterial tonsillitis. International Journal of Microbiology, 2023, Article ID 6474952.

[11] Bakir, S. H., Ali, F. A. (2015). Evaluation of multidrug resistance and ESBL, AmpC, and metallo-beta-lactamase production in gram-negative bacteria causing pharyngotonsillitis. International Journal of Research, 8.