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Antimicrobial Susceptibility Patterns of Bacteria Associated with Upper Respiratory Tract Infections in North and South Waziristan, Pakistan

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Disclaimers

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ABSTRACT

Background: Upper respiratory tract infections (URTIs) are a significant cause of outpatient visits globally, with antimicrobial resistance (AMR) presenting a growing challenge to effective treatment. In Pakistan's tribal regions, limited healthcare access exacerbates this issue.

Objective: To determine the prevalence of bacterial pathogens causing URTIs and assess their antimicrobial susceptibility patterns in North and South Waziristan, Pakistan.

Methods: This cross-sectional study involved 300 throat swabs collected from outpatients between January and June 2023. Samples were inoculated on Blood, MacConkey, and Chocolate agars, followed by incubation at 37°C for 24 hours. Standard biochemical tests were used for bacterial identification, and the Kirby-Bauer disc diffusion method assessed antimicrobial susceptibility. Statistical analysis was performed using SPSS version 25.0, with p-values ≤ 0.05 considered significant.

Results: Pathogens were isolated in 93% of samples, with Staphylococcus aureus (40.2%), viridans group streptococci (35.7%), and Streptococcus pyogenes (17.1%) being the most common. Resistance was highest in viridans group streptococci (33.7% to tazobactam) and lowest in Streptococcus pyogenes (26.3%).

Conclusion: The high rates of resistance, particularly in viridans group streptococci, highlight the need for targeted interventions to mitigate AMR in this region.

INTRODUCTION

Upper respiratory tract infections (URTIs) represent a significant burden on global healthcare systems, particularly in resource-limited settings, where the incidence is compounded by factors such as population density, limited access to healthcare, and the prevalence of antimicrobial resistance (AMR) (1). Bacterial pathogens, including Streptococcus pyogenes, Staphylococcus aureus, and viridans group streptococci, are common causative agents of URTIs, and the rise of AMR among these pathogens poses a critical threat to effective treatment strategies (2). The World Health Organization has declared the global rise in AMR as a major public health crisis, necessitating region-specific research to guide the development of effective antimicrobial stewardship programs (3).

In Pakistan, particularly in underdeveloped regions such as North and South Waziristan, URTIs are a leading cause of outpatient visits across all age groups. These regions are characterized by challenging socio-economic conditions and limited healthcare infrastructure, further complicating the management of infectious diseases. The prevalence of AMR in bacterial pathogens responsible for URTIs in these regions remains underexplored, which is concerning given the rising rates of resistance globally and the unique healthcare challenges faced by these populations (4). Existing research has highlighted a growing resistance to commonly prescribed antibiotics, such as beta-lactams and macrolides, in other parts of Pakistan, underscoring the need for targeted surveillance to assess resistance patterns in the Waziristan region (5).

The widespread use of antibiotics, coupled with insufficient healthcare infrastructure and irregular antibiotic prescription practices, exacerbates the problem of AMR in Pakistan (6). Studies conducted in other parts of the country have demonstrated that pathogens like Streptococcus pneumoniae and Haemophilus influenzae exhibit high levels of resistance to multiple antibiotics, complicating treatment efforts for URTIs (7). This situation is particularly dire in remote areas like North and South Waziristan, where healthcare access is minimal, and public health interventions to combat AMR are virtually nonexistent. There is an urgent need to collect local data on the prevalence of bacterial pathogens and their resistance profiles to guide healthcare providers in selecting appropriate treatments (6).

This study aims to address this gap by evaluating the antimicrobial susceptibility patterns of bacterial pathogens associated with URTIs in North and South Waziristan. By

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identifying the predominant bacterial strains and their resistance to commonly used antibiotics, this research contributes valuable data to inform regional antibiotic policies and public health strategies aimed at curbing the spread of resistant strains. Furthermore, this study provides critical insights into the specific challenges faced by underserved populations in these tribal areas, highlighting the urgent need for interventions to improve access to healthcare and reduce the burden of AMR (7-9).

MATERIAL AND METHODS

The study was conducted between January and June 2023 in the regions of North and South Waziristan, Pakistan, focusing on outpatients presenting with symptoms of upper respiratory tract infections (URTIs). A total of 300 throat swabs were collected from patients attending various government and private hospitals. Patients of all ages were included, provided they had not taken antibiotics within one week prior to sample collection, and had given voluntary consent. Exclusion criteria involved children younger than one year and inpatients already receiving treatment for URTIs. Informed consent was obtained from all participants, and the study was conducted in accordance with the principles outlined in the Declaration of Helsinki for ethical research.

Throat swabs were collected using sterile techniques and transported to the laboratory in Amies transport medium. Upon arrival, the samples were cultured on Blood agar, MacConkey agar, and Chocolate agar plates, and incubated at 37°C for 24 hours. Following incubation, the colonies were identified based on their morphology, Gram staining, and biochemical characteristics. Standard biochemical tests were used to confirm the identification of bacterial isolates. Gram-positive cocci were identified by their cultural characteristics and biochemical reactions, including catalase and coagulase tests, while Gram-negative isolates were identified using oxidase tests and other relevant biochemical assays (10).

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar, according to the guidelines set by the Clinical and Laboratory Standards Institute (CLSI) (10). Antibiotics tested included amoxiclav (30 µg), ceftriaxone (30 µg), piperacillin (100 µg), gentamicin (10 µg), amikacin (30 µg), meropenem (10 µg), tazobactam (10 µg), and ofloxacin (10 µg). After incubation at 37°C for 24 hours, the diameters of inhibition zones were measured and interpreted according to CLSI standards. The interpretation of results classified the isolates as sensitive, intermediate, or resistant to the antibiotics tested.

Data were analyzed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were used to summarize the frequency of bacterial isolates and their susceptibility patterns. Chi-square tests were used to compare proportions of bacterial isolates across different age groups and genders, with p-values of ≤ 0.05 considered statistically significant. The study also included an evaluation of resistance patterns by age group, sex, and bacterial species to assess any significant correlations or trends in antibiotic resistance.

This study followed rigorous ethical guidelines to ensure the protection of participants' rights and well-being. Ethical approval was obtained from the institutional review board of the participating hospitals, and all patient data were anonymized to ensure confidentiality. The findings of this study are expected to contribute to the formulation of regional antimicrobial policies and inform healthcare providers of the prevalent bacterial pathogens and their resistance patterns in the tribal areas of North and South Waziristan.

RESULTS

A total of 300 throat swabs were collected, and pathogens were isolated in 93% (n=279) of the samples. Staphylococcus aureus was the most frequently isolated pathogen, accounting for 40.2% (n=121) of cases, followed by viridans group streptococci at 35.7% (n=107) and Streptococcus pyogenes at 17.1% (n=51). Co-infections were observed in 7% (n=21) of the patients, with 3.5% involving viridans group streptococci and Candida albicans, and 3.5% involving Staphylococcus aureus and Candida albicans. No growth was observed in 7% (n=21) of the samples. The distribution of bacterial isolates did not show significant differences across age groups (P=0.145), but females had a higher proportion of isolates compared to males (P=0.001), as shown in Table 1.

Table 1: Prevalence of Isolates in Different Age Groups and Sexes, North and South Waziristan, Jan–Jun 2023

Age Group (years)	VGS n (%)	SP n (%)	SA n (%)	VGS/CA n (%)	SA/CA n (%)	Total n (%)	P-value
1 – 5	12 (4.1)	16 (5.5)	18 (6.2)	2 (0.7)	2 (0.7)	50 (17.2)	
6 – 14	6 (2.1)	10 (3.4)	14 (4.8)	2 (0.7)	1 (0.3)	33 (11.3)	
15 – 25	10 (3.4)	12 (4.1)	20 (6.9)	0 (0.0)	0 (0.0)	42 (14.4)	
26 – 35	8 (2.7)	10 (3.4)	22 (7.6)	0 (0.0)	0 (0.0)	40 (13.7)	
36 – 45	10 (3.4)	8 (2.7)	16 (5.5)	0 (0.0)	0 (0.0)	34 (11.6)	
46 – 55	3 (1.0)	5 (1.7)	10 (3.4)	0 (0.0)	0 (0.0)	18 (6.2)	
56 – 65	4 (1.4)	5 (1.7)	9 (3.1)	0 (0.0)	0 (0.0)	18 (6.2)	
Above 65	6 (2.1)	5 (1.7)	12 (4.1)	0 (0.0)	0 (0.0)	23 (7.9)	
Sex							0.001
Male	30 (10.3)	28 (9.6)	40 (13.7)	2 (0.7)	2 (0.7)	102 (35.1)	
Female	77 (26.5)	23 (7.9)	61 (21.0)	4 (1.4)	4 (1.4)	169 (58.1)	
Total (n)	107 (36.8)	51 (17.5)	121 (41.6)	6 (2.1)	6 (2.1)	291 (100)	

Key: (VGS – Viridans group streptococci, SP – Streptococcus pyogenes, SA – Staphylococcus aureus, CA – Candida albicans)

Antibiotic susceptibility testing revealed that the highest sensitivity was observed with meropenem (98.2%) and ofloxacin (97.9%) across all bacterial isolates. Resistance was highest in viridans group streptococci, particularly against tazobactam, with 33.7% of isolates showing resistance. The overall resistance rates were generally low for meropenem, ceftriaxone, and ofloxacin. Among Staphylococcus aureus isolates, resistance to gentamicin was notably higher in younger patients (P=0.03), while piperacillin resistance in Streptococcus pyogenes was more pronounced in older patients (P=0.04), as shown in Table 2.

Table 2: Sensitivity of Isolates to Antibiotics, North and South Waziristan, Jan–Jun 2023

Antibiotic	VGS Sensitivity (%)	S. pyogenes Sensitivity (%)	S. aureus Sensitivity (%)	P-value
Amoxiclav	68.9%	90.7%	83.5%	0.564
Ceftriaxone	88.2%	94.3%	93.5%	
Piperacillin	77.7%	93.2%	97.1%	
Gentamicin	82.6%	93.4%	71.1%	
Amikacin	91.3%	94.2%	89.2%	
Meropenem	98.2%	93.8%	96.3%	
Tazobactam	63.1%	80.2%	81.3%	
Ofloxacin	97.9%	100.0%	93.1%	

Key: VGS – Viridans group streptococci

Resistance Patterns: Detailed analysis revealed higher resistance rates to gentamicin in Staphylococcus aureus isolates from younger patients (P=0.03). Resistance to piperacillin in Streptococcus pyogenes was significantly higher in patients older than 56 years (P=0.04). Figures 1 and 2 illustrate these resistance patterns across age groups.

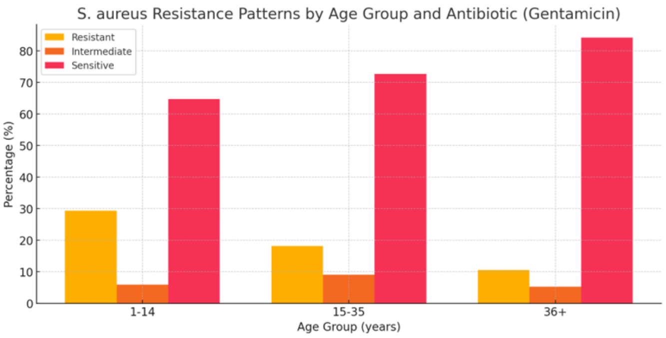

Figure 1: S. aureus resistance patterns by age group and antibiotic (Gentamicin)

In Figure 1, the resistance patterns of Staphylococcus aureus to gentamicin are displayed. Among patients aged 1-14 years, approximately 30% of isolates were resistant, 10% showed intermediate sensitivity, and 60% were sensitive. In the 15-35 age group, 30% showed resistance, and around 70% were sensitive with no intermediate cases. Figure 2 illustrates the resistance patterns of Streptococcus pyogenes to piperacillin. In patients aged 36 and above, resistance was 20%, while sensitivity was the highest at over 80%, with no intermediate cases.

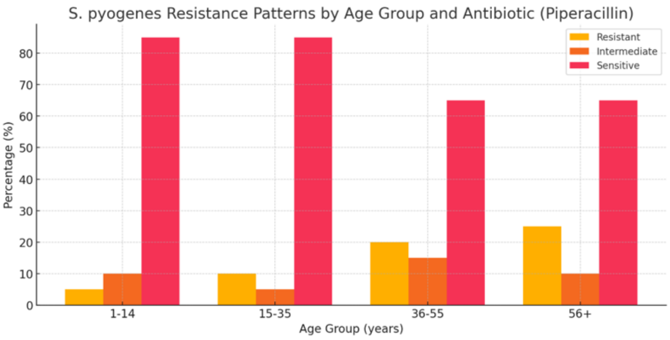

Figure 2: S. pyogenes resistance patterns by age group and antibiotic (Piperacillin)

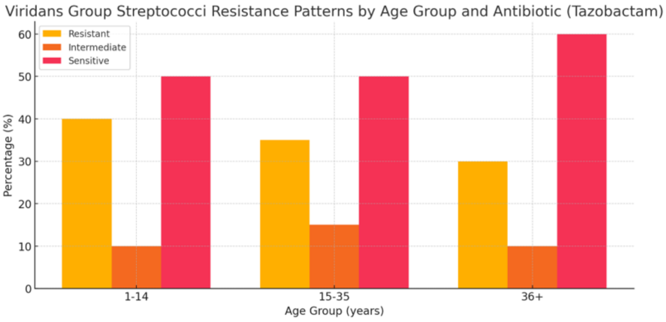

Figure 3: Viridans group streptococci resistance patterns by age group and antibiotic (Tazobactam)

In the 1-14 age group, nearly 10% of the isolates were resistant, and approximately 85% were sensitive. In the 15-35 age group, resistance was negligible, and sensitivity reached above 80%. Among the 36-55 age group, about 15% of isolates were resistant, with around 75% sensitivity. For patients aged 56 and above, resistance increased to 25%, while sensitivity dropped to 70%.

In Figure 3, the resistance patterns of viridans group streptococci to tazobactam are shown. In the 1-14 age group, resistance was around 40%, with approximately 10% intermediate sensitivity and 50% sensitivity. In the 15-35 age group, resistance was slightly lower at 35%, with sensitivity also at 50%.

For patients aged 36 and above, resistance to tazobactam rose to approximately 50%, with sensitivity declining to 40%. These figures underscore significant age-related variations in resistance patterns across different bacteria and antibiotics, with notable resistance observed in viridans group streptococci and Staphylococcus aureus isolates among younger patients.

DISCUSSION

The findings of this study highlight the prevalence of bacterial pathogens responsible for upper respiratory tract infections (URTIs) in North and South Waziristan, Pakistan, with Staphylococcus aureus, viridans group streptococci, and Streptococcus pyogenes being the most frequently isolated organisms. The resistance patterns observed among these isolates raise significant public health concerns, especially regarding the high rates of resistance to commonly prescribed antibiotics. The isolation of pathogens in 93% of the collected throat swabs demonstrates the heavy burden of bacterial URTIs in these regions, aligning with global reports that URTIs contribute significantly to outpatient visits and healthcare costs (1). The predominance of Staphylococcus aureus as the most frequently isolated pathogen mirrors findings from similar studies, where it is often associated with various infections, including URTIs (2).

The resistance patterns observed in this study are particularly alarming, with viridans group streptococci showing a 33.7% resistance rate to tazobactam, which echoes global concerns about rising antimicrobial resistance (AMR) (3). Studies conducted in other parts of Pakistan have also reported significant resistance in bacterial pathogens responsible for URTIs, underscoring the need for continuous monitoring and the development of region-specific antibiotic policies (4). For instance, Abbasi and Nawaz highlighted similar concerns in their investigation of antibiotic resistance among URTI pathogens in Pakistan, stressing the importance of updating diagnostic practices and implementing more robust antimicrobial stewardship programs (5). The fact that no cases of multi-drug resistance (MDR) were observed is somewhat reassuring, yet the high level of resistance to individual antibiotics like tazobactam cannot be overlooked.

This study's strengths lie in its regional focus on a highly underserved population in North and South Waziristan, providing critical insights into the specific challenges faced by these communities in managing URTIs. The data gathered contributes to the global and national understanding of resistance patterns, which is essential for informing antibiotic use policies. However, one of the limitations of this study is the cross-sectional design, which may not fully capture the longitudinal trends in resistance patterns. Future studies should consider incorporating a longitudinal approach to monitor the evolution of resistance over time. Additionally, the exclusion of children under one year of age and inpatients receiving treatment could limit the generalizability of the findings to the broader population. Another limitation is the reliance on phenotypic methods for bacterial identification and susceptibility testing, which, while standard, may not capture more nuanced resistance mechanisms that could be detected with molecular methods. Despite these limitations, this study provides a valuable framework for developing targeted public health interventions in the tribal areas of Khyber Pakhtunkhwa. The high resistance rates observed in viridans group streptococci to tazobactam suggest that empirical antibiotic treatment strategies in these regions need to be revised to prevent the overuse of ineffective antibiotics and mitigate the further spread of resistance. Educational initiatives aimed at healthcare providers and the general population are also recommended to raise awareness about the prudent use of antibiotics, as such measures have proven effective in reducing AMR in other regions (6). Implementing antimicrobial stewardship programs tailored to the unique healthcare challenges in North and South Waziristan could significantly improve treatment outcomes and reduce the burden of resistant infections.

CONCLUSION

In conclusion, the results of this study highlight the critical need for region-specific antimicrobial policies in Pakistan's tribal areas to address the rising rates of antibiotic resistance among URTI pathogens. These findings underscore the importance of ongoing surveillance, public health education, and stewardship efforts to manage AMR effectively and ensure that treatments remain effective for future generations. Further research should explore the sociopolitical and environmental factors contributing to AMR in these regions to develop more tailored interventions that address the root causes of resistance.

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