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Antimicrobial susceptibility patterns in Karaikal district, South India: a three-year surveillance analysis

S.R. Swarna[1], K.B. Laksham[2], D. Jeyakumari[1], R. Bharathi[3]

[1]Department of Microbiology, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Karaikal, Puducherry, India
[2]Department of Community Medicine, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Karaikal, Puducherry, India
[3]District Public Health Laboratory, Karaikal, Puducherry, India

ABSTRACT:

— ***Objective:*** Antimicrobial resistance (AMR) is a major global public health challenge that contributes to increased morbidity and mortality from infectious diseases. In India, AMR surveillance data are largely derived from tertiary care hospitals, while evidence from secondary care settings remains limited. District-level data are particularly scarce, restricting the utility of national antibiograms for local clinical decision-making.
The aim of this study was to describe the antimicrobial susceptibility patterns of aerobic bacterial isolates in a resource-constrained secondary care hospital in Karaikal, India, over three years (2020-2022).

— ***Patients and Methods:*** This retrospective observational study analyzed data from 1,989 clinical samples processed at the Government General Hospital, Karaikal. Of these, 763 clinically significant aerobic bacterial isolates were identified and subjected to antimicrobial susceptibility testing using standard disc diffusion methods. Susceptibility data were retrieved from microbiology laboratory registers and analyzed descriptively.

— ***Results:*** The most frequently isolated organisms were *Escherichia coli* (24.8%), *Staphylococcus aureus* (18.7%), *Pseudomonas aeruginosa* (11.3%), and *Klebsiella pneumoniae* (8.4%). *E. coli* showed low susceptibility to ampicillin (6%), ceftriaxone (13%), and ciprofloxacin (24%). *S. aureus* showed ciprofloxacin susceptibility of 39%, while susceptibility to linezolid remained high (98%). *P. aeruginosa* demonstrated low susceptibility to gentamicin (29%). *K. pneumoniae* showed moderate susceptibility to piperacillin-tazobactam (56%) and meropenem (43%).

— ***Conclusions:*** This study demonstrates low susceptibility to several commonly used antibiotics in a secondary care hospital setting. These findings underscore the need for strengthened local AMR surveillance, development of hospital-specific antibiograms, and implementation of context-appropriate antimicrobial stewardship and infection control strategies to guide rational antibiotic use in this region.

— ***Keywords:*** Antimicrobial resistance, Aerobic bacterial isolates, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, Antibiotic susceptibility, Karaikal, India.

Corresponding Author: Karthik Balajee Laksham, MD; e-mail: dr.balajeelaksham@gmail.com

INTRODUCTION

Antimicrobial resistance (AMR) is a growing global health issue that leads to significant morbidity and mortality[1]. A recent review[2] highlights a 43% global increase in multidrug-resistant infections, with sharp rises in healthcare-associated (67%) and community-acquired (38%) infections, particularly in regions with high antibiotic misuse. In 2019, AMR was responsible for an estimated 1.27 million deaths worldwide, with low- and middle-income countries bearing a disproportionate burden, and the Methicillin-Resistant *Staphylococcus aureus* (MRSA) was associated with more than 100,000 deaths globally[3]. India is the largest consumer of antibiotics, and the rising use of antibiotics contributes to the rapid development of resistance[4]. MRSA prevalence in India ranges from 25% to 50%[5-7]. Increasing resistance to drugs like methicillin, vancomycin, carbapenems, and other key antibiotics is a significant public health concern[8]. The WHO[9] has launched a Global Action Plan (GAP) to combat AMR by improving surveillance, promoting antimicrobial stewardship, and reducing the spread of resistant infections. Additionally, the Sustainable Development Goals (SDGs) have now recognized AMR as a priority area for global health.

The Indian Council of Medical Research (ICMR) generates annual antibiogram data in collaboration with tertiary care institutions. However, these national datasets may not be directly applicable to smaller regions, as antibiotic susceptibility patterns can vary significantly across locations due to differences in medical practices and prescribing behaviors. Recent evidence from secondary-care hospitals in central India reported that over 78% of inpatients received antibiotics, while only 21.9% underwent culture-based confirmation[10]. This highlights the reliance on empirical antibiotic use and the diagnostic capacity gap in secondary-level hospitals, a setting that is crucial in India's healthcare delivery structure.

District-level data are scarce, limiting the utility of national antibiograms for guiding local treatment decisions. In Karaikal, a district in the Union Territory of Puducherry, no systematic local data on susceptibility patterns are available. Establishing local surveillance is essential to guide targeted treatment strategies, strengthen antimicrobial stewardship, and support the objectives of India's National Action Plan on Antimicrobial Resistance by providing locally relevant susceptibility data.

This study addresses that gap by describing the distribution of aerobic bacterial isolates and their antibiotic susceptibility patterns from clinical specimens processed at Government General Hospital (GH), Karaikal (2020-2022). The findings aim to provide a baseline reference for developing local antibiograms, strengthening infection control, and contributing to national AMR surveillance efforts.

PATIENTS AND METHODS

Study Design and Settings

The study was conducted at the Government General Hospital (GH) in Karaikal, India, from 2020 to 2022. Karaikal (latitude 10.9254° N, longitude 79.8380° E) is a coastal district in Puducherry UT with a population of approximately 200,000. The GH Karaikal is a 400-bed secondary care hospital with departments including Medicine, Surgery, Obstetrics and Gynecology, ENT, Pediatrics, and Orthopedics. The GH is the district's referral center and provides various medical services. The samples were obtained for routine diagnostic procedures from various departments, transported to the microbiology laboratory under proper handling protocols, and processed using standard procedures[11].

The antimicrobial susceptibility testing of isolated pathogens was performed manually using the Kirby-Bauer disk diffusion method recommended by the Clinical and Laboratory Standards Institute guidelines, 31st edition (CLSI M100, 2021)[12]. Antibiotic discs were stored and handled as per the manufacturer's recommendations, ensuring appropriate temperature and humidity conditions. Automated antimicrobial susceptibility testing (AST) (e.g., VITEK 2) was not available during the study period due to resource limitations. The tested antibiotics included commonly used agents for aerobic bacterial pathogens. The antibiotic panel, grouped by class and disk content, is summarized in Supplementary Table 1. The antibiotic panels tested varied depending on the organism identified and specimen type as part of routine laboratory practice. Antibiotics were selected according to internal laboratory testing guidelines followed in the microbiology laboratory, aligned with CLSI recommendations, and the availability of antibiotic discs during the study period. In selected cases, additional antibiotics were tested on the clinician's request when considered clinically relevant. The core routine testing panel remained largely unchanged during the study period, although not every organism-antibiotic combination was tested for all isolates.

All isolates of *Staphylococcus aureus* and *Staphylococcus* spp. were screened for methicillin resistance using cefoxitin (30 µg) by the conventional disc diffusion method[11]. Species-level identification of *Enterococcus* isolates (i.e., *E. faecalis*, *E. faecium*) was not routinely performed due to resource limitations. All isolates were reported as *Enterococcus* spp., and antibiotic susceptibility was reported for ampicillin and vancomycin. Phenotypic confirmatory testing for extended-spectrum β-lactamase (ESBL) production was not performed. For Enterobacterales, isolates demonstrating a cefotaxime zone diameter ≤27 mm or a ceftriaxone zone diameter ≤25 mm were categorized as non-susceptible to third-generation cephalosporins based on CLSI breakpoints[12]. Similarly, non-susceptibility to carbapenems (e.g., meropenem) was identified using disc diffusion screening. No phenotypic or molecular confirmatory tests for carbapen-

emase production were performed, and carbapenem non-susceptibility was interpreted only at the phenotypic susceptibility level.

For descriptive reporting, *Pseudomonas aeruginosa* and *Acinetobacter* spp. were presented separately. The category "non-fermentative Gram-negative bacilli" refers to other non-fermenters excluding *Pseudomonas aeruginosa* and *Acinetobacter* spp. The category "*Pseudomonas* spp." refers to non-*aeruginosa Pseudomonas* isolates as recorded in the laboratory register. Routine quality control (QC) for antimicrobial susceptibility testing (AST) was performed weekly using standard American Type Culture Collection (ATCC) strains: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 25923. Results were interpreted within the acceptable QC ranges specified in CLSI M100 (31st edition) to ensure test validity and reproducibility.

Demographic details (age, sex, and clinical department), specimen types (blood, urine, pus, sputum, and others), and AST results were retrieved from laboratory registers for analysis. Entries with incomplete data, isolates without clinical significance (e.g., normal commensal flora from pus, sputum, or stool), and cultures identified as contaminants due to improper specimen collection or environmental contamination (e.g., skin flora in blood) were excluded from the study.

Statistical Analysis

Data entry and analysis were performed using Epi Info version 7 (Centers for Disease Control and Prevention, ATL, USA) and R software version 4.1.2 (R Foundation for Statistical Computing, Vienna, Austria). Categorical variables, including organism distribution and antimicrobial susceptibility results, were summarized using frequencies and percentages. This study was designed as a descriptive antimicrobial susceptibility surveillance analysis. No inferential statistical tests were performed, as the primary objective was to describe antimicrobial susceptibility patterns in a secondary care hospital setting rather than to assess associations or causality. Susceptibility proportions were calculated for organisms with ≥30 isolates to ensure stable estimates. Percent susceptibility was calculated using the number of isolates tested for each organism-antibiotic combination as the denominator. For stratified analyses by specimen type (urine *vs.* pus) and patient-care setting (inpatient *vs.* outpatient), susceptibility (%) was reported only when the number tested within each specimen stratum for a given organism-antibiotic combination was ≥30; otherwise, results were reported as "NR" (not reported).

Year-wise trend analysis was not attempted because data collection was disrupted during 2021 due to the COVID-19 pandemic, resulting in incomplete monthly data that could lead to misleading trend interpretations. Heatmaps depicting antimicrobial susceptibility patterns were generated using the *ggplot2* package in R software to facilitate visual comparison across organisms and antibiotic classes.

Ethical Considerations

This study was approved by the JIPMER Institutional Ethics Committee (IEC) (JIP/IEC/2022/067 dated 03.06.2022). Patient confidentiality was maintained by anonymizing data. All ethical guidelines for human research were followed.

RESULTS

A total of 1,989 clinical samples were processed from various clinical departments at the Government General Hospital (GH), Karaikal, during the period 2020-2022. After excluding normal flora and contaminants, 763 clinically significant bacterial isolates were included in the analysis. The majority of isolates were obtained from pus samples (54.5%, n=416), followed by urine samples (26.9%, n=205). Other specimen types included ear swabs (6.2%), sputum (5.4%), and blood (2.9%) (Supplementary Table 2). The patient age ranged from newborns to 90 years, with a median age of 45 years (IQR 32-61 years). The sex distribution was nearly equal (51% male, 49% female). More than half of the isolates were from inpatients (54%), while 46% were from outpatients. Most isolates originated from the Surgery department (16.9%), followed by Obstetrics and Gynecology (15.1%), ENT (10%), and Medicine (3.1%).

The predominant organisms isolated were *Escherichia coli* (24.8%), *Staphylococcus aureus* (18.7%), *Pseudomonas aeruginosa* (11.3%), and *Klebsiella pneumoniae* (8.4%) (Supplementary Table 3). Other notable isolates included *Enterococcus* spp. (6.7%) and non-fermentative Gram-negative bacilli excluding *Pseudomonas aeruginosa* and *Acinetobacter* spp. (5.6%).

Table 1 presents inpatient-outpatient stratified susceptibility estimates for major organisms and selected antibiotics. Antimicrobial susceptibility patterns of the major organisms demonstrated variable susceptibility across antibiotic classes, as summarized in the heatmap (Figure 1). Among *E. coli* isolates, susceptibility to ceftriaxone was 13% based on CLSI disc diffusion breakpoints, while susceptibility among *K. pneumoniae* isolates was 50%. Susceptibility to meropenem was 84% among *E. coli* and 43% among *K. pneumoniae* isolates based on disc diffusion screening; no phenotypic or molecular confirmation of resistance mechanisms was performed.

Among *S. aureus* isolates, susceptibility was high for linezolid (98%), clindamycin (81%), and trimethoprim-sulfamethoxazole (77%). Of the 143 *S. aureus* isolates, susceptibility to cefoxitin was 54%, indicating methicillin resistance in 46% of isolates. *Enterococcus* spp. demonstrated susceptibility to ampicillin (74%), vancomycin (93%), and linezolid (95%).

Table 1 . Antimicrobial susceptibility of major pathogens stratified by inpatient and outpatient status.

Organism	Antibiotic	Inpatient susceptibility % (n)	Outpatient susceptibility % (n)
Escherichia coli	Amikacin	77.1 (96)	85.9 (78)
	Ampicillin	5.2 (77)	6.6 (61)
	Ciprofloxacin	15.1 (86)	33.3 (66)
	Gentamicin	58.5 (94)	71.8 (78)
Klebsiella pneumoniae	Amikacin	75.0 (36)	NR
	Ciprofloxacin	51.5 (33)	NR
	Gentamicin	67.6 (34)	NR
Pseudomonas aeruginosa	Amikacin	82.5 (40)	83.3 (36)
	Ciprofloxacin	60.5 (38)	66.7 (36)
Staphylococcus aureus	Cefoxitin	56.0 (50)	52.5 (61)
	Ciprofloxacin	35.6 (45)	45.7 (46)
	Clindamycin	78.1 (64)	85.1 (67)
	Gentamicin	65.2 (46)	69.8 (53)
	Linezolid	95.7 (47)	100 (52)

NR = not reported because fewer than 30 isolates were tested for that organism-antibiotic combination within the specified patient-care stratum. Percent susceptibility was calculated using the number of isolates tested for each organism-antibiotic combination within inpatient or outpatient groups as the denominator.

The susceptibility patterns of *P. aeruginosa* and non-fermentative Gram-negative bacilli (NFGNB) varied across antibiotics. *P. aeruginosa* showed susceptibility to cefepime (70%), ciprofloxacin (64%), imipenem (76%), and piperacillin-tazobactam (80%), with notably lower susceptibility to gentamicin (29%). NFGNB isolates demonstrated susceptibility to cefepime (33%), ciprofloxacin (46%), gentamicin (76%), imipenem (100%), and piperacillin-tazobactam (62%).

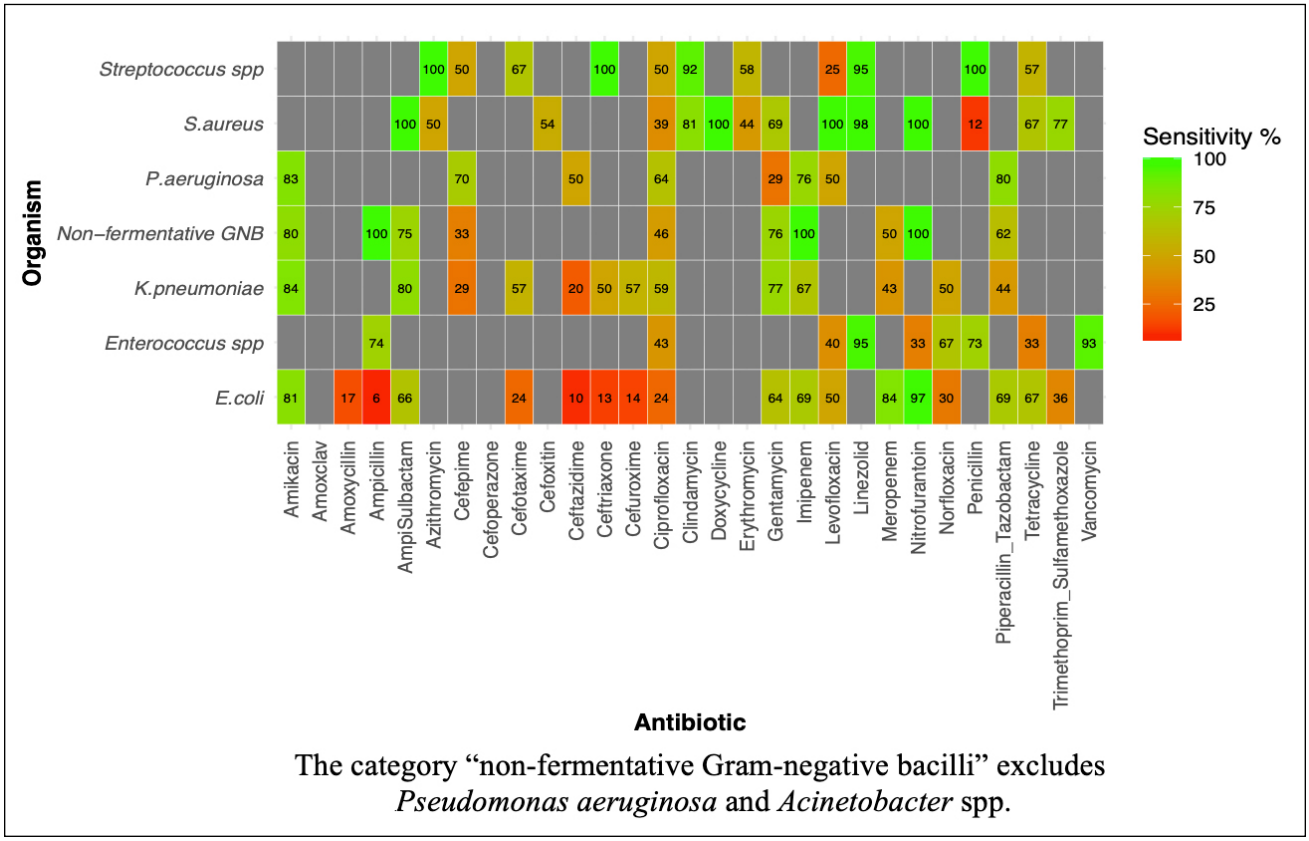

Figure 1. Heatmap showing the percentage susceptibility of major bacterial isolates to tested antimicrobial agents.

In specimen-stratified analysis (Supplementary Table 4), *E. coli* susceptibility was broadly similar in urine and pus for aminoglycosides, while susceptibility to ciprofloxacin remained low in both specimen types. In the inpatient-outpatient comparison, *E. coli* showed lower ciprofloxacin susceptibility among inpatients than outpatients (15.1% *vs.* 33.3%), while *S. aureus* susceptibility patterns were broadly similar across care settings, with persistently high susceptibility to linezolid in both groups. Nitrofurantoin demonstrated high susceptibility among urinary *E. coli* isolates (96.7%). For *S. aureus*, specimen-stratified susceptibility estimates were reportable only for pus isolates due to smaller numbers in urine.

Key Findings

- *E. coli* showed low susceptibility to commonly prescribed oral antibiotics, limiting empirical options.
- *P. aeruginosa* demonstrated low susceptibility to gentamicin, limiting aminoglycoside monotherapy.
- *K. pneumoniae* showed moderate susceptibility to piperacillin-tazobactam and carbapenems, raising concern even at the secondary-care level.
- Nearly half of *S. aureus* isolates were methicillin-resistant, but susceptibility to linezolid remained high.
- Nitrofurantoin retained good activity against urinary *E. coli* isolates.

DISCUSSION

Antimicrobial resistance is a growing concern because it limits treatment options, prolongs hospital stays, and increases morbidity and mortality. Regular monitoring of local antimicrobial susceptibility patterns is therefore essential, particularly in secondary care hospitals where empirical antibiotic use is common. This study provides district-level data on antimicrobial susceptibility from a resource-constrained secondary care hospital in South India, a setting for which published data are limited.

In this study, the most frequently isolated organisms were *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. This distribution is similar to findings reported from rural Karnataka and other secondary care settings in India[13]. Among *E. coli* isolates, susceptibility to ampicillin and ciprofloxacin was low, consistent with reports from other secondary care and rural hospital settings in India[13,14]. Nitrofurantoin demonstrated high susceptibility among urinary *E. coli* isolates, comparable to observations from Ujjain, supporting its continued use for uncomplicated urinary tract infections in similar secondary care settings[15].

Klebsiella pneumoniae isolates in this study showed good susceptibility to ampicillin-sulbactam and gentamicin, with moderate susceptibility to ciprofloxacin. Comparable gentamicin susceptibility has been reported from Vellore, although higher ciprofloxacin susceptibility was noted in that study[16]. Moderate susceptibility to piperacillin-tazobactam was observed for both *E. coli* and *K. pneumoniae*, indicating declining effectiveness of this commonly used agent even in secondary care settings[13]. Similar variability has been reported across Indian hospital settings, suggesting that local prescribing practices influence susceptibility patterns[17].

Susceptibility to third-generation cephalosporins was low among *E. coli* and *K. pneumoniae* isolates in this study. However, confirmatory testing for resistance mechanisms was not performed, and these findings should be interpreted as phenotypic resistance patterns only. Other Indian studies[18] have reported wide variation in susceptibility to third-generation cephalosporins across hospital settings. Previous research[19] has shown that low susceptibility to cephalosporins and fluoroquinolones often parallels patterns of antibiotic consumption. Susceptibility to meropenem was higher among *E. coli* than *K. pneumoniae*, and the lower susceptibility observed in *K. pneumoniae* is a concern even at the secondary-care level. Despite this, aminoglycosides such as amikacin and gentamicin retained good activity against Enterobacterales, supporting their role in selected severe infections, as reported in other Indian studies[20,21].

Methicillin-resistant *Staphylococcus aureus* (MRSA) accounted for 46% of *S. aureus* isolates, a proportion similar to that reported from rural Karnataka but lower than rates observed in some other Indian studies[13]. High susceptibility of *S. aureus* to linezolid and clindamycin was observed, indicating their continued usefulness. In contrast, susceptibility to ciprofloxacin was low, reflecting widespread fluoroquinolone use in both outpatient and inpatient settings[22].

Enterococcus spp. constituted a modest proportion of isolates, consistent with reports from northeast and central India[6,23]. In this study, *Enterococcus* isolates demonstrated high susceptibility to vancomycin and linezolid, while susceptibility to ciprofloxacin was low. Similar patterns have been reported from rural hospitals in central India[20]. Species-level identification was not performed, and no species-specific conclusions were drawn.

Among non-fermenting Gram-negative organisms, *P. aeruginosa* showed moderate-to-good susceptibility to piperacillin-tazobactam, amikacin, imipenem, and cefepime, but low susceptibility to gentamicin. Non-fermentative Gram-negative bacilli excluding *Pseudomonas aeruginosa* and *Acinetobacter* spp. showed variable susceptibility, with preserved activity of imipenem and aminoglycosides. These findings are broadly consistent with reports from other Indian secondary and tertiary care hospitals[24-26].

Overall, this study highlights low susceptibility to several commonly used antibiotics in a district-level secondary care hospital setting. These findings are particularly relevant for Karaikal, where empirical therapy is frequently initiated in the absence of culture confirmation. Local susceptibility data such as these can directly inform empirical treatment guidelines, support development of hospital-specific antibiograms, and guide early antimicrobial stewardship interventions. The ongoing antimicrobial stewardship initiatives at Government General Hospital, Karaikal, provide an important opportunity to integrate these findings into routine clinical practice and strengthen rational antibiotic use at the district level[27].

Limitations and Future Research

While this study provides valuable insights into antimicrobial susceptibility patterns in Karaikal, several limitations must be considered. The study was conducted at a single hospital, and the results may not fully represent the broader population in the region. Future studies should include more hospitals and outpatient clinics to provide a comprehensive view of the AMR landscape in Karaikal. Most positive cultures originated from pus specimens, reflecting the surgeons' frequent reliance on culture and sensitivity data in this study setting. Other clinical departments commonly initiate empirical therapy and submit fewer specimens. Additionally, molecular analysis of resistance mechanisms, such as the identification of ESBL- and carbapenemase-producing strains, would provide a deeper understanding of the genetic basis of resistance in pathogens isolated in this region.

CONCLUSIONS

This study demonstrates low susceptibility to several commonly used antibiotics among common bacterial pathogens, including *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, in a secondary care hospital setting in Karaikal. The findings highlight the importance of strengthening local antimicrobial resistance surveillance, developing hospital-specific antibiograms, and supporting antimicrobial stewardship and infection control practices. Coordinated efforts among healthcare providers and health system administrators are essential to promote rational antibiotic use and inform local strategies to manage antimicrobial resistance in this region.

Informed Consent:
Informed consent was not applicable for this study as it involved retrospective analysis of anonymized laboratory data collected during routine diagnostic procedures. No individual patient data that could identify participants was used.

ORCID ID:
Karthik Balajee Laksham: 0000-0003-3379-3265

Conflict of Interest:
The authors declare no conflicts of interest related to this study.

Authors' Contributions:
Swarna SR: conceptualization, data collection, analysis, manuscript drafting, and final approval.
Karthik Balajee Laksham: study design, data analysis and interpretation, manuscript editing, and final approval.
Jeyakumari Duraipandian: study design, laboratory analysis, data curation, manuscript review, and final approval.
Bharathi Rajkumar: study design, data collection, laboratory analysis, manuscript review, and final approval.

Ethics Approval:
This study was approved by the JIPMER Institutional Ethics Committee (IEC) (JIP/IEC/2022/067 dated 03.06.2022). Patient confidentiality was maintained by anonymizing data. All ethical guidelines for human research were followed.

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Availability of Data and Materials:
The datasets analyzed during this study are available from the corresponding author upon reasonable request and are subject to ethical and institutional approval.

AI Disclosure:
No AI tools were used to generate the manuscript's scientific content, data analysis, or primary writing.

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