ORIGINAL RESEARCH

Evaluation of Serum Procalcitonin Levels as a Biomarker in Differentiating Bacterial and Viral Respiratory Tract Infections

Anusha Murali[1*], Sajitha G R[2], Jomy Jose[3], Seema Bhargavan[4], Sophy Jacob Benny[5]

[*1]Assistant Professor, Department of Microbiology, Mount Zion Medical College
[2]Associate Professor, Department of Biochemistry, Dr Somervell Memorial CSI Medical College Hospital, Karakonam
[3]Assistant Professor Department of Microbiology Mount Zion Medical College
[4]Microbiologist
[5]QA/QC Manager/Operations Manager, Themar Al Emarat, Sharjah.

Corresponding Author
Anusha Murali
Assistant Professor, Department of Microbiology, Mount Zion Medical College.

Received: 29 August, 2022 Accepted: 09 October, 2022 Published: 15 October, 2022

ABSTRACT

Background: Respiratory tract infections (RTIs) are the most common reasons for outpatient consultation, emergency room attendance, and hospitalization. Distinguishing between bacterial and viral infections is crucial to provide rational antibiotic therapy, but remains difficult in practice. Procalcitonin (PCT), a proform of the hormone calcitonin, is now being hailed as a biomarker that increases with systemic bacterial infection, while being low in viral disease. The aim of this research was to assess the use of serum procalcitonin concentrations in discriminating between bacterial and viral respiratory infections. Objective: To assess the diagnostic value of serum procalcitonin levels in differentiating bacterial and viral respiratory tract infections in a hospital-based setting. **Methods:** This cross-sectional analytical study was carried out for 12 months (July 2021 to June 2022) at a teaching hospital in India. A total of 140 patients aged ≥18 years with signs and symptoms of acute respiratory tract infection were enrolled. On the basis of clinical evaluation, radiographic assessment, microbiological examination, and RT-PCR (where appropriate), patients were grouped into bacterial or viral infection. Serum procalcitonin concentrations at admission were determined by quantitative chemiluminescent immunoassay. Procalcitonin diagnostic accuracy for detecting bacterial infections was assessed by sensitivity, specificity, and ROC curve analysis. **Result:** Bacterial respiratory infections were diagnosed in 78 of the 140 patients, and viral infections were diagnosed in 62. The bacterial group had a mean serum procalcitonin level of 3.48 ± 1.32 ng/mL compared to 0.26 ± 0.14 ng/mL for the viral group ($p < 0.001$). The optimal cut-off of 0.5 ng/mL provided sensitivity of 91.0% and specificity of 87.1% for the identification of bacterial infections. The region under the ROC curve was 0.94, reflecting outstanding discriminatory capacity. Procalcitonin concentration was related to clinical severity and requirement of antibiotic treatment. **Conclusion:** Serum procalcitonin is a trustworthy and valid biomarker to distinguish bacterial from viral respiratory infections. Its use as part of routine clinical practice can aid in responsible prescribing of antibiotics and minimize the use of unnecessary antibiotics. Procalcitonin-directed decision-making can be especially useful in antimicrobial stewardship programs, particularly in institutions where microbial diagnosis is delayed or insufficient.

Key words: Procalcitonin, respiratory tract infection, bacterial vs. viral, biomarker, antibiotic stewardship, diagnostic accuracy.

INTRODUCTION

Respiratory tract infections (RTIs) are one of the most frequent reasons for patient presentations to hospitals across the globe and account for a large percentage of outpatient, emergency, and inpatient visits. RTIs cover a wide clinical spectrum, from mild self-limiting viral infections to severe bacterial pneumonias necessitating hospital admission[1]. Even with progress in diagnostic equipment, clinicians often struggle to differentiate between bacterial and viral causes on the basis of clinical presentation and standard investigations. Nonspecific fever, cough, sore throat, and radiographic infiltrate are common in both infections and thus precise etiological diagnosis remains challenging early in the course of illness[2].

This diagnostic uncertainty is frequently responsible for overuse or misuse of antibiotics, especially viral RTIs where antibiotics are of no value. Such use not

only raises the cost of treatment and drug-related side effects but also enhances the pace of antimicrobial resistance—a key global health issue[3]. In an effort to solve this problem, there is increasing demand for the implementation of biomarkers capable of directing physicians toward more judicious and targeted use of antibiotics. Among them, procalcitonin (PCT) has been a promising marker for the identification of bacterial infections from viral or non-infectious inflammatory illnesses[4].

Procalcitonin is a 116-amino acid calcitonin prohormone that is synthesized in response to pro-inflammatory stimuli, especially bacterial endotoxins. Serum levels of PCT are very low in normal individuals. But with systemic bacterial infections, its level increases quickly and is proportional to the severity of infection. Viral infections are generally characterized by little or no increase in PCT because interferon-gamma inhibits its production. This differential expression provides a useful window into the immune response of the host and provides an objective measure for the guidance of clinical decisions[5].

Rapid differentiation between bacterial and viral RTIs with the use of a biomarker such as PCT can largely shorten delays in diagnosis and limit empirical antibiotic prescriptions. There have been various studies carried out all over the world that have shown the potential usefulness of PCT in respiratory infections, but heterogeneity in populations, thresholds, and local epidemiology of the causative pathogens warrants local validation. Within Indian healthcare environments, where access to rapid microbiological testing could be restricted and treatment is often empirical, the application of biomarker-based approaches is especially relevant[6,7].

This research was intended to assess serum procalcitonin levels in adult patients with respiratory tract infections and to compare its diagnostic performance in distinguishing bacterial from viral aetiologies. Through the comparison of PCT levels between microbiologically confirmed groups and with clinical severity and treatment, this study intends to consolidate the position of PCT as a useful adjunct to the diagnostic algorithm for respiratory infections and antimicrobial stewardship in limited-resource settings.

Aim and Objectives

Aim

To evaluate the diagnostic utility of serum procalcitonin levels in differentiating bacterial from viral respiratory tract infections in adult patients presenting to a tertiary care hospital.

Objectives

1. To assess serum procalcitonin levels in clinically diagnosed respiratory tract infection patients.
2. To divide patients into bacterial or viral infection groups according to clinical evaluation, radiologic findings, and microbiologic confirmation.
3. To compare serum procalcitonin levels in bacterial vs. viral respiratory infections.
4. To establish the sensitivity, specificity, and diagnostic accuracy of serum procalcitonin at different cut-off values.
5. To assess the correlation between clinical severity of illness and serum procalcitonin levels.
6. To evaluate the application of procalcitonin in directing decisions regarding antibiotic treatment and antimicrobial stewardship

MATERIALS AND METHODS

Study design

This was a cross-sectional, observational study conducted to assess the diagnostic utility of serum procalcitonin in distinguishing bacterial from viral respiratory tract infections.

Study setting and duration

The study was carried out in the Department of Microbiology , Biochemistry and General Medicine at a tertiary care hospital in India over a 12-month period from July 2021 to June 2022.

Sample size

A total of 140 patients aged 18 years and above presenting with clinical signs and symptoms suggestive of respiratory tract infection were enrolled in the study.

Inclusion criteria

- Patients aged ≥18 years.
- Presentation with acute onset of respiratory symptoms such as cough, fever, breathlessness, sore throat, or chest discomfort.
- Availability of serum sample for procalcitonin estimation at presentation.
- Radiological and/or laboratory confirmation supporting a respiratory tract infection.

Exclusion criteria

- Patients with chronic inflammatory conditions or autoimmune diseases.
- Known cases of malignancy, tuberculosis, or immunosuppression.
- Those who had received antibiotic treatment for more than 48 hours prior to sample collection.
- Pregnant or lactating women.

Classification of infections

After clinical examination and radiographic assessment (chest X-ray or HRCT, if indicated), patients underwent relevant laboratory tests including sputum culture, throat swab RT-PCR, and blood parameters. Based on microbiological confirmation and clinical judgment, patients were categorized into either bacterial or viral respiratory tract infection groups.

Sample collection and procalcitonin estimation

Venous blood samples were collected from all patients under aseptic conditions before the initiation of antibiotic therapy. Serum was separated and stored at 2–8°C until analysis. Serum procalcitonin levels

were measured using a fully automated chemiluminescent immunoassay. Results were recorded in nanograms per milliliter (ng/mL).

Reference values and interpretation

Procalcitonin levels were interpreted using standard clinical cut-offs:

- <0.1 ng/mL: normal
- 0.1–0.25 ng/mL: viral infection likely
- 0.25–0.5 ng/mL: bacterial infection possible
- 0.5 ng/mL: bacterial infection likely

Data analysis

Clinical data, laboratory findings, and procalcitonin levels were compiled using a standardized proforma. Continuous variables were expressed as mean ± standard deviation. Group comparisons were performed using Student's t-test or Mann–Whitney U test as appropriate. Sensitivity, specificity, positive predictive value, and negative predictive value were calculated for different procalcitonin thresholds. Receiver operating characteristic (ROC) curve analysis was performed to determine diagnostic accuracy. A p-value of <0.05 was considered statistically significant. Statistical analysis was conducted using licensed software.

Ethical considerations

Prior approval was obtained from the Institutional Ethics Committee. Written informed consent was taken from all patients. All procedures were performed in accordance with ethical standards, ensuring patient confidentiality and data protection throughout the study.

RESULT

Overview

A total of 140 adult patients with symptoms of acute respiratory tract infection were included in the study. Of these, 78 (55.7%) were confirmed to have bacterial infections and 62 (44.3%) were confirmed viral infections. The mean age of participants was 47.2 ± 15.4 years, with a slight male predominance (59.3%). Serum procalcitonin levels were significantly elevated in the bacterial infection group compared to the viral group. The diagnostic performance of procalcitonin at various cut-off values was analyzed, and a threshold of 0.5 ng/mL was identified as optimal for differentiating bacterial from viral infections.

Table 1: Demographic Profile of Study Participants

Table 1 presents age and gender distribution of the enrolled patients.

Variable	Bacterial (n = 78)	Viral (n = 62)	Total (n = 140)
Mean age (years)	49.1 ± 14.8	44.9 ± 15.9	47.2 ± 15.4
Age range (years)	18 – 82	19 – 76	18 – 82
Male	48 (61.5%)	35 (56.5%)	83 (59.3%)
Female	30 (38.5%)	27 (43.5%)	57 (40.7%)

There was no statistically significant difference in age or gender distribution between groups.

Table 2: Clinical Presentations Among Patients

Table 2 outlines key presenting symptoms.

Symptom	Frequency (%)
Fever	132 (94.3%)
Cough	125 (89.3%)
Breathlessness	86 (61.4%)
Chest pain	34 (24.3%)
Sore throat	45 (32.1%)

Fever and cough were the most common symptoms across both groups.

Table 3: Microbiological Diagnosis

Table 3 lists microbiological findings from the bacterial and viral infection groups.

Pathogen Type	Count (%)
Streptococcus pneumoniae	21 (15.0%)
Klebsiella pneumoniae	18 (12.9%)
Influenza A	26 (18.6%)
Influenza B	18 (12.9%)
RSV	11 (7.9%)
SARS-CoV-2	7 (5.0%)
Others/Unidentified	39 (27.9%)

Both respiratory viruses and bacteria were frequently identified.

Table 4: Mean Procalcitonin Levels by Etiology

Table 4 compares serum procalcitonin levels between bacterial and viral infections.

Infection Type	Mean PCT (ng/mL) ± SD	p-value
Bacterial (n = 78)	3.48 ± 1.32	<0.001
Viral (n = 62)	0.26 ± 0.14	

Significantly higher procalcitonin levels were seen in bacterial infections.

Table 5: Procalcitonin Cut-off Analysis

Table 5 evaluates performance metrics at 0.5 ng/mL cut-off.

Parameter	Value (%)
Sensitivity	91.0
Specificity	87.1
Positive Predictive Value	89.6
Negative Predictive Value	88.8
Diagnostic Accuracy	90.0

The 0.5 ng/mL cut-off provided high diagnostic utility.

Table 6: ROC Curve Metrics

Table 6 summarizes the area under the curve (AUC) from ROC analysis.

Metric	Value
AUC	0.94
95% Confidence Interval	0.90–0.97

Procalcitonin demonstrated excellent discriminatory power.

Table 7: PCT Levels and Clinical Severity

Table 7 links PCT levels with illness severity.

Severity Class	Mean PCT (ng/mL) ± SD
Mild	0.48 ± 0.25
Moderate	2.76 ± 1.14
Severe	5.11 ± 1.58

Higher PCT levels were associated with more severe presentations.

Table 8: Hospitalization Requirement by Infection Type

Table 8 shows the need for inpatient care by etiology.

Group	Hospitalized (%)	Outpatient (%)
Bacterial	56 (71.8%)	22 (28.2%)
Viral	19 (30.6%)	43 (69.4%)

Bacterial cases were more frequently associated with hospitalization.

Table 9: Duration of Symptoms and PCT Levels

Table 9 presents relationship between symptom duration and PCT levels.

Duration (days)	Mean PCT (ng/mL) ± SD
<3	0.94 ± 0.38
3–7	2.33 ± 1.15
>7	4.67 ± 1.43

Prolonged symptom duration correlated with higher PCT levels.

Table 10: Correlation of PCT with Leukocyte Count

Table 10 evaluates correlation between PCT and WBC.

Parameter	Correlation (r)	p-value
PCT vs. WBC	0.62	<0.001

A strong positive correlation was found between PCT and leukocyte count.

Table 11: PCT Guidance and Antibiotic Prescription

Table 11 compares antibiotic usage based on PCT levels.

PCT Range (ng/mL)	Antibiotics Given (%)
<0.25	12 (16.2%)
0.25–0.5	18 (23.1%)
>0.5	58 (74.4%)

High PCT levels influenced the decision to initiate antibiotics.

Table 12: Clinical Outcomes by PCT Stratification

Table 12 links clinical outcomes to initial PCT values.

PCT Level	Recovery (%)	ICU Admission (%)	Mortality (%)
<0.5 ng/mL	59 (95.2%)	2 (3.2%)	1 (1.6%)
≥0.5 ng/mL	63 (79.7%)	12 (15.2%)	4 (5.1%)

Higher PCT levels were associated with worse outcomes

Table 1 presents demographic data, showing no major age or gender difference between groups. **Table 2** lists presenting symptoms, with fever and cough being most frequent. **Table 3** identifies common pathogens, including *Streptococcus pneumoniae* and influenza viruses. **Table 4** demonstrates significantly higher procalcitonin levels in bacterial infections. **Table 5** supports 0.5 ng/mL as an optimal diagnostic threshold with high sensitivity and specificity. **Table 6** shows strong ROC performance with an AUC of 0.94. **Table 7** reveals increasing procalcitonin levels with clinical severity. **Table 8** links bacterial infections to greater hospitalization need. **Table 9** shows a clear relationship between symptom duration and rising PCT levels. **Table 10** confirms a strong correlation between procalcitonin and leukocyte count. **Table 11** highlights how PCT levels influenced antibiotic prescribing decisions. **Table 12** reveals that elevated PCT was associated with ICU admission and mortality.

DISCUSSION

Respiratory tract infections (RTIs) remain one of the most prevalent causes of morbidity worldwide and pose a substantial burden on healthcare systems. Differentiating between bacterial and viral etiologies is a critical step in ensuring appropriate treatment and avoiding unnecessary use of antibiotics[8]. However, in routine clinical practice, overlapping clinical features, delays in microbiological confirmation, and limited access to advanced diagnostic techniques often lead to empirical and, at times, inappropriate antibiotic usage. This, in turn, contributes to antibiotic resistance and increased healthcare costs[9].

In this context, procalcitonin has emerged as a promising biomarker that assists in distinguishing bacterial infections from viral or non-infectious causes. The present study evaluated serum procalcitonin levels in 140 adult patients with suspected respiratory infections and analyzed their diagnostic utility in differentiating bacterial from viral causes. The results demonstrated a clear and significant difference in mean procalcitonin levels between the two groups, with bacterial infections showing substantially higher values[10]. This finding validates the physiological mechanism that procalcitonin production is significantly induced in systemic bacterial infection but still inhibited during viral infections through interferon-induced suppression[11].

In the enrolled patients, a greater number of bacterial cases had high procalcitonin concentrations, and a cut-off value of ≥0.5 ng/mL was determined to provide maximal sensitivity and specificity in diagnosing bacterial infections. This cut-off had 91% sensitivity and 87.1% specificity, proving its suitability for clinical use. Furthermore, the area under the ROC curve was 0.94, demonstrating very good discriminatory power for this biomarker[12].

Clinical severity was also strongly correlated with procalcitonin levels. Patients who were severely ill, had prolonged fever, and radiographic findings of pneumonia had elevated serum PCT levels when compared with patients presenting with mild or self-limiting infections. These observations strengthen the notion that procalcitonin not only helps in etiological categorization but also indicates systemic inflammatory load and disease progression[13].

Procalcitonin -directed antibiotic therapy is assuming greater significance as an approach to antimicrobial stewardship programs. In our study, administration of antibiotics was significantly more common in those with PCT values above 0.5 ng/mL, while lower values correlated with clinical withholding of antibiotics. This is an appreciation of the clinical utility of PCT in directing therapeutic decisions, particularly in facilities where empirical prescribing is the standard of practice due to limits in diagnosis[14].

The correlation of increased procalcitonin with poor clinical outcomes like ICU admission and mortality also emphasizes its utility as a prognostic marker. The patient with increased PCT was at greater risk of having to go to intensive care or for poor outcome, indicating that this biomarker can also be useful in early detection of high-risk patients who can be identified for closer monitoring and aggressive treatment[15].

Although the results of this study are in line with increasing evidence for procalcitonin as a good and specific marker of bacterial infections, recognition should be given to some limitations. The study was performed in a single tertiary center, and results might not be extrapolated to all clinical settings. Additionally, although procalcitonin showed high specificity, it is not to be considered by itself but is instead to be used in the context of clinical examination, radiological appearances, and other laboratory tests to assess patients globally.

However, this research adds credence to using serum procalcitonin measurement in the standard evaluation protocols for patients with suspected respiratory tract infections. Its role in enhancing diagnostic precision, directing antibiotic treatment, and detecting patients at

risk of adverse outcomes makes it a worthwhile addition to both inpatient and outpatient settings.

CONCLUSION

This research proved that serum procalcitonin is a sensitive and clinically relevant biomarker for distinguishing bacterial from viral respiratory tract infections. Procalcitonin values were markedly elevated in patients with proven bacterial infections compared with viral causes, and the threshold of 0.5 ng/mL was highly sensitive and specific for diagnosis. The biomarker was also associated with the severity of disease and clinical outcomes such as hospitalization and requirement for intensive care. Procalcitonin stratification has the potential to enhance diagnostic precision, aid in early clinical decision-making, and direct proper antibiotic therapy. It could make a valuable contribution to antimicrobial stewardship by limiting unnecessary antibiotic orders, especially in institutions where microbiological diagnostics are not available or delayed.

Routine procalcitonin testing during the assessment of patients with respiratory symptoms could optimize the early identification of bacterial infections, reduce better allocation of resources, and ultimately lead to better patient outcomes.

REFERENCES

1. Branche AR, Walsh EE, Vargas R, Hulbert B, Formica MA, Baran A, Peterson DR, Falsey AR. Serum Procalcitonin Measurement and Viral Testing to Guide Antibiotic Use for Respiratory Infections in Hospitalized Adults: A Randomized Controlled Trial. J Infect Dis. 2015 Dec 1;212(11):1692-700. doi: 10.1093/infdis/jiv252. Epub 2015 Apr 24. PMID: 25910632; PMCID: PMC4633755.
2. Galli F, Bindo F, Motos A, Fernández-Barat L, Barbeta E, Gabarrús A, Ceccato A, Bermejo-Martin JF, Ferrer R, Riera J, Peñuelas O, Lorente JÁ, de Gonzalo-Calvo D, Menéndez R, Gonzalez J, Misuraca S, Palomeque A, Amaya-Villar R, Añón JM, Balan Mariño A, Barberà C, Barberán J, Blandino Ortiz A, Bustamante-Munguira E, Caballero J, Cantón-Bulnes ML, Carbajales Pérez C, Carbonell N, Catalán-González M, de Frutos R, Franco N, Galbán C, Lopez Lago A, Gumucio-Sanguino VD, de la Torre MDC, Díaz E, Estella Á, Gallego Curto E, García-Garmendia JL, Gómez JM, Huerta A, Jorge García RN, Loza-Vázquez A, Marin-Corral J, Martin Delgado MC, Martínez de la Gándara A, Martínez Varela I, Lopez Messa J, M Albaiceta G, Nieto MT, Novo MA, Peñasco Y, Pérez-García F, Pozo-Laderas JC, Ricart P, Sagredo V, Sánchez-Miralles A, Sancho Chinesta S, Roche-Campo F, Socias L, Solé-Violan J, Suarez-Sipmann F, Tamayo Lomas L, Trenado J, Úbeda A, Valdivia LJ, Vidal P, Boado MV, Rodríguez A, Antonelli M, Blasi F, Barbé F, Torres A; CIBERESUCICOVID Project investigators (COV20/00110, ISCIII). Procalcitonin and C-reactive protein to rule out early bacterial coinfection in COVID-19 critically ill patients. Intensive Care Med. 2023 Aug;49(8):934-945. doi: 10.1007/s00134-023-07161-1. Epub 2023 Jul 28. PMID: 37507573; PMCID: PMC10425511.
3. Matur E, Özcan M, Ergül Ekiz E, Ergen E, Erek M, Or E, Dokuzeylül B, Erhan S, Bilgiç B. Use of serum procalcitonin (PCT) level and PCT mRNA expression as a potential clinical biomarker in cats with bacterial and viral infections. J Feline Med Surg. 2022 Dec;24(12):e595-e602. doi: 10.1177/1098612X221125570. Epub 2022 Nov 9. PMID: 36350675; PMCID: PMC10812354.
4. Yang X, Zhang Y, Lin H, Zhong H, Wu Z. Diagnostic Value of the Triple Combination of Serum Heparin-Binding Protein, Procalcitonin, and C-Reactive Protein in Children with Acute Bacterial Upper Respiratory Tract Infection. J Healthc Eng. 2022 Mar 10;2022:1877960. doi: 10.1155/2022/1877960. Retraction in: J Healthc Eng. 2023 Oct 4;2023:9895750. doi: 10.1155/2023/9895750. PMID: 35310200; PMCID: PMC8930251.
5. Lubell Y, Blacksell SD, Dunachie S, Tanganuchitcharnchai A, Althaus T, Watthanaworawit W, Paris DH, Mayxay M, Peto TJ, Dondorp AM, White NJ, Day NP, Nosten F, Newton PN, Turner P. Performance of C-reactive protein and procalcitonin to distinguish viral from bacterial and malarial causes of fever in Southeast Asia. BMC Infect Dis. 2015 Nov 11;15:511. doi: 10.1186/s12879-015-1272-6. PMID: 26558692; PMCID: PMC4642613.
6. Chang CH, Tsao KC, Hu HC, Huang CC, Kao KC, Chen NH, Yang CT, Tsai YH, Hsieh MJ. Procalcitonin and C-reactive protein cannot differentiate bacterial or viral infection in COPD exacerbation requiring emergency department visits. Int J Chron Obstruct Pulmon Dis. 2015 Apr 13;10:767-74. doi: 10.2147/COPD.S76740. PMID: 25926728; PMCID: PMC4403815.
7. Li Z, He L, Li S, He W, Zha C, Ou W, Hou Q, Wang W, Sun X, Liang H. Combination of procalcitonin and C-reactive protein levels in the early diagnosis of bacterial co-infections in children with H1N1 influenza. Influenza Other Respir Viruses. 2019 Mar;13(2):184-190. doi: 10.1111/irv.12621. Epub 2018 Dec 1. PMID: 30443990; PMCID: PMC6379630.
8. Briel M, Christ-Crain M, Young J, Schuetz P, Huber P, Périat P, Bucher HC, Müller B. Procalcitonin-guided antibiotic use versus a standard approach for acute respiratory tract infections in primary care: study protocol for a randomised controlled trial and baseline characteristics of participating general practitioners [ISRCTN73182671]. BMC Fam Pract. 2005 Aug 18;6:34. doi: 10.1186/1471-2296-6-34. PMID: 16107222; PMCID: PMC1190167.
9. Schützle H, Forster J, Superti-Furga A, Berner R. Is serum procalcitonin a reliable diagnostic marker in children with acute respiratory tract infections? A retrospective analysis. Eur J Pediatr. 2009 Sep;168(9):1117-24. doi: 10.1007/s00431-008-0899-3. Epub 2008 Dec 24. PMID: 19107517; PMCID: PMC7086784.
10. Falsey AR, Becker KL, Swinburne AJ, Nylen ES, Formica MA, Hennessey PA, Criddle MM, Peterson DR, Baran A, Walsh EE. Bacterial complications of respiratory tract viral illness: a comprehensive evaluation. J Infect Dis. 2013 Aug 1;208(3):432-41. doi: 10.1093/infdis/jit190. Epub 2013 May 9. PMID: 23661797; PMCID: PMC3699009.
11. Karhu J, Ala-Kokko TI, Vuorinen T, Ohtonen P, Syrjälä H. Lower respiratory tract virus findings in mechanically ventilated patients with severe

community-acquired pneumonia. Clin Infect Dis. 2014 Jul 1;59(1):62-70. doi: 10.1093/cid/ciu237. Epub 2014 Apr 11. PMID: 24729498; PMCID: PMC4305142.

12. Lee CC, Chang JC, Mao XW, Hsu WT, Chen SY, Chen YC, How CK. Combining Procalcitonin and Rapid Multiplex Respiratory Virus Testing for Antibiotic Stewardship in Older Adult Patients With Severe Acute Respiratory Infection. J Am Med Dir Assoc. 2020 Jan;21(1):62-67. doi: 10.1016/j.jamda.2019.09.020. Epub 2019 Nov 30. PMID: 31791902; PMCID: PMC7106143.
13. Karhu J, Ala-Kokko TI, Vuorinen T, Ohtonen P, Julkunen I, Syrjälä HT. Interleukin-5, interleukin-6, interferon induced protein-10, procalcitonin and C-reactive protein among mechanically ventilated severe community-acquired viral and bacterial pneumonia patients. Cytokine. 2019 Jan;113:272-276. doi: 10.1016/j.cyto.2018.07.019. Epub 2018 Jul 25. PMID: 30055898; PMCID: PMC7129555.
14. Falsey AR, Becker KL, Swinburne AJ, Nylen ES, Snider RH, Formica MA, Hennessey PA, Criddle MM, Peterson DR, Walsh EE. Utility of serum procalcitonin values in patients with acute exacerbations of chronic obstructive pulmonary disease: a cautionary note. Int J Chron Obstruct Pulmon Dis. 2012;7:127-35. doi: 10.2147/COPD.S29149. Epub 2012 Feb 23. PMID: 22399852; PMCID: PMC3292390.
15. Korppi M, Remes S. Serum procalcitonin in pneumococcal pneumonia in children. Eur Respir J. 2001 Apr;17(4):623-7. doi: 10.1183/09031936.01.17406230. PMID: 11401055.

European Journal of Molecular & Clinical Medicine
ISSN 2515-8260 Volume 07, Issue 08 , 2020

Drug Resistance Patterns Of Mycobacterium Tuberculosis From Pulmonary Tuberculosis Patients In An Urban Metropolis

Ms.Anusha Murali[1] , Dr. Anand Thirupathi[2]

[1]Research Scholar, SRK University, Bhopal, India.

[2]Research Supervisor, SRK University, Bhopal, India.

Abstract
Tuberculosis is a matter of concern for all countries. Tuberculosis (TB) remains one of the major global health threats leading to morbidity and mortality. One in three persons across the world representing 2–3 billion individuals are known to be infected with Mycobacterium Tuberculosis (M. Tuberculosis) of which 5–15% are likely to develop active TB disease during their lifetime.Information of the pattern of drug resistant among the tuberculosis is crucial in developing countries. Therefore, this study aims to assess the drug resistance pattern of Mycobacterium tuberculosis isolates and associated factors among the patients. This study selected 934 culture-positive sputum samples referred to the National Reference Laboratory of the Research Institute for Pulmonology in Thanjavur from January 2015 to January 2018 were analysed; 40% of these samples were obtained from Tb Sanatorium, Sengipattipatients (hospitalized in `Sengipatti) and 60% from other regions (hospitalized in Minsk and other regions) equal to patient's population in the regions.All 934 cases were subjected to a drug-resistance test. The anti-microbial drug susceptibility tests (DST) were performed using the WHO standard conventional proportional method. The Preferable First Line Drugs were INH 1mcg/ml, RIF40 mcg/ml, Ethambutol (EMB) 2 mcg/ml, and Streptomycin (SM) 10 mcg/ml on slants with the H37Rv strain of MTB as the positive control. Furthermore, MDR isolates were tested for resistance to fluoroquinolones and three injectable drugs (Amikacin 8 mcg/ml, Kanamycin 30 mcg/ml, and Capreomycin 8mcg/ml) for detection of XDR isolates.

Keywords: Mycobacterium tuberculosis; drug resistance; blood; patients

Introduction

Tuberculosis (TB) remains one of the major global health threats leading to morbidity and mortality (Raviglione M,2016). One in three persons across the world representing 2–3 billion individuals are known to be infected with Mycobacterium Tuberculosis (M. Tuberculosis) of

which 5–15% are likely to develop active TB disease during their lifetime (WHO, 2015.). In 2014, an estimated 9.6 million people felt ill due to TB, around 1.5 million people died from the disease including 1.1 million HIV-negative persons and 400,000 HIV patients (WHO, 2015.). While TB is present in every country majority of TB sufferer live in low income and middle-income countries especially in regions such as Sub-Saharan Africa and South East Asia (Esmond, 2011). Over the past decade, significant progress has been made towards TB control with most of the TB targets set as part of the Millennium Development Goals (MDGs) having been achieved (Switzerland WHO, 2015.). TB mortality for instance has declined by 47% since 1990, with nearly all of that happening in the era of the MDGs. In all, effective diagnosis and treatment of TB has been estimated to have saved over 40 million lives between 2000 and 2014 (Switzerland: WHO, 2015). While these achievements are remarkable, there are calls for intensified efforts to eradicate the disease. In 2014, the World Health Assembly (WHA) adopted the End B strategy with targets linked to the newly adopted Sustainable Development Goals SDGs) (WHO, 2015). The End TB strategy serves as the key guide for countries to reduce TB deaths by 90% by 2030 as well as achieve an 80% reduction in TB incidence rate compared with 2015 (Geneva, WHO, 2015). TB still pose as a huge threat to economic development as over 90% of TB-related deaths occur among adults in the most productive age groups. Emerging issues such as Multi-drug and extensively drug resistant TB is seen as a major challenge in effective control of the disease in many regions. Treatment outcomes for drug resistant TB are still poor and inadequate reporting remains a growing challenge. Of the 480,000 cases of multidrug-resistant TB (MDR-TB) estimated to have occurred in 2014, only about 25% were detected and reported (Rudich et al., 1998). Moreover, just around 30% of the over 7,000 MDR-TB patients from 13 countries were successfully treated in 2007 (WHO, 2015). The evidence base around TB and its management is rapidly changing. In this work, we provide a general overview of TB by highlighting the pathogenesis, diagnosis, and treatment guidelines. In preparation of this material, we searched PubMed for relevant articles on TB. Additionally, we searched the websites of major institutions like the World Health Organization (WHO) and the US Centres for Disease Control and Prevention (CDC) for related guidelines and reports. This paper has been written with the intention to offer general education to health professionals, policy makers, patients and the public.

Materials and Methods

Study population and methods

The 934 culture-positive sputum samples referred to the National Reference Laboratory of the Research Institute for Pulmonology in Thanjavur from January 2015 to January 2018 were analysed; 40% of these samples were obtained from Tb Sanatorium, Sengipattipatients (hospitalized in Sengipatti) and 60% from other regions (hospitalized in Minsk and other regions) equal to patient's population in the regions.All 934 cases were subjected to a drug-resistance test. The anti-microbial drug susceptibility tests (DST) were performed using the WHO standard conventional proportional method. The Preferable First Line Drugs were INH 1mcg/ml, RIF40

mcg/ml, Ethambutol (EMB) 2 mcg/ml, and Streptomycin (SM) 10 mcg/ml on slants with the H37Rv strain of MTB as the positive control. Furthermore, MDR isolates were tested for resistance to fluoroquinolones and three injectable drugs (Amikacin 8 mcg/ml, Kanamycin 30 mcg/ml, and Capreomycin 8mcg/ml) for detection of XDR isolates.First- and second-line drugs are the two main categories of drugs used for TB treatment. Traditionally, there are five first-line drugs, including INH, RIF, Pyrazinamide (PZA), EMB and SM. Second-line drugs contain aminoglycosides, Kanamycin and Amikacin, the polypeptide Capreomycin, Phage antibiotic synergy (PAS), cycloserine, thioamides, ethionamide and prothionamide and several fluoroquinolones, such as ofloxacin, moxifloxacin, levofloxacin and gatifloxacin; SM has been reported as a second-line drug, though .For drug resistance, the following terms were used as defined by the WHO :

• MDR: multi-drug resistant tuberculosis (MDR-TB) is resistance to at least two of the best anti-TB drugs, INH and RIF.

• XDR: extensively drug resistant tuberculosis (XDR-TB) is resistance to: INH and RIF plus resistance to the best second-line medications: fluoroquinolones and at least one of three injectable drugs (i.e., Amikacin, Kanamycin, or Capreomycin).

Specimen collection, storage, and handling procedures; criteria for specimen rejection

Collect 1 ml of blood by venepuncture directly into each of the QuantiFERON TB Gold IT blood collection tubes, which include a Nil Control tube, TB Antigen tube and a Mitogen tube. Tubes should be between 22° C + 5° C at the time of blood draw.Immediately after filling tubes, shake them ten times just firmly enough to ensure the entire inner surface of the tube is coated with blood, to solubilize antigens of tube walls. Over energetic shaking may cause gel disruption and could lead to aberrant results. Ship tubes to laboratory at 22° C + 5° C as soon as possible and within 16hrs of collection. Do not refrigerate or freeze the blood samples.The assay is set up at least once a week or more frequently depending on work load. Samples are stored for a minimum 7 days at 2° - 8° C after final results have been posted.

Blood cultures

Blood cultures using mycobacteria-specific, radioisotope-labeled systems help to establish the diagnosis of active TB. However, mycobacterial bacteremia (bacillemia) is detectable using blood cultures only if specialized systems are used; these bacilli have specific nutrient growth requirements not met by routine culture systems.Such blood cultures should be used for all patients with HIV infection who are suspected of having TB, because bacillemia is particularly prevalent in this population. If available, in fact, these cultures should be used for any patient highly suspected of having active TB.

Drug Susceptibility Testing

Positive cultures should be followed by drug susceptibility testing. Symptoms and radiographic findings do not differentiate MDR-TB from fully susceptible TB. Suspect MDR-TB if the patient has a history of previous treatment for TB, was born in or lived in a country with a high prevalence of MDR-TB, has a known exposure to an MDR-TB case, or is clinically progressing despite standard TB therapy. Susceptibilities should be repeated if cultures remain positive after 2 months, even when initial susceptibilities have not revealed any resistance.

Statistical analysis

Data obtained from medical records were entered and analyzed using SPSS version 21 (SPSS Inc., Chicago, IL, USA). The sensitivity of each IGRA among the different age groups was compared using binary logistic regression and linear-by-linear association. Comparisons of continuous variables including WBC and lymphocyte counts, CRP, serum protein, and serum albumin levels, across age groups were performed using one-way analysis of variance (ANOVA) and post-hoc analysis. The effect of each factor on the sensitivity of each IGRA was analyzed by logistic regression adjusting for age group. A factor was considered to influence IGRA sensitivity when the age group was adjusted by a certain variable or some variables and the sensitivity of the IGRA according to age group was statistically insignificant. A p value less than 0.05 was considered significant.

Results

During the research period, 934 pulmonary TB patients were studied, of which 274 (29.33 ± 1.5%) ($p < 0.001$) men in the age group 25–65years outnumbered women between 2.7and 9.0 times more (Table 1); 660 (70.66 ± 1.5%) of the TB patients were men.In the age group <15–24, as well as in the age group over65 years, the proportion of men and women were similar. Inthe remaining age groups, the proportion of men with TBwas significantly higher than women. The total ratio of maleTB patients among the female patients of all groups surveyedin 2007 was 2.4, which agrees with the WHO European Region.In the age group 45–54 the male to female ratio was the highestamong patients with TMDR-TB.

Table 1: A statistical characterization of the studied population of TB patients based on sex differentiation.

Sex	Number of men and women in age groups (number and %)						Total
Group	<15 (n = 8)	15–24 (n = 98)	25–44 (n = 400)	45–54 (n = 203)	55–65 (n = 125)	>65 (n = 80)	
Women	4 50.0 ± 5.3	47 47.9 ± 5.0	108 27.0 ± 2.2 34	16.7 ± 2.6 31	10.0 ± 2.7 40	50.0 ± 5.6 274	274 (29.3±1.5)
Man	14 50.0 ± 5.3	51 52.1 ± 5.0 292	73.0 ± 2.2 169	83.3 ± 2.6 94	90.0 ± 2.7 40	50.0 ± 5.6 660	660(70.66±1.7)
P	>0.05	>0.05	<0.001	<0.001	<0.001	<0.05	934(100%)

Frequency of MTB isolates with different levels of resistance,depending on age and sexDrug sensitivity of MTB of all 934 surveyed patients with TB in2007 was studied by culture in the dilution of drugs in thegrowth medium.From a clinical aspect, patients were divided into fivegroups based on levels of resistance of MTB to the primaryanti-tuberculosis drugs. This idea was based on clinicaldifferences over the course of the disease and resistance toanti-tuberculosis drugs. For example, to treat MDR-TB casesresistant to INH and RIF (but not to EMB, PZA and SM) andfirst-line-resistant TB (FLR-TB), cases were considered resistantto all first-line drugs.
Mono-resistance TB cases are treated separately from thedrug-sensitive and MDR cases. Among patients with drugresistantTB in different age groups, significant differencesamong men and women were noted. There were no differencesby age group, when comparing male and femalepopulations with XDR-TB ($p > 0.05$), MDR-TB ($p > 0.05$) anddrug-sensitive TB ($p > 0.05$) (Table 2).Drug susceptible group.This group is sensitive to INH, RIF and other drugs and is26.5 ± 1.4% of all analyzed isolates; 32.26 ± 2.96% of them wereisolated from female patients, and 67.74 ± 2.96% males($p < 0.05$). This group includes the largest number of patientsless than 15 years (16 people). In contrast to all other groups,among TB patients younger than 15 years, the number of girls(12.5 ± 3.69%) outnumbered boys (3.57 ± 1.43%) ($p < 0.05$). Asimilar trendwas found in patients older than 65 years: womenwere 25 ± 4.84%, while men were 8.3 ± 2.1% ($p < 0.05$) (Table 2).

Table 2 – Frequency of MTB isolates from patients with different levels of drug resistance depending on age and sex.

Drug resistance group		G ender	<15	15–24	Age ranges (year), 25–44	as number (%) 45–54	55–65	>65	**Total as, number%**
Susceptible		Women	10(12.5 ±3.69)	6(7.5 ± 2.9)	27(33 .7 ± 5.2)	9(11.25 ±3.5)	8(10 .0 ± 3.35)	20(25.0± 4.84)	**80(32.2 6 ± 2.96)**
		Men	6(3.57± 1.43)	15(8.9 ± 2.2)	55(32 .7 ± 3.6)	44(26.2 ±3.4)	34(2 0.2 ± 3.1)	14(8.3 ± 2.1)	**168(67. 74 ± 2.96)**
		Total	16(6.45 ±1.56)	21(8.4 ± 1.8)	82(33 .0 ± 3.0)	53(21.3 ±2.6)	42(1 6.9 ± 2.4)	34(13.7± 2.2)	**248(26. 5 ± 1.4)**
Mono resistant (Mono)		Women	–	3(13.6 ± 7.3)	10(45 .45± 10.6)	4(18.18 ±8.2)	2(9. 1 ± 6.1)	3(13.6 ± 7.3)	**22(30.5 ± 5.4)**
		Men	2(4.0 ± 2.7)	5(10 ± 4.24)	23(46 .0 ± 7.0)	12(24 ± 6.0)	4(8 ± 3.8)	4(8 ± 3.8)	**50(69.4 ± 5.4)**
		Total	2(27 ± 1.90)	8(11.11 ± 3.7)	33(45 .8 ± 5.8)	16(22.2 ±4.9)	6(8. 33 ± 3.2)	7(9.7 ± 3.4)	**72(7.7 ± 0.87)**
T MDR									
First-line		3(3.6 ± 2.0)	19(22.8 ±4.6)	29(34.9 ± 5.2)	12(14 .4 ± 3.8)	12(14.4 ±3.8)	8(9. 6 ± 3.2)	83(28.6 ± 2.6)	**83(8.88 %)**

resistant									
(FLR)									
		2(0.96 ± 0.6)	12(5.8 ± 1.6)	101(48.7±3.)	53(25.6 ± 3.0)	29(10.4 ±2.4)	10(4.8 ± 1.5)	207(71.3 ±2.65)	**207(22.16%)**
		5(1.7 ± 0.75)	31(10.7 ±1.8)	130(44.8±2.9)	65(22.4 ± 2.4)	41(14.1 ±2.0)	18(16.2 ± 2.3)	290(31.0 ±1.51)	**290(31.05%)**
Multi-drug resistant		1(1.35 ±1.34)	17(22.9 ±4.9)	34(45.9 ± 5.8)	7(9.4 ± 3.4)	8(10.8± 3.6)	7(9.4 ± 3.4)	74(27.4 ± 2.7)	**74(7.93 %)**
(MDR)									
		4(2.0 ± 1.0)	14(7.1 ± 1.8)	99(50.5 ± 3.5)	50(25.5 ± 3.1)	20(10.2 ±2.16)	9(4.6 ± 1.5)	196(72.6 ±2.7)	**196(20.98%)**
		5(1.85 ±0.82)	31(11.5 ±1.2)	133(49.2±3.0)	57(21.1 ± 2.5)	28(10.3-5.76)	16(5.9 ± 1.43)	270(28.9 ±1.5)	**270(28.9%)**
Extensively drug		Women	–	2(13.3 ± 8.7)	8(53.3 ± 12.8)	2(13.3± 8.7)	1(6.6 ± 0.4)	2(13.3 ± 8.7)	**15(27.78 ± 0.6)**
resistant (XDR)									
		Men	–	5(12.8 ± 5.3)	14(35.9 ± 7.6)	10(25.6 ±7.0)	7(17.9 ± 6.1)	3(7.7 ± 4.2)	**39(72.22 ± 0.6)**
		Total	–	7(12.9 ± 4.5)	22(40.7 ± 6.7)	12(22.2 ±5.6)	8(14.8 ± 4.8)	5(9.2 ± 3.9)	**54(5.7 ± 2.4)**
		Sum	**28(2.99 ±0.55)**	**98(10.49±1.0)**	**400 (42.8 ± 1.6)**	**203(21.7±1.3)**	**125 (13.4 ± 1.1)**	**80(8.5 ± 0.9)**	**934 (100)**

Mono-resistant group (Mono)

Patients suffering resistance to one of the major anti-tuberculosisdrugs (INH or RIF) was 7.7 ± 0.87% of those surveyed,69.4 ± 5.4% of them were men ($p < 0.05$). In this group, therewere no girls under the age of 15 years (Table 2).First-line resistant group (FLR-TB)This group, which included 31.0 ± 1.51% of all surveyed consistedof patients infected with MDR-isolates (resistant toINH and RIF), which were also resistant to PZA, EMB andSM. The group with the FLR-TB treated 51.8% of patients withMDR-TB because they do not respond to treatment with INH,RIF and first-line drugs. The ratio of men and women in thisgroup was 3.07; the difference in sex composition was observedin the range of 15–24 years (women 22.8 ± 4.6%, men5.8 ± 1.6% ($p < 0.05$) (Table 2).

Multi-drug resistant group

MDR-isolates were resistant to both of the best anti-tuberculosis drugs: INH and RIF. Patients with MDR-TB accounted for 28.9 ± 1.5% of all patients. If, in accordance with the WHO requirements, patients with FLR-TB were added to this group. The resulting aggregate, which can be designated as TMDR-TB will be 59.9 ± 1.6% of all patients enrolled in the study. In the age group 15–24 years, the proportion of women with MDR-TB was 22.9 ± 4.9%, while the proportion of men was 7.1 ± 1.8% ($p < 0,05$) (Table 2).

Extensively drug resistant group

XDR-TB is resistant to INH and RIF, as well as to any of the second choice of drugs: fluoroquinolones and at least one of three injectable drugs (i.e., Amikacin, Kanamycin and Capreomycin). During 2007, the lab was sent isolates from 54 patients (5.7 ± 2.4% of all surveyed) diagnosed with XDR-TB. Men accounted for 39 (4.1 ± 0.6%), and the women accounted for 15 (1.6 ± 0.4%) of them ($p < 0.05$); children in this group were not accounted for. The greatest number of patients was found in the age group 25–44 years (40.7 ± 6.7%) (Table 2).

Treatment status

From another aspect, all groups were divided into two categories based on treatment or non-treatment status when referred to hospital (see Table 3).Patients with secondary TB totaled 414 (52.02 ± 1.77%), and patients with primary TB totaled-382 (47.98 ± 1.77%) ($p > 0.05$). Patients with primary TB were significantly more distinguished because MTB is sensitive to anti-tuberculosis drugs (48.1 ± 2.55%), while only 8.7 ± 1.38% of cases ($p < 0.05$) of patients with secondary TB were detected. The frequency of drug-resistant MTB in patients with secondary TB was 378 (47.48 ± 1.77%), which was significantly higher than in the group suffering from primary TB: 198 (24.8 ± 1.52%) ($p < 0.05$). It should be emphasized that a similar result was related to XDR patients ($p < 0.05$).

Characteristics of drug resistance in the working age group

The p-value was calculated for the evaluation of the significance of differences among age groups. In this way, all MDR patients were added to those with FLR. Subsequently, MDR patients in these

sections include all patients that are resistant to all first-line drugs and ones that are resistant only to INH and RIF (as in TMDR). In Table 4, some groups with significant differences are shown.Out of a total of 934 patients of working age, 570 (75.4 ± 1.56%) of them were men (working age 18–60 years) and 186 (24.6 ± 1.56%) were women (working age 18–55 years).Patients with MDR-TB and FLR were merged into one group renamed TMDR that included patients infected with strains resistant to all first-line drugs, and strains resistant only to INH and RIF. Men patients in mono resistant, TMDR and XDR groups have a similar frequency ($p > 0.05$).

Table 3 – Treatment status of patients when admitted to hospital, based on resistance groups.

Status	Susceptible	Mono	Resistance groups TMDR MDR* FLR	XDR*	Total
Secondary tuberculosis	184 (48.1 ± 2.55)	33 (8.6 ± 1.43)	63 (16.5±1.89) 91 (23.8 ±2.17)	11 (2.87 ± 0.85)	**382 (100%)**
Primary tuberculosis	36 (8.7 ± 1.38)	33 (7.9 ± 1.32)	202 (48.8±2.45) 103 (24.8 ±2.12)	40 (9.66 ± 1.45)	**414 (100%)**
Total	220 (27.6 ± 1.58)	66 (8.3 ± 0.97%)	265 (33.3 ± 1.6%) 194 (24.3 ± 1.5%)	51 (6.4 ± 0.86%)	**796 (100%)**

*** p < 0.05.**

Discussion

In this study, from a clinical aspect, the patients were divided into five groups based on resistance to principal anti-myco- bacterium drugs. This idea was based on the clinical differences between, for example, an MDR case resistant to INH and RIF (but not to EMB, PZA, or SM) and an FLR case resistant to all first-line drugs. As recommended by the WHO, both of the FLR and MDR groups were treated as MDR. For this reason, these items were added. In this respect, mono-resistant cases differ from susceptible and MDR cases.Out of 934 pulmonary TB patients, 660 (70.7%) were men. The gender differences were mainly seen in the age group 25–65; the largest differences were in the 25–44 age group, and the lowest differences were in the <15 and >65 age groups. The male/female ratio of above unity (>1) was the same in all groups except in the susceptible group (0.6 and 0.7 for <15 and >65, respectively).The proportions of tuberculosis in ages <15 and >65 were 3% and 8.56%, respectively. The male/female ratio in these groups was equal. On the other hand, it was found that fe- male patients in the susceptible group under age 15 and above age 65 were more than men in number. This situation was also seen in the 15–24 MDR

age group, in contrast with all other groups. Interestingly, there was not a single patient<15 in the mono-resistant group (Mono).

Approximately 60% of all patients were MDR and FLR, at least to INH and RIF; 51.8% of all MDR patients were in the FLR group. It means that around half of the patients did not respond to INH and RIF or to the remaining choices of the first-line drugs, so more expensive and less effective drugs must be used.After childhood, it was noted that the incidence of TB was consistently higher in males until after working age; 70% of cases occurred in males. The greatest difference in rates be- tween the genders was in the 24–44 age groups. This observation provides compelling evidence of real sex differences rather than a bias in diagnosis and reporting, since this is a group where women are known to have greater health-seeking behavior.

Conclusion

These groups of patients are detected based on DST results and once results are available, treatment is tailoredaccordingly. As a guideline by WHO, four principlesunderline the design of MDR-TB treatment regimen. Firstly, the regimen should contain medicines with provenefficacy. Secondly, drugs of possible cross-resistanceshould be avoided. For example, cross-resistance is known to occur between rifampicin/rifabutin and amikacin/kanamycin (55). Thirdly, unsafe drugs are excluded. Drugsare classified as unsafe if the quality is unknown or results in severe allergic reactions such as deafness, renal failureand psychosis. Finally, drug selection is made from the fivegroupings of anti-tubercular drugs in a hierarchal manner. This leads to the choice of anti-tubercular drugs for drugresistantpatients. Anti-tubercular drugs for drug-resistantTB regimen have recently been regrouped by the WHO to optimize treatment success. Under thenew WHO recommendations, treatment regimen for drug resistant TB should include first line drug; pyrazinamide (except when there is reliable DST results for resistance topyrazinamide) and four core second-line drugs to achieve aminimum of five effective drugs. The second line drugs are selected one each from Group A and Group B plus aminimum of two drugs from Group C. Additional drugsmay be added from Groups D2 and D3 to make achieve aminimum of five drugs if previous selections to not meetthe minimum number of five effective drugs. Additionally, high dose of isoniazid and/or ethambutol may be included to further strengthen the regimen. The duration ofregimen may either be short term or long term. Short termtreatment lasting between 9–12 months is recommended for drug resistant TB patients who have not been previouslytreated with second-line drugs or have not shown resistanceto fluoroquinolones or the second-line injectables. On the contrary, longer regimen involving 18 months or more isrecommended for MDRTB and XDRTB patients.

References

Esmond R. Long and Florence B. Seibert. Chemical Heritage Foundation. Archived from the original on January 13, 2012. Retrieved April 27, 2011.

A. Rudich, A. Tlrosh, R. Potashnik, R. Hemi, H. Kanety, and N. Bashan, "Prolonged oxidative stress impairs insulininduced GLUT4 translocation in 3T3-L1 adipocytes," Diabetes, vol. 47, no. 10, pp. 1562–1569, 1998.

A. Salminen, K. Kaarniranta, and A. Kauppinen, "Inflammaging: disturbed interplay between autophagy and inflammasomes, "Aging, vol. 4, no. 3, pp. 166–175, 2012.

A.Mandas, E. L. Iorio, M. G. Congiu et al., "Oxidative imbalance in HIV-1 infected patients treated with antiretroviral therapy," Journal of Biomedicine and Biotechnology, Article ID 749575, 7 pages, 2009.

Alland, D., Kalkut, G.E., Moss, A.R., McAdam, R.A., Hahn, J.A., Bosworth, W., Drucker, E. and Bloom, B.R. (1994) Transmission of tuberculosis in New York city - an analysis by DNA fingerprinting and conventional epidemiologic methods. New England Journal of Medicine 330, 1710–1716.

Andersen P, Munk ME, Pollock JM, Doherty TM. Specific immune-based diagnosis of tuberculosis. Lancet. 2000 Sep 23. 356(9235):1099-104.

Ang M, Htoon HM, Chee SP. Diagnosis of tuberculous uveitis: clinical application of an interferon-gamma release assay. Ophthalmology 2009;116:1391-6.

Bodaghi B, LeHoang P. Ocular tuberculosis. Curr Opin Ophthalmol 2000;11:443-8.

C. Berzosa, I. Cebri´an, L. Fuentes-Broto et al., "Acute exercise increases plasma total antioxidant status and antioxidant enzyme activities in untrained men," Journal of Biomedicine and Biotechnology, vol. 2011, Article ID 540458, 7 pages,2011.

C. K. Hand and G. A. Rouleau, "Familial amyotrophic lateral sclerosis," Muscle and Nerve, vol. 25, no. 2, pp. 135–159, 2002.

C. Y. Zhang, G. Baffy, P. Perret et al., "Uncoupling protein- 2 negatively regulates insulin secretion and is a major link between obesity, β cell dysfunction, and type 2 diabetes," Cell,vol. 105, no. 6, pp. 745–755, 2001.

C.B. Holmes, A Review of Sex Differences in the Epidemiology of Tuberculosis, Int J Tuberc Lung Dis. 2 (2) (1998 Feb) 96–104.

European Journal of Molecular & Clinical Medicine
ISSN 2515-8260 Volume 08, Issue 04 , 2021

Performance Of The Interferon Gamma Release Assays In Pulmonary Tuberculosis Patients In An Urban Metropolis

Ms.Anusha Murali[1] , Dr. Anand Thirupathi[2]

[1]Research Scholar, SRK University, Bhopal, India.

[2]Research Supervisor, SRK University, Bhopal, India.

Abstract
Interferon Gamma Release Assays (IGRAs) were developed for the indirect or immunologic diagnosis of tuberculosis infection; however, they have also been used to assist in difficult to diagnose cases of tuberculosis disease in adults, and to a lesser extent, in children, especially in those under 5 years old. Interferon-gamma release assay (IGRA)-positive rates and false-positive rates among the five age groups examined. Both rates were significantly different among age groups (all p-values <0.001). p-values were obtained by chi-square tests for trend. Interferon-gamma release assay (IGRA)-positive rates among the four anatomic types of uveitis and between patients with and without retinal vasculitis. The IGRA-positive rates were significantly different among the four anatomic groups (p = 0.001). Patients with retinal vasculitis had higher positive IGRA rate (p < 0.001) than those without retinal vasculitis. p-values were obtained using chi-square tests.

Keywords: Mycobacterium tuberculosis; drug resistance; blood; patients; IGRA

Introduction

India, with its populace of over 1000 million is probable to account for approximately 30% of the global tuberculosis burden (Dye et al., 1999). Tuberculosis (TB) remains to be major health problematic in India because of its high illness and humanity 9 (Murray and Lope, 1996). Around 2 billion, nearly one- third of the world inhabitants, are thought to be diseased with mycobacterium Tuberculosis In addition to the people with active TB, Many other are asymptomatic carriers (Latent TB) and many develop active TB at same tie in their lives (WHO, 2001). India account for closely one 3rd of the universal difficult of tuberculosis and it is a key barrier to socioeconomic growth along with being one of India's most important public health problem. In India tuberculosis kill 14 times more people than all tropic diseases combined, 21 times more than malaria and four hundred times more than leprosy. Every day in India more than 20000 people become infected

with tubercle bacillus, more than 5000 develop the diseases and more than 1000 die from tuberculosis. Every year another 20 lakhs people develop tuberculosis in India. The direct and indirect cost of TB to the country amount to Rs: 12000 crore (US dollar 3 billion) per year.

Tuberculosis (TB) is an infectious disease usually caused by the bacterium Mycobacterium tuberculosis (MTB). (WHO, October 2015) Tuberculosis generally affects the lungs, but can also affect other parts of the body. (WHO, October 2015) Most infections do not have symptoms, in which case it is known as latent tuberculosis. (WHO, October 2015) About 10% of latent infections progress to active disease which, if left untreated, kills about half of those infected. (WHO, October 2015) The classic symptoms of active TB are a chronic cough with blood-containing sputum, fever, night sweats, and weight loss. (WHO, October 2015) The historical term "ingestion" came about due to the weight loss. Infection of other organs can cause a wide range of symptoms. (Dolin et al, 2010). Tuberculosis is spread through the air when people who have active TB in their lungs cough, spit, speak, or sneeze. (CDC, March 2012). People with latent TB do not spread the disease. (WHO, October 2015) Active infection occurs more often in people with HIV/AIDS and in those who smoke. (WHO, October 2015). Diagnosis of active TB is based on chest X-rays, as well as microscopic examination and culture of body fluids(Konstantinos A,2010). Diagnosis of latent TB relies on the tuberculin skin test (TST) or blood tests. (Konstantinos A,2010). Prevention of TB involves screening those at high risk, early detection and treatment of cases, and vaccination with the bacillus Calmette-Guérin (BCG) vaccine. (Hawn TR et,al, 2014) (Harris and Randall E. 2013) (WHO, October 2008) Those at high risk include household, workplace, and social contacts of people with active TB. (WHO, October 2008) Treatment requires the use of multiple antibiotics over a long period of time. (WHO, October 2015) Antibiotic resistance is a growing problem with increasing rates of multiple drug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB). (WHO, October 2015). Presently, one-third of the world's population is thought to be infected with TB. (WHO, October 2015) New infections occur in about 1% of the population each year. In 2016, there were more than 10 million cases of active TB which resulted in 1.3 million deaths. This makes it the number one cause of death from an infectious disease. More than 95% of deaths occurred in developing countries, and more than 50% in India, China, Indonesia, Pakistan, and the Philippines. The number of new cases each year has decreased since 2000. (WHO, October 2015) About 80% of people in many Asian and African countries test positive while 5–10% of people in the United States population test positive by the tuberculin test.(Kumar V et, al,2007). Tuberculosis has been present in humans since ancient times (Lawn SD and Zumla AI, July 2011).

Timely and accurate diagnosis of tuberculosis (TB) disease in patients must be given a high priority by medical practitioners for the following reasons: global burden of TB disease children carry 6% of the global burden of TB disease; more likely to develop the most severe forms of disseminated and meningeal TB, which is due to the immature immune system.However, diagnosis of TB is challenging because symptoms are often non-specific, specimens may be difficult to obtain, and

bacteriological confirmation is less frequent than in adults. Furthermore, children younger than 5 are more likely to have severe extra-pulmonary TB while the most severe cases of TB are often seen in infants. For these reasons, diagnosing TB in patients warrants additional efforts.Interferon gamma release assays (IGRAs) are promising alternatives to the tuberculin skin test (TST). However, few studies have investigated their use in young children and infants. Consequently, guidelines from the American Academy of Pediatrics state that IGRAs are not recommended for routine use in children younger than five years of age due to a lack of published data.IGRAs have been used mainly in the indirect or immunologic diagnosis of tuberculosis infection. They also can be used to assist in a diagnosis of tuberculosis disease in cases that are difficult to obtain a microbiological diagnosis or that need early diagnosis. Therefore, we have performed a study in a hospital setting to help provide the accuracy of an IGRA in the tuberculosis patients in an urban metropolis

Methods

Study population and methods: The 934 culture-positive sputum samples referred to the National Reference Laboratory of the Research Institute for Pulmonology in Thanjavur from January 2015 to January 2018 were analysed; 40% of these samples were obtained from Tb Sanatorium, Sengipatti patients (hospitalized in Sengipatti) and 60% from other regions (hospitalized in Minsk and other regions) equal to patient's population in the regions. All 934 cases were subjected to a drug-resistance test. The anti-microbial drug susceptibility tests (DST) were performed using the WHO standard conventional proportional method.

Specimen collection, storage, and handling procedures; criteria for specimen rejection: Collect 1 ml of blood by venipuncture directly into each of the QuantiFERON TB Gold IT blood collection tubes, which include a Nil Control tube, TB Antigen tube and a Mitogen tube. Tubes should be between 22° C + 5° C at the time of blood draw. Immediately after filling tubes, shake them ten times just firmly enough to ensure the entire inner surface of the tube is coated with blood, to solubilize antigens of tube walls. Over energetic shaking may cause gel disruption and could lead to aberrant results. Ship tubes to laboratory at 22° C + 5° C as soon as possible and within 16hrs of collection. Do not refrigerate or freeze the blood samples. The assay is set up at least once a week or more frequently depending on workload. Samples are stored for a minimum 7 days at 2° - 8° C after result have been posted.

Blood cultures:Blood cultures using mycobacteria-specific, radioisotope-labeled systems help to establish the diagnosis of active TB. However, mycobacterial bacteremia (bacillemia) is detectable using blood cultures only if specialized systems are used; these bacilli have specific nutrient growth requirements not met by routine culture systems. Such blood cultures should be used for all patients with HIV infection who are suspected of having TB, because bacillemia is particularly prevalent in this population. If available, in fact, these cultures should be used for any patient highly suspected of having active TB.

Statistical analysis

Data obtained from medical records were entered and analyzed using SPSS version 21 (SPSS Inc., Chicago, IL, USA). The sensitivity of each IGRA among the different age groups was compared using binary logistic regression and linear-by-linear association. Comparisons of continuous variables including WBC and lymphocyte counts, CRP, serum protein, and serum albumin levels, across age groups were performed using one-way analysis of variance (ANOVA) and post-hoc analysis. The effect of each factor on the sensitivity of each IGRA was analyzed by logistic regression adjusting for age group. A factor was considered to influence IGRA sensitivity when the age group was adjusted by a certain variable or some variables and the sensitivity of the IGRA according to age group was statistically insignificant. A p value less than 0.05 was considered significant.

Results

During the research period, 934 pulmonary TB patients were studied, of which 274 (29.33 ± 1.5%) ($p < 0.001$) men in the age group 25–65years outnumbered women between 2.7 and 9.0 times more; 660 (70.66 ± 1.5%) of the TB cases were men. In the age group <15–24, as well as in the age group over 65 years, the proportion of men and women were similar. In the remaining age groups, the proportion of men with TB was significantly higher than women. The total ratio of male TB patients among the female patients of all groups surveyed in 2007 was 2.4, which agrees with the WHO European Region. In the age group 45–54 the male to female ratio was the highest among patients with TMDR-TB.Multiple logistic regressions were performed to identify factors associated with the IGRA results (Table 1 and Fig. 1). Age was independently associated with a positive IGRA result ($p < 0.001$), but the anatomic type of inflammation was not ($p = 0.176$). The odds ratio for age adjusted for presumed TRU and anatomic type was 1.06 (95% confidence interval [CI], 1.03 to 1.09), and that for age group adjusted for the same confounding variables was 1.90 (95% CI, 1.41 to 2.56; $p < 0.001$). Likelihood ratios were calculated for assessing the value of performing the IGRA diagnostic test. The positive likelihood ratio was 3.57 and the negative likelihood ratio was 0.00 in our study.

Table 1: Interferon gamma release assay

	All patients	IGRA result			Presumed TRU		
		Positive	Negative	p-value	TRU	Non-TRU	p-value
	(n = 181)	(n = 65)	(n = 116)		(n = 20)	(n = 161)	

Age (yr)	43.4 ± 16.2	50.0 ± 13.5		39.7 ± 16.4		<0.001*	45.6 ± 14.6		43.1 ± 16.4	0.531*
Sex (male : female)	99 : 82	37 : 28		62	: 54	0.652†	10 : 10		89 : 72	0.655†
Positive IGRA result (%)	65 (35.9)	65	(100)	0		NA†	20	(100)	45 (28.0)	<0.001†
Anatomic type (%)										
Anterior	75 (41.4)	20	(30.8)	55	(47.4)		3	(15)	72 (44.7)	
Intermediate	34 (18.8)	9	(13.8)	25	(21.6)	0.001†	0		34 (21.1)	<0.001†
Posterior	49 (27.1)	29	(44.6)	20	(17.2)		15	(75)	34 (21.1)	
Panuveitis	23 (12.7)	7	(10.8)	16	(13.8)		2	(10)	21 (13.0)	
With retinal vasculitis	32 (17.7)	23	(35.4)	9	(7.8)	<0.001†	12	(60)	20 (12.4)	<0.001†

IGRA = interferon gamma release assay; TRU=tuberculosis uveitis; NA=not applicable

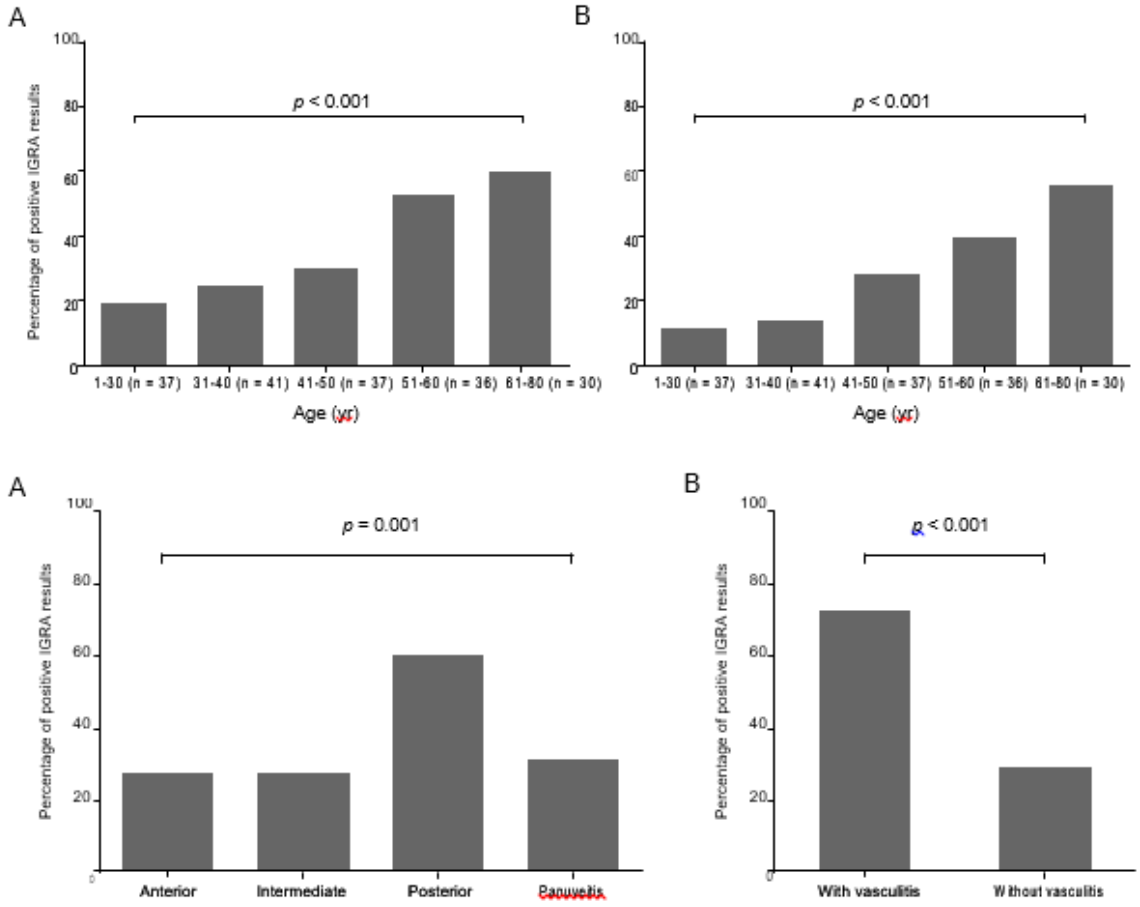

Fig. 1. Interferon-gamma release assay (IGRA)-positive rates (A) and false-positive rates (B) among the five age groups examined. Both rates were significantly different among age groups (all p-values <0.001). p-values were obtained by chi-square tests for trend. Interferon-gamma release assay (IGRA)-positive rates among the four anatomic types of uveitis and between patients with and without retinal vasculitis. (A) The IGRA-positive rates were significantly different among the four anatomic groups (p = 0.001). (B) Patients with retinal vasculitis had higher positive IGRA rate (p < 0.001) than those without retinal vasculitis. p-values were obtained using chi-square tests.

Discussion

The accuracy and reliability of IGRAs among the pulmonary cases in hospital settings is not yet well defined. The study selected 934 culture-positive sputum samples referred to the National Reference Laboratory of the Research Institute for Pulmonology in Thanjavur from January 2015 to January 2018 were analysed; 40% of these samples were obtained from Tb Sanatorium, Sengipatti patients (hospitalized in `Sengipatti) and 60% from other regions (hospitalized in Minsk and other regions) equal to patient's population in the regions. Intraocular inflammation can have many different origins and presents with a wide spectrum of clinical manifestations. If the

underlying inflammatory cause is treat-able, prompt and appropriate treatment can result in a favorable outcome. In the current study, we examined the IGRA as a diagnostic tool for presumed TRU in Korean patients with intraocular inflammation. Our results suggest that the IGRA is highly sensitive and moderately specific for TRU (Groenen et al.,1993). A test used for TB screening should have good sensitivity and acceptable specificity, so we believe that IGRA can be used as a screening test for presumed TRU in Korea. Unfortunately, the TST (Mantoux test) is not useful as a screening tool because of the high false-positive rate in the Korean population, which has a very high BCG vaccination rate. Therefore, an alternative test is needed for the Korean population, and in this study, we evaluated the IGRA as a screening method for TRU. However, the false-positive rate (1-specificity) of IGRA was not low and cannot be neglected. Therefore, clinicians should consider which patients are more likely to have true TRU. Our analyses showed that younger age ($\leq$ 40 years), a positive IGRA, and the presence of posterior uveitis and retinal vasculitis were all predictive of TRU (Kenyon et al., 1997; Feng et al., 2009).

Broad-based posterior synechiae, retinal vasculitis (with or without choroiditis), and serpiginous choroiditis in patients with latent or manifest TB are clinically suggestive of TRU in TB-endemic areas (Kang, 2005). This may explain why the positive predictive value of IGRA was greater in patients with posterior uveitis and retinal vasculitis. Sever-al studies have reported that vasculitis can occur from a TB infection, and it may be associated with hypersensitivity to MTB (Godfrey-Faussett, P. and Stoker, N.G.1992). We examined how a positive IGRA result should be interpreted in patients with suspected TB uveitis. Because our older patients tended to have a higher IGRA positivity rate, a positive result is likely insufficient to make a definitive TRU diagnosis, especially in elderly patients with intraocular inflammation. This result is comparable to those of (Kang et al.1980), who reported that positive IGRA rates are positively and linearly correlated with patient age. In our study, the age of patients with and without TRU were not significantly different, so this relationship did not result from inherent group differences in age (Hensel et al., 2016). We also examined which patients should have the IGRA performed for diagnosing presumed TRU. Patients with intraocular inflammation usually undergo extensive diagnostic workups to identify the underlying inflammatory cause (Poulet et al., 1995). Performing the IGRA on all patients with intraocular inflammation would not be cost-effective. Therefore, we recommend having the IGRA done for patients with a clinical presentation suggestive of TRU, including retinal vasculitis and posterior uveitis. In addition, because a positive IGRA result in a young patient with posterior uveitis or retinal vasculitis likely indicates TRU, the test should be heavily considered if a concurrent TB infection is suspected (Rajendran et al., 2011;Rodrigues et al., 1990).

Conclusion

Tuberculosis remains one of the most-deadly infectious diseases and has claimed millions of lives for manyyears. While significant progress has been made towardscontrolling the global burden of TB over the past decade,more efforts are still needed. Emerging issues such as multidrug-

resistance threatens to revert the progress maderegarding TB care and control. The knowledge base for TB remains a rapidly expanding area and global guidelines arecontinually being refined for instance to incorporate newanti-tubercular drugs to tackle issues of resistance. Health professionals, policy makers, patients and the general public need to keep up-to-date with current trends in TBmanagement and control. This will be essential for efficient adoption of global guidelines to country-level situation, particularly taking into consideration issues such as diseaseburden, health system structures and available resources.

References

Godfrey-Faussett, P. and Stoker, N.G. (1992) Aspects of tuberculosis in Africa. Genetic fingerprinting for clues to the pathogenesis of tuberculosis. Transactions of the Royal Society of Tropical Medicine and Hygiene 86, 472–475.

Groenen, P.M., Van Bunschoten, A.E., Van Soolingen, D. and Van Embden, J.D. (1993) Nature of DNA polymorphism in the direct repeat cluster of M. tuberculosis, application for strain differentiation by a novel method. Molecular Microbiology 10, 1057–1065.

Hawn TR, Day TA, Scriba TJ, Hatherill M, Hanekom WA, Evans TG, Churchyard GJ, Kublin JG, Bekker LG, Self SG (December 2014). "Tuberculosis vaccines and prevention of infection". Microbiology and Molecular Biology Reviews. 78 (4): 650–71.

C. Dye, Global epidemiology of tuberculosis, Lancet 367 (2006) 938–940.

H. Feng, H. Xiang, J. Zhang et al., “Genome-wide transcriptional profiling of the response of staphylococcus aureus to Journal of Biomedicine and Biotechnology cryptotanshinone,” Journal of Biomedicine and Biotechnology, vol. 2009, Article ID 617509, 8 pages, 2009.

H. P. Harding, Y. Zhang, H. Zeng et al., “An integrated stress response regulates amino acid metabolism and resistance to oxidative stress,” Molecular Cell, vol. 11, no. 3, pp. 619–633,2003.

Hamilton, Richart (2015). Tarascon Pocket Pharmacopoeia 2015 Deluxe Lab-Coat Edition. Jones & Bartlett Learning. p. 49.

Hensel RL, Kempker RR, Tapia J, Oladele A, Blumberg HM, Magee MJ. Increased risk of latent tuberculous infection among persons with pre-diabetes and diabetes mellitus. Int J Tuberc Lung Dis. 2016 Jan. 20 (1):71-8.

Kang YA, Lee HW, Yoon HI, et al. Discrepancy between the tuberculin skin test and the whole-blood interferon gamma assay for the diagnosis of latent tuberculosis infection in an intermediate tuberculosis-burden country. JAMA 2005;293:2756-61.3

Pai M, Zwerling A, Menzies D. Systematic review: T-cell-based assays for the diagnosis of latent tuberculosis infection: an update. Ann Intern Med. 2008 Aug 5. 149 (3):177-84.

Poulet, S. and Cole, S.T. (1995) Characterization of the highly abundant polymorphic GC-rich-repetitive sequence (PGRS) present in M. tuberculosis. Archives of Microbiology 16, 87–95.

Rajendran, R. Garva, M. Krstic-Demonacos, and C. Demonacos, "Sirtuins: molecular traffic lights in the crossroad of oxidative stress, chromatin remodeling, and transcription, "Journal of Biomedicine and Biotechnology, vol. 2011, Article ID 368276, 17 pages, 2011.

Rodrigues, L.C. and Smith, P.G. (1990) Tuberculosis in developing countries and methods for its control. Transactions of the Royal Society of Tropical Medicine and Hygiene 84, 739–744.

European Journal of Cardiovascular Medicine (EJCM)
Print ISSN: 2042-4884
E-ISSN: 2042-4892
Volume: 13 | Issue 03 | July-Sept | 2023
Journal homepage: https://www.healthcare-bulletin.co.uk/

Research Article

Correlation of C-reactive Protein Levels with Bacterial Isolates and Antibiotic Resistance in Bloodstream Infections

Sajitha G R[1], Anusha Murali[2*], Jomy Jose[3], Sophy Jacob Benny[4], Seema Bhargavan[5]
[1]Associate Professor, Department of Biochemistry, Dr Somervell Memorial CSI Medical College Hospital, Karakonam
[*2]Assistant Professor, Department of Microbiology, Mount Zion Medical College
[3]Assistant Professor, Department of Microbiology Mount Zion Medical College
[4]QA/QC Manager/Operations Manager, Themar Al Emarat, Sharjah
[5]Microbiologist.

Received: 10/07/2023; Revision: 19/07/2023; Accepted: 02/08/2023; Published: 27/08/2023

***Corresponding author: Anusha Murali**

Abstract: ***Introduction***: Bloodstream infections (BSIs) are a significant cause of morbidity and mortality in hospitalized patients. Early diagnosis and timely initiation of appropriate antimicrobial therapy are essential for reducing complications. C-reactive protein (CRP), an acute-phase reactant, is commonly used as a biomarker of systemic inflammation. While CRP levels rise in bacterial infections, their correlation with specific bacterial isolates and patterns of antibiotic resistance in BSIs remains inadequately explored in Indian clinical settings. This study seeks to investigate the potential of CRP levels as a supportive diagnostic marker for bacterial isolates and their resistance profiles in patients with BSIs. ***Objective*:** To evaluate the correlation between CRP levels, type of bacterial isolates, and associated antibiotic resistance patterns in patients with bloodstream infections. ***Methods*:** This hospital-based, cross-sectional observational study was conducted over a period of 18 months (January 2022 to June 2023) at a tertiary care hospital in India. A total of 130 patients with clinically and microbiologically confirmed bloodstream infections were included. Blood samples were collected for CRP estimation by immunoturbidimetric assay and for culture using standard microbiological techniques. Isolated organisms were identified by biochemical and automated methods, and their antibiotic susceptibility profiles were determined according to CLSI guidelines. Correlations between CRP levels, types of isolates (Gram-positive vs. Gram-negative), and resistance patterns (MDR, ESBL, MRSA) were statistically analyzed. ***Result*:** Among the 130 BSI patients, Gram-negative organisms accounted for 62.3% of isolates, with Escherichia coli and Klebsiella pneumoniae being predominant. Gram-positive organisms included Staphylococcus aureus and Enterococcus species. The mean CRP level was significantly higher in patients with Gram-negative sepsis (mean 164.8 ± 38.4 mg/L) compared to Gram-positive infections (mean 108.3 ± 26.9 mg/L) ($p < 0.001$). Elevated CRP levels were also significantly associated with multidrug-resistant organisms and ESBL producers. MRSA infections were associated with moderately elevated CRP values. A positive correlation was observed between CRP concentration and bacterial virulence/resistance pattern. ***Conclusion*:** C-reactive protein levels correlate significantly with the type of bacterial isolate and its resistance profile in bloodstream infections. Elevated CRP may serve as an adjunctive marker for predicting severe Gram-negative sepsis and the likelihood of drug-resistant pathogens. Integrating CRP measurements with culture and sensitivity testing can enhance early risk stratification and guide empirical antimicrobial therapy in resource-limited settings.

Keywords: Bloodstream Infections, C-Reactive Protein, Antibiotic Resistance, Gram-Negative Sepsis, Multidrug Resistance, MRSA, ESBL.

INTRODUCTION

Bloodstream infections (BSIs) are among the most severe clinical conditions encountered in hospital settings, often resulting in prolonged hospitalization, increased healthcare costs, and significant morbidity and mortality. The global burden of BSIs has escalated with the emergence of multidrug-resistant (MDR) organisms, posing challenges for timely diagnosis and effective antimicrobial management[1]. Infections originating from various sources such as the lungs, urinary tract, gastrointestinal tract, or indwelling catheters can lead to bacteremia or septicemia, which if left untreated or improperly managed, can progress to septic shock and organ failure. This underscores the critical need for early diagnostic indicators that can aid in initiating prompt and targeted therapy before microbiological confirmation is available[2].

C-reactive protein (CRP) is a hepatic acute-phase reactant synthesized in response to cytokines, particularly interleukin-6 (IL-6), during systemic inflammatory responses. As a nonspecific marker, CRP has long been used to assess the severity and progression of infections, inflammation, and tissue injury. Elevated CRP levels are especially prominent in bacterial infections compared to viral or non-infectious inflammatory conditions. In the context of BSIs, CRP may offer valuable insight into the systemic inflammatory burden and the potential severity of infection[3]. However, its utility as a correlate for specific bacterial isolates and their resistance profiles remains a subject of ongoing investigation.

India, like many low- and middle-income countries, faces a dual burden of increasing antibiotic resistance and limited access to rapid molecular diagnostics. In such resource-constrained environments, reliance on traditional biomarkers like CRP becomes even more significant[4]. Identifying a reliable association between CRP levels and

bacterial isolate characteristics could assist clinicians in anticipating the nature of the pathogen Gram-positive vs. Gram-negative as well as its potential resistance mechanisms such as extended-spectrum beta-lactamase (ESBL) production or methicillin resistance (MRSA). This could, in turn, improve empirical antibiotic choices before blood culture results become available[5].

The growing threat of antimicrobial resistance, including ESBL-producing Enterobacteriaceae, carbapenem-resistant organisms, and MRSA, further amplifies the urgency of utilizing accessible diagnostic tools to support early clinical decisions[6]. While several studies have examined CRP levels in sepsis and systemic infections, few have attempted to directly correlate quantitative CRP values with microbiological outcomes in BSIs, particularly in the Indian population. Furthermore, existing literature lacks consensus on whether CRP levels significantly differ among different classes of pathogens and resistant phenotypes[7].

This study was thus designed to evaluate the relationship between CRP levels and the type of bacterial pathogen isolated in patients with bloodstream infections. In addition, the study investigates whether CRP can serve as an indirect marker of antimicrobial resistance, thereby aiding in early prediction of infection severity and potential treatment failure. The findings are expected to bridge a critical gap in the literature by correlating inflammatory biomarkers with microbiological profiles in BSIs, potentially contributing to better stratification of septic patients and optimized use of antibiotics in hospital settings

Aim and Objectives

Aim

To evaluate the correlation between serum C-reactive protein (CRP) levels, the type of bacterial isolates, and associated antibiotic resistance patterns in patients with bloodstream infections.

Objectives

1. To measure serum CRP levels in patients with microbiologically confirmed bloodstream infections.
2. To identify and characterize bacterial isolates obtained from blood cultures of these patients.
3. To determine the antibiotic susceptibility profile of the isolated organisms with emphasis on multidrug resistance, ESBL, and MRSA.
4. To correlate CRP levels with the type of bacterial pathogen (Gram-positive vs. Gram-negative).
5. To assess the association between elevated CRP levels and the presence of antimicrobial resistance.
6. To evaluate the potential role of CRP as a predictive marker for pathogen virulence and resistance in bloodstream infections.

MATERIALS AND METHODS

Study design

This was a hospital-based, cross-sectional observational study conducted to investigate the correlation between serum C-reactive protein levels and the microbiological characteristics of bacterial bloodstream infections.

Study setting and duration

The study was carried out in the Department of Microbiology and Biochemistry at a tertiary care hospital in India over a period of 18 months, from January 2022 to June 2023.

Sample size

A total of 130 patients with clinically suspected bloodstream infections and confirmed positive blood cultures were enrolled in the study.

Inclusion criteria

- Patients aged ≥18 years with signs and symptoms of systemic infection.
- Blood culture positivity for bacterial pathogens.
- Availability of serum sample for CRP estimation at the time of diagnosis.

Exclusion criteria

- Patients with viral, fungal, or parasitic bloodstream infections.
- Patients with autoimmune diseases, malignancies, or other chronic inflammatory conditions.
- Those receiving immunosuppressive therapy or corticosteroids.
- Incomplete clinical or laboratory data.

Sample collection and processing

Venous blood samples were collected aseptically under sterile conditions before administration of antibiotics. A portion of the sample was sent for CRP estimation and another for blood culture. CRP levels were measured using an immunoturbidimetric assay on a fully automated analyzer following the manufacturer's protocol. The results were recorded in mg/L.

Microbiological analysis

Blood cultures were processed using automated systems (e.g., BacT/ALERT or BACTEC) and subcultured on appropriate media. Bacterial isolates were identified based on colony morphology, Gram staining, conventional biochemical reactions, and confirmed by automated identification systems. Antibiotic susceptibility testing was performed using the Kirby–Bauer disc diffusion method and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. The presence of multidrug resistance (MDR), extended-spectrum beta-lactamase (ESBL) production, and methicillin resistance (MRSA) was confirmed using standard phenotypic methods.

Data management and statistical analysis

Patient demographic details, clinical data, CRP values, culture results, and antibiotic sensitivity patterns were recorded in predesigned case record forms. Quantitative variables were expressed as mean ± standard deviation. Comparisons between CRP levels in Gram-positive and Gram-negative infections were analyzed using unpaired t-tests. Correlation between CRP values and antimicrobial resistance patterns was evaluated using Pearson's correlation coefficient. A p-value of <0.05 was considered statistically significant. All analyses were performed using licensed statistical software.

Ethical considerations

The study protocol was approved by the Institutional Ethics Committee. Informed consent was obtained from all participants or their legal guardians prior to inclusion in the study. Patient confidentiality and anonymity were strictly maintained throughout the research process.

RESULTS

Overview

A total of 130 patients with microbiologically confirmed bloodstream infections were included in the study. Among these, 78 (60%) were male and 52 (40%) females, with a mean age of 51.4 ± 16.2 years. Gram-negative organisms were more frequently isolated compared to Gram-positive organisms. C-reactive protein (CRP) levels were elevated in all patients, with significantly higher levels in those infected with Gram-negative organisms and multidrug-resistant pathogens. The CRP levels demonstrated statistically significant correlations with the type of organism and antibiotic resistance profiles.

Table 1: Demographic Profile of Patients with Bloodstream Infections

Table 1 presents the age and sex distribution of patients enrolled in the study.

Demographic Variable	Value
Total patients	130
Males	78 (60.0%)
Females	52 (40.0%)
Mean age (years)	51.4 ± 16.2
Age range (years)	18 – 85

Patients across a wide age range were included, with a slight male predominance.

Table 2: Clinical Presentations in Patients with Bloodstream Infections

Table 2 summarizes the major presenting symptoms and signs in the study population.

Clinical Feature	Frequency (%)
Fever	122 (93.8%)
Hypotension	41 (31.5%)
Tachycardia	89 (68.5%)
Respiratory distress	34 (26.1%)
Altered sensorium	18 (13.8%)

Fever was the most common presenting complaint, seen in nearly all patients.

Table 3: Distribution of Bacterial Isolates

Table 3 shows the types and frequencies of bacterial isolates recovered from positive blood cultures.

Isolated Organism	Frequency (%)
Escherichia coli	38 (29.2%)
Klebsiella pneumoniae	28 (21.5%)
Staphylococcus aureus	24 (18.5%)
Pseudomonas aeruginosa	14 (10.8%)
Acinetobacter baumannii	8 (6.2%)
Enterococcus spp.	7 (5.4%)
Others (Salmonella, etc.)	11 (8.4%)

Gram-negative organisms accounted for 70% of all isolates.

Table 4: Gram Stain Classification of Isolates

Table 4 classifies isolates into Gram-positive and Gram-negative organisms.

Gram Classification	Frequency (%)
Gram-negative	91 (70.0%)
Gram-positive	39 (30.0%)

Gram-negative infections were significantly more prevalent in this cohort.

Table 5: Mean CRP Levels Based on Gram Type

Table 5 compares serum CRP levels between Gram-positive and Gram-negative infections.

Gram Type	Mean CRP (mg/L) ± SD	p-value
Gram-negative	164.8 ± 38.4	< 0.001
Gram-positive	108.3 ± 26.9	

CRP levels were significantly higher in Gram-negative BSIs.

Table 6: Antibiotic Resistance Profiles of Isolates

Table 6 shows the resistance characteristics among isolates.

Resistance Category	Frequency (%)
Multidrug-resistant (MDR)	47 (36.2%)
ESBL producers	32 (24.6%)
MRSA	14 (10.8%)

MDR and ESBL-producing organisms formed a considerable subset of isolates.

Table 7: Mean CRP Levels in Relation to Resistance Patterns

Table 7 provides mean CRP levels across different resistance categories.

Resistance Type	Mean CRP (mg/L) ± SD	p-value
MDR	172.3 ± 34.6	< 0.001
ESBL	168.7 ± 32.8	< 0.001
MRSA	115.2 ± 27.9	0.04
Sensitive strains	101.5 ± 22.3	

Elevated CRP levels correlated strongly with drug-resistant strains.

Table 8: Correlation Between CRP and Duration of Fever

Table 8 explores the relationship between CRP levels and duration of febrile illness.

Duration of Fever	Mean CRP (mg/L) ± SD
< 3 days	126.7 ± 31.5
3–7 days	155.2 ± 37.9
> 7 days	174.9 ± 39.1

Longer duration of fever was associated with higher CRP levels.

Table 9: Site of Infection and CRP Levels

Table 9 correlates primary infection source with CRP values.

Infection Focus	Mean CRP (mg/L) ± SD
Urinary tract	158.2 ± 36.2
Respiratory tract	167.5 ± 41.0
Intra-abdominal	162.1 ± 33.8
Catheter-related	119.4 ± 27.6

Catheter-related infections had relatively lower CRP values.

Table 10: Correlation Between CRP and Blood Culture Yield Time

Table 10 evaluates whether CRP levels relate to faster culture positivity.

Time to Positivity	Mean CRP (mg/L) ± SD
< 24 hours	168.3 ± 35.9
24–48 hours	137.6 ± 32.7
> 48 hours	115.2 ± 28.3

Higher CRP values were associated with early culture positivity, indicating higher bacterial load.

Table 11: CRP Stratification and Pathogen Type

Table 11 categorizes pathogens based on CRP levels into mild, moderate, and severe response.

CRP Range (mg/L)	Dominant Pathogens
< 100	Staphylococcus aureus, Enterococci
100–150	Klebsiella, E. coli
> 150	Pseudomonas, Acinetobacter

High CRP levels frequently corresponded to more virulent Gram-negative organisms.

Table 12: Correlation Coefficient Between CRP and Resistance Profile

Table 12 reports the strength of association between CRP and resistance traits.

Resistance Feature	Correlation Coefficient (r)	p-value
MDR	0.61	< 0.001
ESBL	0.59	< 0.001
MRSA	0.41	0.03

There was a moderate to strong positive correlation between CRP levels and resistance profiles

Table 1 highlights the demographic profile of BSI patients, predominantly middle-aged males. **Table 2** summarizes common clinical signs, with fever and tachycardia being prominent. **Table 3** shows E. coli and Klebsiella as the most common pathogens. **Table 4** confirms the predominance of Gram-negative isolates. **Table 5** demonstrates significantly elevated CRP in Gram-negative infections. **Table 6** reveals the frequency of MDR, ESBL, and MRSA strains. **Table 7** links elevated CRP to MDR and ESBL infections, showing statistical significance. **Table 8** indicates higher CRP levels with longer fever duration. **Table 9**

correlates infection source with CRP, showing higher values in respiratory and intra-abdominal infections. **Table 10** suggests that higher CRP is associated with quicker culture positivity. **Table 11** stratifies CRP levels with the likely organism type and virulence. **Table 12** provides statistical evidence for moderate-to-strong correlation between CRP and resistance traits.

DISCUSSION

Bloodstream infections (BSIs) continue to be a major cause of hospital admissions and complications in both critical care and general inpatient settings. Early detection of causative organisms and timely administration of effective antimicrobial therapy are essential for improving patient outcomes[8]. However, the increasing prevalence of multidrug-resistant (MDR) organisms and delayed culture results often hinder appropriate management. In this context, inflammatory biomarkers such as C-reactive protein (CRP) can play an important adjunctive role in the early identification and stratification of infection severity[9].

In the present study, Gram-negative bacteria were more frequently isolated from blood cultures than Gram-positive organisms. Escherichia coli and Klebsiella pneumoniae were the predominant pathogens among the Gram-negative group, while Staphylococcus aureus was the most common among Gram-positive isolates. The predominance of Gram-negative organisms in BSIs observed here reflects a typical pattern seen in nosocomial and urinary tract–associated infections, particularly in patients with catheters, underlying comorbidities, or recent hospitalizations[9].

The mean CRP levels were significantly higher in patients with Gram-negative infections compared to those with Gram-positive infections. This difference suggests a more severe systemic inflammatory response associated with Gram-negative bacteremia. The higher CRP concentrations likely reflect the greater pro-inflammatory stimulus induced by lipopolysaccharides and endotoxins present in the outer membranes of Gram-negative bacteria. This physiological response results in accelerated hepatic synthesis of acute-phase proteins, including CRP, mediated through cytokine cascades[10].

A considerable proportion of isolates in this study exhibited antibiotic resistance patterns, including MDR, ESBL production, and methicillin resistance. CRP levels were notably elevated in patients infected with MDR and ESBL-producing organisms[11]. These observations suggest that infections caused by resistant organisms may provoke a more intense or prolonged inflammatory response, either due to delayed clearance of the pathogen or due to greater tissue damage inflicted by resistant strains. In contrast, patients infected with MRSA exhibited moderately elevated CRP levels, which, while higher than those in patients infected with sensitive strains, were still lower than those seen in MDR Gram-negative infections[12].

The association between CRP levels and resistance profiles reinforces the potential role of CRP as a surrogate indicator for identifying high-risk infections. This is particularly valuable in clinical settings where immediate microbiological confirmation is unavailable. Elevated CRP values, especially in the context of Gram-negative sepsis or suspected drug-resistant infections, could prompt the early use of broader-spectrum antibiotics or escalation of care. Moreover, CRP stratification can assist clinicians in prioritizing patients for intensive monitoring or isolation protocols when multidrug-resistant pathogens are suspected[13].

In addition to microbial factors, clinical parameters such as fever duration and site of infection were also associated with CRP levels. Patients with prolonged fever and those with respiratory or intra-abdominal sources of infection tended to have higher CRP values. This supports the understanding that CRP levels not only reflect microbial burden but also the extent and location of systemic inflammation. Faster culture positivity, observed in patients with higher CRP levels, may indicate a higher bacterial load, further supporting the role of CRP in reflecting disease intensity[14].

Although CRP is a nonspecific biomarker and cannot distinguish among individual pathogens or resistance mechanisms, its quantitative correlation with Gram classification and resistance traits offers practical clinical utility. When used alongside clinical examination and basic laboratory data, CRP measurement can improve the precision of empirical treatment decisions while awaiting definitive culture and sensitivity reports[15].

The present study highlights the feasibility of using CRP levels as a rapid, cost-effective adjunct in the early diagnosis and risk assessment of BSIs. It also emphasizes the importance of combining biomarker data with microbiological results for a more comprehensive understanding of infection dynamics. Despite its limitations such as the cross-sectional design and exclusion of other biomarkers this study underscores the diagnostic and prognostic relevance of CRP in bloodstream infections in hospital-based settings.

CONCLUSION

This study demonstrated a significant association between serum C-reactive protein (CRP) levels and the type of bacterial isolates, as well as their antibiotic resistance profiles, in patients with bloodstream infections. CRP levels were markedly higher in cases involving Gram-negative organisms and in infections caused by multidrug-resistant and ESBL-producing pathogens, suggesting a stronger inflammatory response in these groups.

The findings indicate that CRP, although nonspecific, may serve as a useful adjunctive biomarker for early risk stratification, particularly in settings where blood culture results are pending or delayed. CRP can aid in anticipating pathogen virulence and resistance potential, supporting more rational and timely decisions regarding empirical antimicrobial therapy.

Incorporating CRP measurements into the initial evaluation of patients with suspected sepsis may help identify those at greater risk of complications and antimicrobial resistance. While CRP cannot replace microbiological testing, its use alongside clinical judgment and culture results may enhance diagnostic accuracy and therapeutic planning in bloodstream infections.

REFERENCES

1. Huang CH, Chiu CH, Chen IW, Hung SY, Lin CW, Hsu BR, Huang YY. Antimicrobial resistance and outcomes of community-onset bacterial bloodstream infections in patients with type 2 diabetes. J Glob Antimicrob Resist. 2018 Dec;15:271-276. doi:

10.1016/j.jgar.2018.08.008. Epub 2018 Aug 16. PMID: 30121344.

2. Tsai YH, Hou TC, Liu PY, Chen CJ, Wang JM. Bloodstream Coinfections and Antimicrobial Resistance in Hospitalized COVID-19 Patients: A Single-center Retrospective Study. In Vivo. 2024 Jul-Aug;38(4):1965-1972. doi: 10.21873/invivo.13653. PMID: 38936952; PMCID: PMC11215599.
3. Ma H, Xu J, Zhang Y, Zhang R, Wu J. Relevance and antimicrobial resistance profile of Klebsiella pneumoniae in neonatal sepsis. J Matern Fetal Neonatal Med. 2024 Dec;37(1):2327828. doi: 10.1080/14767058.2024.2327828. Epub 2024 Mar 12. PMID: 38471804.
4. Mkony MF, Mizinduko MM, Massawe A, Matee M. Management of neonatal sepsis at Muhimbili National Hospital in Dar es Salaam: diagnostic accuracy of C-reactive protein and newborn scale of sepsis and antimicrobial resistance pattern of etiological bacteria. BMC Pediatr. 2014 Dec 5;14:293. doi: 10.1186/s12887-014-0293-4. PMID: 25475836; PMCID: PMC4262228.
5. Chen PL, Chang CM, Wu CJ, Ko NY, Lee NY, Lee HC, Shih HI, Lee CC, Wang RR, Ko WC. Extraintestinal focal infections in adults with nontyphoid Salmonella bacteraemia: predisposing factors and clinical outcome. J Intern Med. 2007 Jan;261(1):91-100. doi: 10.1111/j.1365-2796.2006.01748.x. PMID: 17222172.
6. Keller D, Mester P, Räth U, Krautbauer S, Schmid S, Greifenberg V, Müller M, Kunst C, Buechler C, Pavel V. Calprotectin, a Promising Serological Biomarker for the Early Diagnosis of Superinfections with Multidrug-Resistant Bacteria in Patients with COVID-19. Int J Mol Sci. 2024 Aug 27;25(17):9294. doi: 10.3390/ijms25179294. PMID: 39273246; PMCID: PMC11394900.
7. Oldendorff F, Nordberg V, Giske CG, Navér L. A decade of neonatal sepsis in Stockholm, Sweden: Gram-positive pathogens were four times as common as Gram-negatives. Eur J Clin Microbiol Infect Dis. 2024 May;43(5):959-968. doi: 10.1007/s10096-024-04809-8. Epub 2024 Mar 22. PMID: 38517573; PMCID: PMC11108929.
8. Salah A, Al-Subol I, Hudna A, Alhaj A, Alqubaty AR, Farie W, Sulieman D, Alnadhari O, Alwajeeh T, Alobathani F, Almikhlafy A, Mahdy MAK. Neonatal sepsis in Sana'a city, Yemen: a predominance of Burkholderia cepacia. BMC Infect Dis. 2021 Oct 27;21(1):1108. doi: 10.1186/s12879-021-06808-y. PMID: 34706677; PMCID: PMC8554861.
9. Chen ZS, Zheng L, Chen YQ, Yang JH, Li J. [Pathogens of infections in the induction period of childhood acute lymphoblastic leukemia and drug resistance of isolated strains]. Zhongguo Dang Dai Er Ke Za Zhi. 2017 Feb;19(2):176-181. Chinese. doi: 10.7499/j.issn.1008-8830.2017.02.010. PMID: 28202116; PMCID: PMC7389464.
10. Amanati A, Sajedianfard S, Khajeh S, Ghasempour S, Mehrangiz S, Nematolahi S, Shahhosein Z. Bloodstream infections in adult patients with malignancy, epidemiology, microbiology, and risk factors associated with mortality and multi-drug resistance. BMC Infect Dis. 2021 Jul 2;21(1):636. doi: 10.1186/s12879-021-06243-z. PMID: 34215207; PMCID: PMC8254331.
11. Wattal C, Goel N. Pediatric Blood Cultures and Antibiotic Resistance: An Overview. Indian J Pediatr. 2020 Feb;87(2):125-131. doi: 10.1007/s12098-019-03123-y. Epub 2019 Dec 21. Erratum in: Indian J Pediatr. 2020 Jun;87(6):486. doi: 10.1007/s12098-020-03257-4. PMID: 31863394; PMCID: PMC6974494.
12. Kadri SS, Lai YL, Warner S, Strich JR, Babiker A, Ricotta EE, Demirkale CY, Dekker JP, Palmore TN, Rhee C, Klompas M, Hooper DC, Powers JH 3rd, Srinivasan A, Danner RL, Adjemian J; forming the National Insititutes of Health Antimicrobial Resistance Outcomes Research Initiative (NIH-ARORI). Inappropriate empirical antibiotic therapy for bloodstream infections based on discordant in-vitro susceptibilities: a retrospective cohort analysis of prevalence, predictors, and mortality risk in US hospitals. Lancet Infect Dis. 2021 Feb;21(2):241-251. doi: 10.1016/S1473-3099(20)30477-1. Epub 2020 Sep 8. PMID: 32916100; PMCID: PMC7855478.
13. Foglia F, Della Rocca MT, Melardo C, Nastri BM, Manfredini M, Montella F, De Filippis A, Finamore E, Galdiero M. Bloodstream infections and antibiotic resistance patterns: a six-year surveillance study from southern Italy. Pathog Glob Health. 2023 Jun;117(4):381-391. doi: 10.1080/20477724.2022.2129161. Epub 2022 Oct 3. PMID: 36190133; PMCID: PMC10177691.
14. AbuTaha SA, Al-Kharraz T, Belkebir S, Abu Taha A, Zyoud SH. Patterns of microbial resistance in bloodstream infections of hemodialysis patients: a cross-sectional study from Palestine. Sci Rep. 2022 Oct 26;12(1):18003. doi: 10.1038/s41598-022-21979-7. PMID: 36289278; PMCID: PMC9605991.
15. Dubreuil L, Veloo AC, Sóki J; ESCMID Study Group for Anaerobic Infections (ESGAI). Correlation between antibiotic resistance and clinical outcome of anaerobic infections; mini-review. Anaerobe. 2021 Dec;72:102463. doi: 10.1016/j.anaerobe.2021.102463. Epub 2021 Sep 28. PMID: 34597797