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ORIGINAL ARTICLE

Frequency of microorganisms detected in patients with empyema thoracis at Department of Pulmonology, Sheikh Zayed Medical College/Hospital, Rahim Yar Khan.

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ABSTRACT… Objective: To determine the frequency and types of microorganisms isolated from pleural fluid samples of patients with empyema thoracis, in order to aid empirical antibiotic selection and improve microbiological diagnosis in the local clinical setting. **Study Design:** Cross-sectional study. **Setting:** Department of Pulmonology, Sheikh Zayed Medical College/Hospital, Rahim Yar Khan. **Period:** March 2024 to February 2025. **Methods:** A total of 102 patients aged 13–70 years with clinically and radiologically confirmed empyema thoracis were enrolled through non-probability consecutive sampling. Pleural fluid was aseptically aspirated and subjected to microbiological culture and sensitivity testing. Data were recorded on a structured proforma and analyzed using SPSS version 26.0, with statistical significance set at $p < 0.05$. **Results:** Out of 102 patients, the mean age was 44.7 ± 13.4 years, with a mean symptom duration of 20.9 ± 7.4 days. Males comprised 68.6% and rural residents 58.8%. Right-sided effusion was most common (60.8%). Comorbidities included hypertension (25.5%) and diabetes (21.6%). Culture revealed monomicrobial growth in 54.9%, polymicrobial in 17.6%, and sterile fluid in 27.5%. Staphylococcus aureus was the most frequently isolated organism with significant male predominance ($p = 0.041$). Gram-positive organisms accounted for 39.2%, Gram-negative for 33.3%. **Conclusion:** Empyema thoracis in our region showed predominance of Gram-positive organisms, especially Staphylococcus aureus, with a significant male association, emphasizing the need for tailored empirical antimicrobial strategies.

Key words: Empyema Thoracis, Gram-positive Bacteria, Microbial Spectrum, Pleural Fluid Culture.

INTRODUCTION

Empyema thoracis is defined as the pathological accumulation of purulent fluid in the pleural cavity, typically as a complication of pneumonia, thoracic surgery, trauma or systemic infections.[1] Epidemiologically, empyema thoracis affects over 65,000 patients annually in the United States and the United Kingdom, with mortality rates ranging between 10% and 20%.[2] In Pakistan, its burden is notably higher among young and middle-aged adults, where tuberculosis, pneumonia, and delayed medical care contribute to both its incidence and complications.[3] The clinical sign and symptoms of empyema include; pleuritic chest pain, cough, fever, chills, weight loss, anorexia, dyspnea and night sweats. The etiological profile of empyema thoracis has undergone significant regional and temporal variation, with recent studies indicating a rising prevalence of Gram-negative organisms and methicillin-resistant Staphylococcus aureus (MRSA), particularly in nosocomial and immunocompromised settings.[4,5]

The accurate identification of microbial etiology through culture and sensitivity testing is essential not only for guiding targeted antimicrobial therapy but also for informing local antibiotic stewardship policies.[6] However, culture-negative cases still represent a significant proportion—ranging from 33% to 67% across studies—underscoring the need for early aspiration, proper specimen handling, and adjunctive molecular techniques where available.

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The major aim of empyema management is to eliminate the infection and re-expansion of the lungs which is usually achieved by eradicating the bacterial growth from the pleural fluid by the use of appropriate antibiotic therapy along with drainage process.[7,8]

Multiple regional studies have consistently demonstrated the predominance of Staphylococcus aureus, Streptococcus pneumoniae, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Escherichia coli in culture-positive pleural samples. For instance, Atif et al. reported Pseudomonas aeruginosa as the leading isolate in 33.3% of cases, followed by Klebsiella spp. and S. aureus, with high resistance to third-generation cephalosporins and fluoroquinolones.[9] In contrast, pediatric populations have shown a higher burden of S. aureus and Mycobacterium tuberculosis, with recovery rates exceeding 98% when treated with antibiotics and tube thoracostomy, as demonstrated by Dhal et al. and Chatterjee et al.[10,11] Furthermore, systematic reviews have highlighted that up to 13% of empyema cases are polymicrobial, often involving anaerobes such as Bacteroides and viridans streptococci, which complicate empirical treatment decisions.[12]

Given the evolving microbial landscape and regional variations in pathogen distribution and resistance patterns, it is imperative to continuously update local data to inform empiric therapy. The present study was therefore designed to determine the frequency and pattern of microorganisms isolated from patients with empyema thoracis, along with relevant demographic and clinical characteristics, to contribute to the growing body of evidence guiding effective clinical management of this potentially life-threatening condition.

METHODS

A cross-sectional study was executed at Sheikh Zayed Medical Hospital, Rahim yar khan, in the Pulmonology Department from March 2024 to February 2025. Ethical approval was obtained from the Institutional Review Board (IRB No. 606/IRB/SZMC/SZH) before research initiation and informed consent was taken from patients before enrollment. A sample size of 102 patients was computed by using OpenEpi calculator keeping 95% confidence level, 6% absolute precision and 10.7% previously reported expected frequency of organism growth.[12] A non-probability consecutive sampling technique was utilized for participant enrollment.

Inclusion and Exclusion Criteria

This study included patients aged 13 to 70 years of either gender who were diagnosed with empyema thoracis, confirmed through clinical evaluation, radiological findings and the presence of purulent fluid on pleural aspiration. Patients were excluded if they had received systemic antibiotics for more than 72 hours prior to pleural fluid collection, had post-surgical empyema or trauma-related pleural infections, or were known cases of malignant pleural effusion or pleural malignancy. Individuals with an immunocompromised status, including HIV/AIDS, those undergoing chemotherapy or long-term immunosuppressive therapy, and those with microbiologically or clinically confirmed tuberculosis at presentation were also excluded.

Data Collection Procedure

Once eligibility was confirmed, baseline demographic and clinical information was recorded for each participant, including age, gender, duration of symptoms in days, residential status (urban or rural), laterality of pleural effusion (right, left, or bilateral), smoking status (categorized as never smoker, former smoker, or current smoker), and comorbid conditions such as hypertension, diabetes mellitus, asthma, and chronic obstructive pulmonary disease (COPD). Pleural fluid sampling was performed under strict aseptic conditions through ultrasound guided pleural tap, which was carried out in a designated procedure room. The procedural site was identified based on imaging findings, typically at the midaxillary line (if the patient is supine) or the posterior midscapular line (if the patient is seated) or over the area of maximal dullness on percussion. After sterile preparation of the skin with povidone-iodine and alcohol, local anesthesia was administered using 1% lidocaine infiltrated into the skin, subcutaneous tissues and parietal pleura. A sterile 20G needle was inserted

just above the upper border of the rib to avoid the neurovascular bundle and pleural fluid was aspirated into a sterile syringe. The aspirated fluid was immediately transferred into sterile, properly labeled containers and transported to the microbiology laboratory without delay to ensure sample integrity and microbial viability.

In the microbiology laboratory, each sample was first subjected to macroscopic examination for characteristics such as color, turbidity, odor, and viscosity. Microscopic analysis was performed using Gram staining to identify the presence of bacterial cells and to assess the predominant inflammatory cells. When anaerobic or polymicrobial infection was clinically suspected, samples were cultured on appropriate anaerobic media and incubated in anaerobic jars or chambers. Bacterial identification was carried out using conventional biochemical tests, including catalase, coagulase, oxidase, and standard sugar fermentation assays, depending on the preliminary Gram stain findings. Each sample was then categorized based on microbial growth as monomicrobial, polymicrobial, or no growth (sterile). All findings were meticulously recorded in a structured data collection form designed specifically for the study.

Statistical Analysis

Data were entered and analyzed using IBM SPSS Statistics version 26.0. Quantitative variables were expressed as mean ± standard deviation (SD). Categorical variables were presented as frequencies and percentages. Chi-square test was applied to qualitative variables. A p-value of < 0.05 was considered as statistically significant.

RESULTS

The mean age of patients with empyema thoracis was 44.7 ± 13.4 years, while the mean duration of symptoms prior to presentation was 20.9 ± 7.4 days. Among the 102 patients included in the study, 26 (25.5%) were aged 13–30 years, 46 (45.1%) were between 31–50 years, and 30 (29.4%) were in the 51–70 years age group. There were 70 (68.6%) males and 32 (31.4%) females. Most patients resided in rural areas 60 (58.8%), while 42 (41.2%) were from urban settings. Regarding smoking status, 64 (62.7%) were never smokers, 22 (21.6%) were former smokers, and 16 (15.7%) were current smokers. Right-sided pleural effusion was observed in 62 (60.8%) patients, left-sided in 26 (25.5%), and bilateral effusion in 14 (13.7%). Comorbid conditions included hypertension in 26 (25.5%) patients, diabetes mellitus in 22 (21.6%), asthma in 12 (11.8%), and COPD in 16 (15.7%) (Table-I).

Characteristic	n (%)
Age Group	
13–30 years	26 (25.5)
31–50 years	46 (45.1)
51–70 years	30 (29.4)
Gender	
Male	70 (68.6)
Female	32 (31.4)
Residence	
Rural	60 (58.8)
Urban	42 (41.2)
Smoking Status	
Never Smoker	64 (62.7)
Former Smoker	22 (21.6)
Current Smoker	16 (15.7)
Presence of Pleural Effusion	
Right	62 (60.8)
Left	26 (25.5)
Bilateral	14 (13.7)
Comorbidities	
Hypertension	26 (25.5)
Diabetes Mellitus	22 (21.6)
Asthma	12 (11.8)
COPD*	16 (15.7)

Table-I. Baseline characteristics of patients with empyema thoracis (n = 102)
*** COPD = Chronic Obstructive Pulmonary Disease**

Among the 102 pleural fluid samples analyzed, 56 (54.9%) yielded monomicrobial growth, 18 (17.6%) showed polymicrobial growth, while 28 (27.5%) had no microbial growth (sterile cultures) (Figure-1).

Staphylococcus aureus was isolated in 16 (22.9%) males and 2 (6.3%) females, which was

statistically significant (p = 0.041). Streptococcus pneumoniae was found in 4 (5.7%) males and 4 (12.5%) females (p = 0.237), while Streptococcus pyogenes was isolated in 4 (5.7%) males and 2 (6.3%) females (p = 0.915). Pseudomonas aeruginosa was reported in 6 (8.6%) males and 4 (12.5%) females (p = 0.536), Klebsiella pneumoniae in 6 (8.6%) males and 2 (6.3%) females (p = 0.686), and Escherichia coli in 4 (5.7%) males and 2 (6.3%) females (p = 0.915). Bacteroides was isolated in 8 (11.4%) males and 2 (6.3%) females (p = 0.414), whereas Viridans streptococci were identified in 6 (8.6%) males and 2 (6.3%) females (p = 0.686) (Table-II).

Microorganism	Category	Male n (%)	Female n (%)	P-Value
Staphylococcus aureus	Yes	16 (22.9)	2 (6.3)	0.041*
	No	54 (77.1)	30 (93.8)	
Streptococcus pneumoniae	Yes	4 (5.7)	4 (12.5)	0.237
	No	66 (94.3)	28 (87.5)	
Streptococcus pyogenes	Yes	4 (5.7)	2 (6.3)	0.915
	No	66 (94.3)	30 (93.8)	
Pseudomonas aeruginosa	Yes	6 (8.6)	4 (12.5)	0.536
	No	64 (91.4)	28 (87.5)	
Klebsiella pneumoniae	Yes	6 (8.6)	2 (6.3)	0.686
	No	64 (91.4)	30 (93.8)	
Escherichia coli	Yes	4 (5.7)	2 (6.3)	0.915
	No	66 (94.3)	30 (93.8)	
Bacteroides	Yes	8 (11.4)	2 (6.3)	0.414
	No	62 (88.6)	30 (93.8)	
Viridans streptococci	Yes	6 (8.6)	2 (6.3)	0.686
	No	64 (91.4)	30 (93.8)	

Table-II. Association between gender and microorganisms isolated in patients with empyema thoracis (n = 102)

Chi-square test was applied to evaluate the association between gender and the isolation of specific microorganisms in empyema thoracis patients. A p-value ≤ 0.05 was considered statistically significant. The association between gender and isolation of Staphylococcus aureus was statistically significant (p = 0.041).

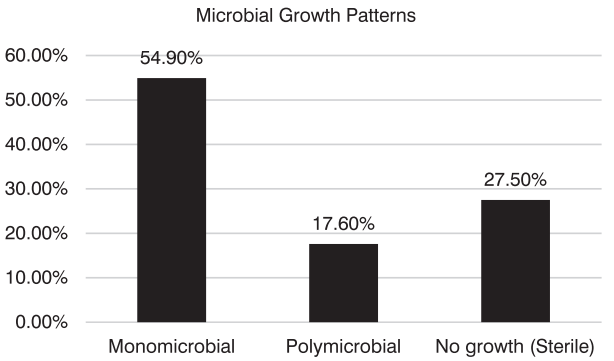

Figure-1. Frequency of microbial growth patterns in pleural fluid samples of patients with empyema thoracis

Among 102 patients with empyema thoracis, Gram-positive organisms were isolated in 40 (39.2%) cases, Gram-negative organisms in 34 (33.3%) cases and no microbial growth in 28 (27.5%) cases (Figure-2).

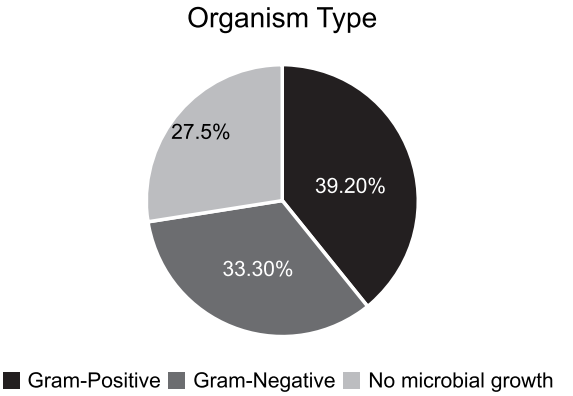

Figure-2. Frequency of organisms type isolated in patients with empyema thoracis

DISCUSSION

This study evaluated the clinical and microbiological profile of patients diagnosed with empyema thoracis at Sheikh Zayed Medical College/Hospital, Rahim Yar Khan, we found that most patients were young-to-middle-aged adults. The mean age of the participants was 44.7 ± 13.4 years, with the majority of cases (45.1%) occurring in the 31–50-year age group. This finding is consistent with the studies conducted by Khan et al. and Khuhawar et al., who reported

mean ages of 41.7 ± 12.9 years among their respective patient populations.[13,14] However, a slightly lower mean age of 39.3 ± 16.6 years was observed in the study by Atif et al., while Hassan et al. reported a higher pooled mean age of 54.4 ± 9.5 years in a systematic review of 10,241 patients.[9,12] In our study, a male predominance (68.6%) was evident, a trend echoed in nearly all previous studies, including those by Salim et al. (76.9%), Khan et al. (67.6%), Chatterjee et al. (66.7%), and Choudhary et al. (71.7%). This male preponderance may reflect higher exposure to occupational and environmental risk factors among males, as noted by Atif et al., who also observed a statistically significant association between male gender ($p = 0.047$).[9,10,13,15]
Rural residency was noted in 58.8% of patients in the present study, similar to the findings by Atif et al. (63.6%). Smoking was also commonly reported, with 37.3% of our participants having a history of smoking, which is closely aligned with Chatterjee et al., who reported a smoking prevalence of 30%.[9,10] This suggests that unlike chronic lung diseases, empyema affects many non-smokers, likely because pneumonia – the usual antecedent of empyema – occurs across the population. Common comorbidities in our series included diabetes mellitus (approximately one-fifth of patients) and chronic lung diseases (such as COPD and bronchiectasis in a minority), comparable to the 22.7% diabetes prevalence reported by Atif et al. These comorbid conditions can impair immunity or lung function and may predispose to complicated pleural infections. Right-sided empyema was more prevalent in our study (60.8%), in agreement with Chatterjee et al. (50%) and Dhal et al., who reported a right-side predominance of 67.6% in pediatric patients.[11] The right lung's larger size and more vertical main bronchus might predispose it to infections and parapneumonic effusions, explaining the slight lateral imbalance.

Culture positivity in this study was observed in 72.5% of cases, comprising 54.9% monomicrobial and 17.6% polymicrobial isolates. These figures are comparable to Atif et al., who reported culture positivity in 52.7%, with 50 monomicrobial and 8 polymicrobial cases.[9] Similarly, Dhal et al. identified 55.2% monomicrobial and 6.7% polymicrobial infections in pediatric empyema.[11] In contrast, Karmakar et al. reported a notably higher culture positivity rate of 87.2%.[16] Such variability likely stems from differences in patient selection and prior antibiotic use, inadequate sampling techniques or omission of anaerobic cultures may further reduce yields. Among the isolates, Gram-positive organisms were detected in 39.2% and Gram-negative organisms in 33.3% of cases. This differs from Salim et al., Choudhary et al., and Karmakar et al., who each reported a dominance of Gram-negative organisms (62.5%, 68.2%, and 73.3%, respectively).+15-17 However, the systematic review by Hassan et al. revealed a balanced distribution, with Gram-positive aerobes accounting for 50.4% and Gram-negative aerobes 37.5%.[12]

Staphylococcus aureus was the most commonly isolated organism in this study (17.6%), predominantly in males ($p = 0.041$), mirroring global data from Hassan et al. (20.7%) and Dhal et al. (36.1%).[11,12] MRSA strains were noted in both Karmakar et al. and Dhal et al., suggesting increasing resistance trends.[16] Streptococcus pneumoniae was isolated in 7.8% of cases in this study, which is consistent with findings by Khan et al. (4.1%), Khuhawar et al. (4.4%), and Hassan et al. (10.8%).[12-14] Streptococcus pyogenes was also isolated in 5.9%, closely matching the rates seen in Chatterjee et al. (6.7%).[10] Among Gram-negative pathogens, Pseudomonas aeruginosa was reported in 9.8% of patients, comparable to Atif et al. (18.8%), Salim et al. (33.3%), and Karmakar et al. (60%). Although variation exists, Pseudomonas consistently appears as a significant nosocomial isolate.[9,15,16] Klebsiella pneumoniae (7.8%) and Escherichia coli (5.9%) were also frequently isolated, consistent with prior findings: Salim et al. reported Klebsiella in 12.5% and E. coli in 8.3%, while Karmakar et al. identified both in approximately 10–12% of isolates.[15,16] These bacteria were often associated with multidrug resistance, as noted by Karmakar et al. and Chatterjee et al. Bacteroides was present in 9.8% of the current cases, similar to Khan et al. (6.8%) and Khuhawar et al. (7.5%), suggesting that anaerobic organisms contribute significantly

to the microbiological landscape of empyema. Viridans streptococci, detected in 7.8%, are also supported by Hassan et al. (18.7%) and Khan et al. (1.8%).[12,13] Their role in polymicrobial infections, especially in community-acquired cases, has been emphasized in prior studies.

This study provides updated, region-specific data on the microbiological spectrum of empyema thoracis, contributing valuable insights for empirical antibiotic protocols. A major strength is the inclusion of a wide range of microorganisms with gender-wise analysis. However, limitations include the single-center design, lack of anaerobic culture facilities, and possible underestimation of tuberculous empyema due to limited molecular testing.

CONCLUSION

This study highlights that empyema thoracis predominantly affects middle-aged males from rural backgrounds, with right-sided involvement being more common. Monomicrobial infections were more frequent than polymicrobial or sterile samples. Gram-positive organisms, particularly Staphylococcus aureus, were the most commonly isolated pathogens, showing a significant gender association. Future studies should adopt multicenter designs, incorporate molecular diagnostics and anaerobic cultures, and evaluate treatment outcomes across microbial categories. Such research can refine management protocols and enhance microbiological surveillance in resource-limited settings like Pakistan.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1. Zettinig D, D'Antonoli TA, Wilder-Smith A, Bremerich J, Roth JA, Sexauer R. **Diagnostic accuracy of imaging findings in pleural empyema: systematic review and meta-analysis.** J Imaging. 2022; 8(1):3. doi:10.3390/jimaging8010003
2. Tobler M. **Empyema Imaging: Practice essentials, radiography, computed tomography [Internet].** Medscape; 2019 [cited 2025 Mar 30]. Available from: https://emedicine.medscape.com/article/355892-overview
3. Ullah H, Ahmed H, Salman A, Iqbal R, Hussaini SJ, Malikzai A. **The rising tide of tuberculosis in Pakistan: factors, impact, and multi-faceted approaches for prevention and control.** Health Sci Rep. 2024; 7(10):e70130. doi:10.1002/hsr2.7013
4. Presti T, Asghar A, Ravikumar N. **Management of pleural infection: A historical review and updates.** J Respir. 2024; 4(2):112-27. doi:10.3390/jrespir4020010
5. Muraji T. **Thoracic empyema. In: Operative General Surgery in Neonates and Infants [Internet].** 2016 [cited 2025 Mar 30]; 125-27. Available from: https://www.ncbi.nlm.nih.gov/books/NBK544279/
6. Uddin TM, Chakraborty AJ, Khusro A, Zidan BRM, Mitra S, Emran TB, et al. **Antibiotic resistance in microbes: History, mechanisms, therapeutic strategies and future prospects.** J Infect Public Health. 2021; 14(12):1750-66. doi:10.1016/j.jiph.2021.10.020
7. Kunisaki SM, Leys CM. **Surgical pulmonary and pleural diseases in children: Lung malformations, empyema, and spontaneous pneumothorax.** Adv Pediatr. 2020; 67:145-69. doi:10.1016/j.yapd.2020.04.006
8. Sorino C, Mondoni M, Lococo F, Marchetti G, Feller-Kopman D. **Optimizing the management of complicated pleural effusion: from intrapleural agents to surgery.** Respir Med. 2022; 191:106706. doi:10.1016/j.rmed.2021.106706
9. Atif M, Naseem M, Sarwar S, Mukhtar S, Malik I, Hassan MRU, et al. **Spectrum of microorganisms, antibiotic resistance pattern, and treatment outcomes among patients with empyema thoracis: A descriptive cross-sectional study from the Bahawal Victoria Hospital Bahawalpur, Punjab, Pakistan.** Front Med. 2021; 8:715033. doi:10.3389/fmed.2021.715033
10. Chatterjee M, Islam N, Rasel MH, Faruque F, Ashraf MA, Saha D, et al. **Bacteriological etiology of empyema thoracis patients admitted in a tertiary care hospital.** Bangladesh J Med. 2024; 35(1):187-94.
11. Dhal S, Champatiray JR, Mohanty DP, Mishra L, Dehury MK, Mishra DN. **Microbiological study of empyema thoracis among children in a tertiary care hospital.** Asian J Med Sci. 2023; 14(7):105-11. doi:10.3126/ajms.v14i7.55555

12. Hassan M, Cargill T, Harriss E, Asciak R, Mercer RM, Bedawi EO, et al. **The microbiology of pleural infection in adults: A systematic review.** Eur Respir J. 2019; 54(3):1900542. doi:10.1183/13993003.00542-2019

13. Khan S, Yasin M, Muhammad M, Tareen S, Adeel M, Jan F. **Culture and sensitivity patterns of the causative organisms isolated from the patient of empyema thoracis.** Pak J Chest Med. 2021; 27(2):80-6.

14. Khuhawar SM, Kumar J, Sanghro KA, Chandio BA, Kukreja M. **Culture and sensitivity patterns of the causative organisms isolated from the patient of empyema thoracis.** J Popul Ther Clin Pharmacol. 2024; 31(3):2152-6. doi:10.47750/jptcp.2024.31.3.19

15. Salim MU, Dilawar SMU, Rehman ST. **Frequency of bacteriological organism in patients with thoracic empyema.** Pak J Med Health Sci. 2021; 15(6):1914-6.

16. Karmakar S, Prasad R, Kant S, Nath A, Mahdi F. **Clinical and microbiological characteristics of thoracic empyema: Retrospective analysis in a tertiary care centre.** Int J Adv Med. 2017; 4(5):1309-14. doi:10.18203/2349-3933.ijam20174238

17. Choudhary B, Choudhary P, Srivastava D, Bajiya VS. **Microbiological prevalence in empyema thoracis in a tertiary care centre in North India.** Int J Acad Med Pharm. 2024; 1(1):182-8.

AUTHORSHIP AND CONTRIBUTION DECLARATION	
1	**Iqra Shaheen:** Conception and design of study, methodology, data collection, and final approval of the manuscript.
2	**Imran Bashir:** Study design methodology, data collection, and final approval of the manuscript.
3	**Mian Muhammad Riaz Qadeer:** Data analysis, methodology, interpretation and final approval of the manuscript.
4	**Muhammad Irfan Jamil:** Data analysis, results formulation, discussion writing and final approval.
5	**Muhammad Shahid Nawaz Khan:** Data analysis, methodology, interpretation and final approval of the manuscript.
6	**Adeel Ahmed:** Study design methodology, data collection, and final approval of the manuscript.