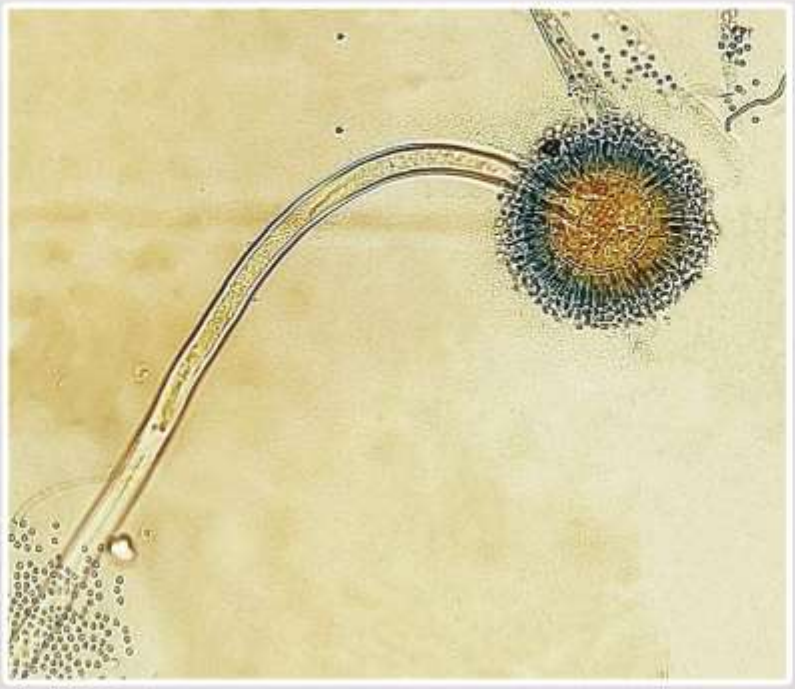
ISSN 1027-0299

INFECTIOUS DISEASES JOURNAL OF PAKISTAN | IDJP

An official journal of
Medical Microbiology & Infectious Diseases
Society of Pakistan



Quarterly

Vol. 33, No. 2, Apr - Jun 2024

Indexed with Indexus Medicus of WHO (IMEMR), EBSCO Host, PASTIC, PakMedinet, Registered with International Standard Serial Number (ISSN-France), Approved by College of Physicians and Surgeons Pakistan (CPSP) Karachi, Recognized by the Pakistan Medical and Dental Council (PM&DC), and Higher Education Commission (HEC) in Category-Y

https://ojs.idj.org.pk/index.php/Files/index

INFECTIOUS DISEASES JOURNAL OF PAKISTAN | IDJP

Vol. 33, No. 2, Apr – Jun 2024

An Official Journal of Medical Microbiology & Infectious Diseases Society of Pakistan

Approved by College of Physicians and Surgeons Pakistan (CPSP) Karachi
Recognized by the Pakistan Medical and Dental Council (PM&DC),
and Higher Education Commission (HEC) in Category-Y

INFECTIOUS DISEASES JOURNAL OF PAKISTAN | IDJP

Vol. 33, No. 2, Apr – Jun 2024

An Official Journal of Medical Microbiology & Infectious Diseases Society of Pakistan

Approved by College of Physicians and Surgeons Pakistan (CPSP) Karachi

Recognized by the Pakistan Medical and Dental Council (PM&DC),

and Higher Education Commission (HEC) in Category-Y

CONTENTS

ORIGINAL ARTICLE

Understanding secondary bloodstream infections in COVID patients: Insights from Karachi, Pakistan

Fareeha Adnan, Nazia Khursheed, Adeel Zafar Nagra, Nida Ghori, Qurat ul Ain Zahid, Moiz Ahmed Khan

Indus Hospital and Health Network, Karachi Pakistan

ABSTRACT

Background: Nosocomial Bloodstream infection (BSI) in COVID patients is an emerging clinical concern for physicians. It can lead to sepsis resulting in rise in morbidity and mortality. In this study, we aimed to assess the prevalence of BSI in COVID patients admitted to Indus Hospital and Health Network in Karachi, Pakistan.

Material and Methods: This retrospective study included the Reverse-transcriptase polymerase chain reaction (RT-PCR) confirmed COVID patients from March 2020 to December 2021. Data of all the patients (n=961) was obtained from electronic medical record of the hospital which included information regarding demographics, BSI, Central line-associated blood stream infections (CLABSI), frequency of pathogens, antimicrobial resistance pattern and clinical outcome.

Results: Our data showed that 217 (22.6%) patients developed BSI from which 44.2% had CLABSI. BSI was higher in males than females (61.8% vs 38.2%) and most patients were 51-64 years of age (n=66, 30.41%). Infections with Gram-negative bacteria were predominant (46.7%), followed by Gram-positive bacteria (17.9%) and yeasts (7.2%). Among the isolates, *Acinetobacter spp.* were the most commonly identified pathogen (17%). Regarding multi drug-resistant organisms, Carbapenem-resistant *Acinetobacter baumanii (CRAB)* was the most frequently isolated (n=37), followed by Methicillin-resistant *S. aureus (MRSA) (*n=16), Carbapenem-resistant *E. coli (CRE) (*n=10), Carbapenem-resistant *Klebsiella spp. (CRE) (*n=8), Vancomycin-resistant *Enterococcus (VRE) (*n=8) and multi drug-resistant *Pseudomonas spp. (*n=3). The mortality among CLABSI in different age groups ranged from 80% to 100% while in BSI ranged from 52.38% to 92.3%.

Conclusion: In order to prevent nosocomial infections from spreading and enhance the prognosis of hospitalized COVID patients, early detection of secondary infections and adherence to appropriate infection control measures are essential.

Keywords: Bloodstream infections, BSI, COVID-19, CLABSI, Multidrug resistance

BACKGROUND

The inception of COVID-19 pandemic posed an intimidating challenge to healthcare systems around the globe.[1-4] COVID patients often need hospitalization and some patients may need intensive care management depending upon the disease severity. ICUs have often been overburdened by COVID patients increasing their susceptibility to developing secondary bacterial and fungal infections especially in patients requiring mechanical ventilation.[5-7]

Bacteria can complicate viral respiratory infections by co-infecting and colonizing respiratory epithelium leading to high morbidity and mortality.[8,9] Sepsis can occur in patients with superimposed bacterial infections which can be prevented by adopting specific measures. Sepsis and COVID-19 share clinical presentations of the disease that include tachycardia, thrombocytopenia, hemolytic anemia, vascular microthrombosis,[10] multiorgan dysfunction syndrome,[11] coagulopathy,[12] septic shock, respiratory failure, fever, leukopenia, hypotension,[13] leukocytosis, high predisposition to opportunistic infections and cytokine storm leading to Systemic inflammatory response syndrome (SIRS). [14,15] Even though several studies have reported secondary bacterial and nosocomial infections in COVID patients, the results are contradictory. However, to date, only a few studies have focused on Bloodstream infections

Correspondence: Dr. Fareeha Adnan, Consultant Microbiology, Microbiology Section, Department of Pathology, Indus Hospital & Health Network, Karachi, Pakistan

Email: fareeha.adnan@tih.org.pk

This article can be cited as: Adnan F, Khursheed N, Nagra AZ, Ghori N, Zahid QA, Khan MA. Understanding secondary bloodstream infections in COVID patients: Insights from Karachi, Pakistan. Infect Dis J Pak. 2024; 33(2): 52-56. DOI: https://doi.org/10.61529/idjp.v33i2.288

Receiving date: **26 Jan 2024** Acceptance Date: **13 May 2024**
Revision date: **30 Mar 2024** Publication Date: **30 Jun 2024**

(BSI) in COVID patients admitted in ICU.[7,16] Approximately 5.2% of ICU-admitted patients are known to develop BSI.[17]

In order to curtail the spread of multi drug-resistant organisms (MDRO) and BSI in the hospital setting, it is essential to have knowledge regarding the burden and outcome of such infections. Hence, the rationale of this study is to assess the prevalence, frequency, and distribution of microorganisms, antimicrobial susceptibility, and clinical outcomes in COVID patients with BSI.

MATERIAL AND METHODS

This retrospective study was carried out at the Indus Hospital and Health Network, Karachi, Pakistan and include COVID patients admitted from March 2020 to December 2021. All patients with confirmed COVID-19 (RT-PCR positive in nasopharyngeal swab samples) were included. A total of 971 blood cultures were enrolled from patients who were admitted for at least 48 hours in the hospital. BSI was defined by the presence of bacterial or fungal organisms in blood demonstrated by the positivity of one or more blood cultures. Moreover, Nosocomial BSI was defined as BSI acquired 48 hours after admission to the hospital. Information regarding patient demographics, antibiotic-sensitivity patterns and outcome measures were collected from electronic media record of the hospital. Contaminated blood samples were excluded from the study. Ethical approval was obtained from the 'Institutional Review Board of the Indus Hospital & Health Network' for the purpose of this study (Ref # IHHN_IRB_2021_12_010).

Blood culture samples were processed according to the standard operating procedures. Isolates were identified on the basis of biochemical reactions and API or Vitek. Central line-associated bloodstream infection (CLABSI) was defined as laboratory confirmed BSI not related to infection at any other site and which developed within 48 hours of central line placement. Carbapenem-resistant Enterobacterale (CRE) was defined with the resistance to any one of the drugs in carbapenem class of antibiotics. Multi drug-resistant organism (MDRO) was defined as an organism with acquired resistance to at least one agent in three or more classes of antimicrobial drugs.

Statistical analysis was carried out using IBM Statistical Package for Social Sciences (SPSS) software version 26. The distribution of data was analyzed by Shapiro Wilk test. Percentages and frequencies were calculated for categorical variables which included age, gender, mortality, BSI, CLABSI and frequency of pathogens. Chi-square test was applied to see any association between BSI, CLABSI and mortality among different age groups and gender. A p value of <0.05 was considered as significant.

RESULTS

During the study period (March 2020 to December 2021), 971 RT-PCR-confirmed COVID patients were admitted to our hospital. Overall, 22.6% (n=217) of hospitalized COVID patients developed BSI while the remaining 77.4 % (n=744) of patients did not develop any BSI, as shown in figure 1. Ten patients were excluded from the study population as their clinical samples were characterized as contaminated hence, the total study population comprised of 961 patients. Among BSI patients, 44.2% (n=96) developed CLABSI.

Patient demographics are summarized in Table-I. Percentage of male patients was greater (61.8%; n=134) than female patients (38.2%; n=83) in BSI group. However, there was no significant difference in mortality with regards to patient's gender in both groups (p=0.6). Patient age groups of 51-64 years and ≥65 years showed the highest frequency of BSI (30.4% and 29%). No statistical significance was observed with regards to difference in mortality among age groups in the BSI and CLABSI groups.

Out of 217 BSI patients, more than one pathogen was found in 41 patients. Most commonly identified pathogens among BSI patients were Gram-negative bacteria (46.7%), followed by Gram-positive bacteria (17.9%) and yeast (7.2%). Coagulase-negative *Staphylococcus spp.* (28%; 72/256) were the most commonly isolated, followed by *Acinetobacter spp.* (17%; 43/ 256), *Staphylococcus aureus* (10%; 26/256), *E. coli* (8%; 20/256), *Pseudomonas spp.* (8%; 20/256), *Klebsiella spp.* (7%; 19/256), *Enterococcus spp.* (7%; 18/256), *Candida spp.* (6%; 16/256) and others (9%; 22/256).

Among the isolated MDROs, Carbapenem-resistant *Acinetobacter baumanii (CRAB)* (37/43) was the most

frequently isolated, followed by Methicillin-resistant *S. aureus (MRSA)* (16/26), Carbapenem-resistant *E. coli (CRE)* (10/20), Carbapenem-resistant *Klebsiella spp. (CRE)* (8/19), Vancomycin-resistant *Enterococcus (VRE)* (8/18) and multi drug-resistant *Pseudomonas spp.* (3/20).

Table-I: Demographics of COVID patients with BSI admitted in the Indus Hospital (Mar 2020 – Dec 2021).

Gender	Total BSI (n=217)	CLABSI (n=96)	BSI (n=121)	p-value	Mortality among BSI		Median (IQR)	Mortality among CLABSI		p-value
					Alive	Expired		Alive	Expired	
Male	134 (61.8%)	58 (60.4%)	76 (62.8%)	0.70	20 (16.52%)	56 (46.28%)		9 (9.37%)	49 (51.04%)	0.13
Female	83 (38.2%)	38 (38.6%)	45 (37.2%)		15 (12.39%)	30 (24.79%)		8 (8.33%)	30 (31.25%)	0.21
Age Group 1-18 years	26 (11.98%)	5 (5.20%)	21 (17.35%)	0.02	10 (47.61%)	11 (52.38%)	8 (3-11.25)	0	5 (100%)	0.12
Age Group 19-40 years	28 (12.90%)	15 (15.62%)	13 (10.74%)		1 (7.69%)	12 (92.30%)	35 (24.25-38)	3 (20%)	12 (80%)	0.35
Age Group 41-50 years	34 (15.66%)	20 (20.83%)	14 (11.57%)		6 (42.85%)	8 (57.14%)	47 (44.75-49)	4 (20%)	16 (80%)	0.15
Age Group 51-64 years	66 (30.41%)	31 (32.29%)	35 (28.92%)		8 (22.85%)	27 (77.14%)	57.50 (54.75-60)	5 (16.12%)	26 (83.87%)	0.49
Age Group ≥65 years	63 (29.03%)	25 (26.04%)	38 (31.40%)		10 (26.31%)	28 (73.64%)	70 (65-73)	5 (20%)	20 (80%)	0.56

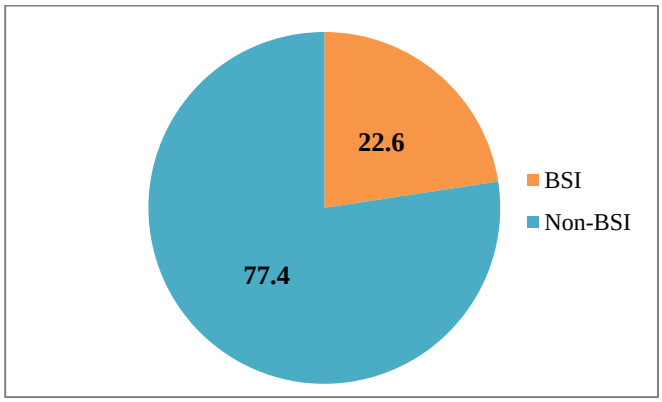


Figure-I: Percentage of COVID patients (n=971) diagnosed with BSI.

DISCUSSION

BSIs are among the significant complications of COVID that worsen the clinical course of disease and increase associated mortality. In our study, it was found that 22.6% of COVID patients developed BSI. However, a major proportion (26.9%) of the identified organisms comprised of coagulase-negative *Staphylococcus spp. (*CoNS), which are skin commensals. Identification of a high percentage of CoNS from blood of hospitalized COVID patients might indicate negligence of adequate skin disinfection before collection of blood cultures. Additionally, the high influx of patients and shortage of critical care resources and relevant healthcare staff might compromise the standards of infection control. A high percentage (15.2%) of CoNS among COVID patients was also reported in a study by Zeno P. *et al*.[18] In our study, 44.2% patients had CLABSI while Kang *et al*. reported 38% of COVID patients with CLABSI.[19] We found a relatively higher prevalence of Gram-negative bacterial infections. Similar observations were reported by Naveenraj P. *et al*. (82.8%)[20] and Vijay S. *et al*. (78%).[21] In contrast, other studies have reported a higher prevalence of Gram-positive pathogens among COVID patients admitted to ICU, varying in prevalence from 44% to 77.6%.[22] This diversity in predominance and frequency of microorganisms may be due to difference in patient set-ups, length of hospital stays, number of patients on mechanical ventilation, and identification of pathogens from different samples such as pus, urine, and respiratory samples.

Studies focusing on secondary infections and BSI in COVID patients have reported a high frequency of *Acinetobacter spp.* We have also observed a relatively higher percentage of *Acinetobacter spp.* (16%) among all the isolated pathogens. Likewise, Montrucchio G *et al*., also reported a higher percentage of *Acinetobacter spp.* among all other isolates in COVID patients admitted in ICU (32.8%).[23] *Acinetobacter spp.* were followed by *Klebsiella spp., E. coli, Pseudomonas spp.*, and *Enterococcus spp.* These pathogens have

increasingly been identified in cases of bacteremia by other studies as well.[24]

We also observed increased mortality ranging from 52% to 100% in COVID patients with BSI and CLABSI admitted in ICU. One of the reasons for increase in mortality rate among these patients may be due to the synergetic effect of the virus and bacteria. Although it is not clear to what extent the burden of mortality could be attributable to infection in our scenario, certain other factors like patient's clinical status, co-morbidities, length of ICU stay should not be undermined. Our findings were consistent with studies conducted in Turkey.[24]

Previously, other studies have reported infections with MDROs among hospitalized COVID patients. Our results also show a high percentage of MDROs in COVID patients. MDRO infections in COVID patients could be attributed to prolonged hospital stay, mechanical ventilation, empirical use of antibiotics, and poor compliance with infection control practices. Moreover, unintended use of carbapenems due to antimicrobial selection pressure, may contribute to these infections. The use of antibiotic combinations in COVID patients also increases the risk of antibiotic resistance.[25] The following factors may attribute to the development of BSI in hospitalized COVID patients:

1) COVID patients are immunocompromised and prone to secondary infections. There are two reasons for the dysregulated immune system. Increased production of cytokines due to viral attacks and a significant decrease in the production of IFN-Ɣ leads to reduction in CD4+ T-cells polarization and cytotoxic activity.
2) Prolonged hospital and ICU stay increases the likelihood of acquiring nosocomial infections among COVID patients.
3) Increased use of immunosuppressive agents such as anti-IL-6 drugs and corticosteroids.

LIMITATIONS

Our study has a few limitations. Firstly, it is a single-center study and hence, data regarding frequency of BSI and prevalence of particular pathogens may represent prevalence in our setting only and may not be generalizable to other healthcare setups in our country. Further multi-center studies are required to accurately assess the scale of BSI and predominant pathogens in the Pakistani population. Secondly, we did not ascertain whether the burden of morbidity and moratlity discussed in our study was associated with any of the factors other than infections such as patient's clinical status, co-morbidities and length of ICU stay. Nonetheless, our data regarding the organisms isolated and the percentage of nosocomial BSI is robust and provide valuable insights from our part of the world.

CONCLUSION

In order to prevent nosocomial infections from spreading and enhance the prognosis of hospitalized COVID patients, early detection of secondary infections using suitable biomarkers and adherence to appropriate infection control measures are essential. Moreover, in order to curtail the ongoing emergence of multi-drug resistant pathogens, it is critical to reinforce proper antimicrobial stewardship practices on a national level.

CONFLICT OF INTEREST

None

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHOR CONTRIBUTION

Fareeha Adnan, Nazia Khursheed: Conceptualization, Writing, Methodology and Overall supervision

Adeel Zafar Nagra, Nida Ghori, Qurat Ul Ain Zahid: Writing and Data analysis

Moiz Ahmed Khan: Writing and Revisions

REFERENCES

1. Eastin C, Eastin T, Guan W, Ni Z, Hu Y. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med. 2019;58(4):711–2. DOI: http://dx.doi.org/10.1056/NEJMoa2002032
2. Lupia T, Scabini S, Pinna SM, Di Perri G, De Rosa FG, Corcione S. 2019 novel coronavirus (2019-nCoV) outbreak: A new challenge. J Glob Antimicrob Resist. 2020; 21: 22–7. DOI: http://dx.doi.org/10.1016/j.jgar.2020.02.021
3. Wu Z, McGoogan JM. Characteristics of and important lessons from the Coronavirus disease 2019 (COVID-19) outbreak in China: Summary of a report of 72 314 cases from the Chinese center for disease control and prevention: Summary of a report of 72 314 cases from the Chinese center for disease control and prevention. JAMA. 2020; 323(13): 1239–42. DOI: http://dx.doi.org/10.1001/jama.2020.2648
4. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, *et al*. Clinical course and risk factors for mortality of adult inpatients

with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020; 395(10229): 1054–62. DOI: http://dx.doi.org/10.1016/S0140-6736(20)30566-3

5. Luyt CE, Sahnoun T, Gautier M, Vidal P, Burrel S, Pineton de Chambrun M, *et al*. Ventilator-associated pneumonia in patients with SARS-CoV-2-associated acute respiratory distress syndrome requiring ECMO: A retrospective cohort study. Ann Intensive Care. 2020; 10(1): 158. DOI: http://dx.doi.org/10.1186/s13613-020-00775-4
6. Rouzé A, Martin-Loeches I, Povoa P, Makris D, Artigas A, Bouchereau M, *et al*. Relationship between SARS-CoV-2 infection and the incidence of ventilator-associated lower respiratory tract infections: A European multicenter cohort study. Intensive Care Med. 2021; 47(2): 188–98. DOI: http://dx.doi.org/10.1007/s00134-020-06323-9
7. Buetti N, Ruckly S, de Montmollin E, Reignier J, Terzi N, Cohen Y, *et al*. COVID-19 increased the risk of ICU-acquired bloodstream infections: a case-cohort study from the multicentric OUTCOMEREA network. Intensive Care Med. 2021; 47(2): 180–7. DOI: http://dx.doi.org/10.1007/s00134-021-06346-w
8. McCullers JA. The co-pathogenesis of influenza viruses with bacteria in the lung. Nat Rev Microbiol. 2014; 12(4): 252–62. DOI: http://dx.doi.org/10.1038/nrmicro3231
9. Metzger DW, Sun K. Immune dysfunction and bacterial coinfections following influenza. J Immunol. 2013; 191(5): 2047–52. DOI: http://dx.doi.org/10.4049/jimmunol.1301152
10. Morris DE, Cleary DW, Clarke SC. Secondary bacterial infections associated with influenza pandemics. Front Microbiol. 2017; 8: 1041. DOI: http://dx.doi.org/10.3389/fmicb.2017.01041
11. Chang JC. TTP-like syndrome: Novel concept and molecular pathogenesis of endotheliopathy-associated vascular microthrombotic disease. Thromb J. 2018;16(1). DOI: http://dx.doi.org/10.1186/s12959-018-0174-4
12. Dempfle CE. Coagulopathy of sepsis. Thromb Haemost. 2004; 91(2): 213-24.
13. Gauer RL. Early recognition and management of sepsis in adults: the first six hours. Am Fam Physician. 2013; 88(1): 44–53.
14. Sepulveda J, Westblade LF, Whittier S, Satlin MJ, Greendyke WG, Aaron JG, *et al*. Bacteremia and blood culture utilization during COVID-19 surge in New York City. J Clin Microbiol. 2020; 58(8). DOI: http://dx.doi.org/10.1128/JCM.00875-20
15. Rothan HA, Byrareddy SN. The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. J Autoimmun. 2020; 109(102433): 102433. DOI: http://dx.doi.org/10.1016/j.jaut.2020.102433
16. Giacobbe DR, Battaglini D, Ball L, Brunetti I, Bruzzone B, Codda G, *et al*. Bloodstream infections in critically ill patients with COVID-19. Eur J Clin Invest. 2020;50(10):e13319. DOI: http://dx.doi.org/10.1111/eci.13319
17. Prowle JR, Echeverri JE, Ligabo EV, Sherry N, Taori GC, Crozier TM, *et al*. Acquired bloodstream infection in the intensive care unit: Incidence and attributable mortality. Crit Care. 2011; 15(2): R100. DOI: http://dx.doi.org/10.1186/cc10114
18. Pasquini Z, Barocci I, Brescini L, Candelaresi B, Castelletti S, Iencinella V, *et al*. Bloodstream infections in the COVID-19 era: Results from an Italian multi-center study. Int J Infect Dis. 2021; 111: 31–6. DOI: http://dx.doi.org/10.1016/j.ijid.2021.07.065
19. Kang M, Conrad Z, Thomas E, Arocha D, Trivedi J. Central-line associated bloodstream infection (CLABSI) in patients hospitalized with COVID-19. Antimicrob Steward Healthc Epidemiol. 2022;2(S1): s32–s32. DOI: http://dx.doi.org/10.1017/ash.2022.116
20. Palanisamy N, Vihari N, Meena DS, Kumar D, Midha N, Tak V, *et al*. Clinical profile of bloodstream infections in COVID-19 patients: A retrospective cohort study. BMC Infect Dis. 2021; 21(1): 933. DOI: http://dx.doi.org/10.1186/s12879-021-06647-x
21. Vijay S, Bansal N, Rao BK, Veeraraghavan B, Rodrigues C, Wattal C, *et al*. Secondary infections in hospitalized COVID-19 patients: Indian experience. Infect Drug Resist. 2021; 14: 1893–903. DOI: http://dx.doi.org/10.2147/idr.s299774
22. Temperoni C, Caiazzo L, Barchiesi F. High prevalence of antibiotic resistance among opportunistic pathogens isolated from patients with COVID-19 under mechanical ventilation: Results of a single-center study. Antibiotics (Basel). 2021; 10(9): 1080. DOI: http://dx.doi.org/10.3390/antibiotics10091080
23. Montrucchio G, Corcione S, Lupia T, Shbaklo N, Olivieri C, Poggioli M, *et al*. The burden of carbapenem-resistant Acinetobacter baumannii in ICU COVID-19 patients: A regional experience. J Clin Med. 2022; 11(17): 5208. DOI: http://dx.doi.org/10.3390/jcm11175208
24. Kurt AF, Mete B, Urkmez S, Demirkiran O, Dumanli GY, Bozbay S, *et al*. Incidence, risk factors, and prognosis of bloodstream infections in COVID-19 patients in intensive care: A single-center observational study. J Intensive Care Med. 2022; 37(10): 1353–62. DOI: http://dx.doi.org/10.1177/08850666221103495
25. Bhargava A, Riederer K, Sharma M, Fukushima EA, Johnson L, Saravolatz L. High rate of multidrug-resistant organisms (MDROs) among COVID-19 patients presenting with bacteremia upon hospital admission. Am J Infect Control. 2021; 49(11): 1441–2. DOI: http://dx.doi.org/10.1016/j.ajic.2021.08.010

ORIGINAL ARTICLE

Analysis of COVID-19 vaccine type and adverse effects following complete vaccination

Sarah Mansoor[1], Muhammad Hashim Nawaz[2], Hira Butt[1], Ayesha Chheena[1], Amina Zahid[1], Maham Nasir[1]

[1]College of Dentistry, Sharif Medical and Dental College, Lahore Pakistan
[2]3 Crown Dental Center, Lahore Pakistan

ABSTRACT

Backgrounds: Immunization is one of the most successful and cost-effective health interventions to prevent infectious diseases. The launch of the COVID-19 vaccine rollout in December 2020 was a landmark for overcoming this pandemic crisis. It is therefore very important to get most of the population vaccinated as definitive cure still does not exist and people must take all required doses on time as required to reach full immunity. To find the association between covid-19 vaccine type and adverse effects following complete vaccination.

Material and Methods: This Cross-sectional study was conducted in Sharif Medical and Dental College, Lahore over a period of one year from January 2022 to January 2023 on a total of 100 individuals. All participants irrespective of their age and gender were included in the study. Participants with any other systemic illness or those on medication for other illnesses were excluded from the study. Data was collected using a pre-validated questionnaire

Results: The association between the type of vaccine and post-vaccination symptom were significant for fever (p=0.018) and cough (p=0.015).

Conclusion: Fever and cough were most prevalent among individuals who received Sinovac. Most of the symptoms were more prevalent in Sinovac recipients as compared to Sinopharm. Post-vaccination COVID-19 infection was also more prevalent in those who received Sinovac.

Keywords: COVID-19 infection, COVID-19 vaccination, Side effects

BACKGROUND

COVID 19 or mostly known as Corona virus pandemic was first detected in Wuhang, China in December 2019 and later spread to more than 150 countries, leaving people economically and socially devastated.[1] WHO declared it a pandemic in January 2020. COVID-19 caused by SARS-CoV-2 is the deadliest communicable healthcare outbreak of the 21st century effecting 500 million people leaving 6.2 million dead as of April 17,2022.[1]

Immunization is one of the most successful and cost-effective health interventions to prevent infectious diseases.[2] The launch of the COVID-19 vaccine rollout in December 2020 was a landmark for overcoming this pandemic crisis. All Covid19 vaccines are approved by WHO after clinical trials and are proven to be effective and safe.[3] It is therefore very important to get most of the population vaccinated as definitive cure still does not exist and people must take all required doses on time as required to reach full immunity.[4]

Pakistan has a population of 184,404,791 out of which 136,187,729 (73.9%) have received their first dose of vaccine and 124,721,404 (67.6%) second dose. Currently available vaccines in Pakistan are Pfizer, Moderna, Astra Zeneca, Sinovac and Sinopharm, and Sputnik[5]. Any available vaccine can be used as a booster dose[6]. Following inoculation with the Sinopharm or Sinovac vaccines, individuals may experience a spectrum of side effects, ranging from mild to moderate in intensity[7]. These symptoms typically manifest within a few hours to days after vaccination and subside spontaneously within a short duration.[8] Now, turning to Sinopharm and its associated adverse reactions, let's provide some details about the Sinopharm BIBP COVID-19 vaccine, also known as BBIBP-CorV.[9] This vaccine, developed by Sinopharm Beijing Institute of Biological Products, is one of two whole inactivated virus COVID-19 vaccines produced by Sinopharm.[9] It is important to emphasize the features of the Sinovac vaccination as we move our attention to it and its negative effects after delivery[10]. Sinovac Biotech, a

Correspondence: Dr. Hira Butt, Demonstrator, Oral Pathology Department, College of Dentistry, Sharif Medical and Dental College, Lahore Pakistan

Email: hira.ah.butt@gmail.com

This article can be cited as: Mansoor S, Nawaz MH, Butt H, Chheena A, Zahid A, Nasir M. Analysis of COVID-19 vaccine type and adverse effects following complete vaccination. Infect Dis J Pak. 2024; 33(2): 57-62.
DOI: https://doi.org/10.61529/idjp.v33i2.266

Receiving date: **20 Feb 2023** Acceptance Date: **01 Apr 2024**
Revision date: **26 Mar 2024** Publication Date: **28 Jun 2024**

Chinese business, created CoronaVac, also known as the Sinovac COVID-19 vaccine, which is a full inactivated virus COVID-19 vaccine.[11] Sinovac is not required to be frozen, in contrast to many other vaccinations, and it can be stored at 2 to 8 degrees Celsius (35.6 to 46.4 degrees Fahrenheit).[12]

Some people have side effects from the vaccine, which are normal signs that their body is building protection. These side effects may affect their ability to do daily activities, but they go away in a few days. Some people have no side effects, and allergic reactions are rare.[7] Major complaints from COVID-19 Vaccine are a combination of pain, redness or swelling (where the shot was given) fever, fatigue, headache, muscle pain, chills, joint pain, nausea, vomiting, swollen lymph nodes, feeling unwell.[5] Most of the symptoms can likely be attributed simply to exuberant production of a cytokine that plays a vital role in potentiating early stages of the immune response, namely, type I interferon (IFN-I).[5]

The aim of this study was to find the association between covid-19 vaccine type and adverse effects following complete vaccination.

MATERIAL AND METHODS

A cross-sectional study was conducted in Sharif Medical and Dental College, Lahore over a period of one year from January 2022 to January 2023. The sample size was calculated to be 100, keeping a precision of 5%, confidence level 95% and prevalence of adverse effects after receiving COVID 19 vaccination to be 7%[13] using an online sample size calculator Scalex Sp 1.0.01.[14] Sampling technique used was convenient sampling. All participants irrespective of their age and gender were included in the study. Participants with any other systemic illness or those on medication for other illnesses were excluded from the study. Data was collected after obtaining ethical clearance from ethical committee of Sharif Medical Research Centre (No. SMDC/SMRC/270-22). Informed consent was taken from the participants before data collection. Data was collected using a pre-validated questionnaire with a Cronbach alpha value of 0.7.[15] It was divided into section 1 which included demographic characteristics (3-items) and section 2 COVID 19 vaccine side effects (30-items) with binary responses. Recorded data was coded and entered using SPSS statistical package version 23. p value ≤ 0.05 was taken as significant. Fisher exact test was used to find the association between development of infection post vaccine and the type of vaccine. Chi square test was used to find the statistical association of the type of vaccination used with side effects of vaccination i.e tiredness and fatigue, decreased sleep quality, fever, headache, haziness and lack of vision, pain at injection site, dizziness, sleepiness and dry throat. Fisher exact test was used to find the association between the type of vaccination and the post vaccine side effects i.e, joint pain, swollen ankles, muscle pain, nausea, abdominal pain, diarrhea, vomiting, bruises, bleeding gums, chills, irritation, body sweats, numbing and tingling sensation, clogged nose, runny nose, dyspnea, chest pain, irregular heartbeat, altered blood pressure and cough.

RESULTS

The study was conducted on 100 individuals with a mean age of 24.81±2.109 years with 25% males and 75% females. It was seen that 79% individuals were never infected with COVID before vaccination while 21% were infected. The types of vaccinations received have been shown in Table-I.

It was reported that majority of the individuals (92%) did not get infected with COVID after receiving the vaccination while only 8% were infected. Table-II shows that there was a non-significant association between type of COVID vaccination and frequency of infection after receiving these vaccines. It was seen that majority of individuals who developed COVID 19 infection were the ones receiving Sinovac.

Table-III shows that the association between type of vaccine and the side effects like fever, and cough were significantly associated with each other.

Table 1: Type of vaccination received by individuals

Type of vaccination	Frequency
PFIZER BioNTech	2
Sinopharm	47
Moderna	2
Sinovac	46
Cansino	3

Table-II: Association between type of COVID vaccines and probability of COVID 19 infection after receiving the vaccine (n=100).

Type covid vaccine received	COVID 19 Infection post vaccination			p value
	Yes n (%)	No n (%)	Total n (%)	
PFIZER BioNTech	0 (0%)	2 (100%)	2 (100%)	0.718
Sinopharm	3(6.4%)	44 (93.6%)	47(100%)	
Moderna	0 (0%)	2(100%)	2(100%)	
Sinovac	5 (10.9%)	41 (89.1%)	46(100%)	
Cansino	0(0%)	3(100%)	3(100%)	

Table-III: Shows that the association between type of vaccine and the side effects like fever, and cough were significantly associated with each other.

Potential side effects		Type of vaccination received			p value
		Sinopharm n(%)	Sinovac n(%)	Total	
Tiredness and fatigue	**Yes**	26 (55.3%)	31(67.4%)	57(61.3%)	0.232
	No	21(44.7%)	15(32.6%)	36(38.7%)	
Decreased sleep quality	**Yes**	8(17%)	10(21.7%)	18(19.4%)	0.565
	No	**39**(83%)	36(78.3%)	75(80.6%)	
Fever	**Yes**	5(10.6%)	14(30.4%)	19(20.4%)	0.018*
	No	42(89.4%)	32(69.6%)	74(79.6%)	
Headache	**Yes**	16(34%)	16(34.8%)	32(34.4%)	0.940
	No	31(66%)	30(65.2%)	61(65.6%)	
Haziness and lack of vision	**Yes**	7(14.9%)	5(10.9%)	12(12.9%)	0.563
	No	40(85.1%)	41(89.1%)	81(87.1%)	
Pain and swelling in injection site	**Yes**	28(59.6%)	22(47.8%)	50(53.8%)	0.256
	No	19(40.4%)	24(52.2%)	43(46.2%)	
Joint pain	**Yes**	1(2.1%)	6(13%)	7(7.5%)	0.059
	No	46(97.9%)	40(87%)	86(92.5%)	
Swollen ankles	**Yes**	1(2.1%)	1(2.2%)	2(2.2%)	1.000
	No	46(97.9%)	45(97.8%)	91(97.8%)	
Muscle pain	**Yes**	14(29.8%)	11(23.9%)	25(26.9%)	0.523
	No	33(70.2%)	35(76.1%)	68(73.1%)	
Nausea	**Yes**	3(6.4%)	4(8.7%)	7(7.5%)	0.714
	No	44(93.6%)	42(91.3%)	86(92.5%)	
Abdominal pain	**Yes**	1(2.1%)	3(6.5%)	4(4.3%)	0.361
	No	46(97.9%)	43(93.5%)	89(95.7%)	
Diarrhea	**Yes**	3 (6.4%)	2(4.3%)	5(5.4%)	1.000
	No	44(93.6%)	44(95.7%)	88(94.6%)	
Vomiting	**Yes**	1(2.1%)	0(0%)	1(1.1%)	1.000
	No	46(97.9%)	45(100%)	91(98.9%)	
Bruises	**Yes**	1(2.1%)	1(2.2%)	2(2.2%)	1.000
	No	46(97.9%)	45(97.8%)	91(97.8%)	
Bleeding gums	**Yes**	1(2.1%)	1(2.2%)	2(2.2%)	1.000
	No	46(97.9%)	45(97.8%)	91(97.8%)	
Chills	**Yes**	4(8.5%)	1(2.2%)	5(5.4%)	0.361
	No	43(91.5%)	45(97.8%)	88(94.6%)	
9Irritation	**Yes**	3(6.4%)	1(2.2%)	4(4.3%)	0.617
	No	44(93.6%)	45(97.8%)	89(95.7%)	
Body sweats	**Yes**	2(4.3%)	0(0%)	2(2.2%)	0.495
	No	44(95.7%)	46(100%)	90(97.8%)	
Numbing and tingling sensation	**Yes**	1(2.1%)	3(6.5%)	4(4.3%)	0.361
	No	46(97.9%)	43(93.5%)	89(95.7%)	
Dizzy	**Yes**	8(17%)	7(15.2%)	15(16.1%)	0.813
	No	39(83%)	39(84.8%)	78(83.9%)	
Clogged nose	**Yes**	3(6.4%)	2(4.3%)	5(5.4%)	1.000
	No	44(93.6%)	44(95.7%)	88(94.6%)	
Runny nose	**Yes**	2(4.3%)	11(2.2%)	3(3.2%)	1.000
	No	45(95.7%)	45(97.8%)	90(96.8%)	
Dyspnea	**Yes**	0(0%)	(2.2%)	1(1.1%)	0.495
	No	47(100%)	45(97.8%)	92(98.9%)	

Chest pain	**Yes**	2(4.3%)	3(6.7%)	5(5.5%)	0.677
	No	44(95.7%)	42(93.3%)	86(94.5%)	
Sleepiness	**Yes**	12(25.5%)	19(41.3%)	31(33.3%)	0.107
	No	435(74.5%)	27(58.7%)	62(66.7%)	
Irregular heartbeat	**Yes**	(8.5%)	5(10.9%)	9(9.7%)	0.740
	No	43(91.5%)	41(89.1%)	84(90.3%)	
Altered blood pressure	**Yes**	5(10.6%)	2(4.3%)	7(7.5%)	0.435
	No	42(89.4%)	44(95.7%)	86(92.5%)	
Dry throat	**Yes**	5(10.6%)	7(15.2%)	12(12.9%)	0.510
	No	42(89.4%)	39(84.8%)	81(87.1%)	
Cough	**Yes**	1(2.1%)	8(17.4%)	9(9.7%)	0.015*
	No	46(97.9%)	38(82.6%)	84(90.3%)	

DISCUSSION

In the global fight against the COVID-19 pandemic, vaccination has emerged as a critical tool in controlling the spread of the virus and reducing the severity of illness.[16] Among the various vaccines available, those developed by Sinopharm and Sinovac have played significant roles, particularly in countries where they are widely administered.[17] However, like any medical intervention, these vaccines can induce a range of post-vaccination symptoms.[18] In this discussion, we delve into the common symptoms experienced by individuals post-Sinopharm and Sinovac vaccinations.[7]

According to our study 55.3% of individuals vaccinated with Sinopharm reported experiencing tiredness and fatigue, while 59.6% reported pain and swelling at the injection site. Other symptoms included muscle pain in 29.8% of participants, fever in 10.6%, and headaches in 34% of participants. The association between type of vaccine and post-vaccine symptom of fever was found to be significant in our study (p=0.018). A separate study focusing on Sinopharm recipients in Pakistan reported results comparable to our study where most people reported with pain at the injection site after first dose, noted by 61.3% of respondents.[19] This study reported a lower percentage of individuals who developed lethargy in (40.6%), myalgia or body pain in 23.9% and headaches in 21%[19] as compared to our study but a higher number of individuals with low-grade fever (22.4%)[19] as compared to our study. Al Kaabi et.al reported that a lower percentage Sinopharm recipients experienced post vaccination symptoms as compared to our study. It was reported that 42% of experienced pain at the vaccination site, 5.1% experienced tenderness, 9.6% reported headaches, and 12.2% experienced fatigue.[20] Additionally, 1.1% reported fever, cough, and allergies.[20] The association between type of vaccine and post-vaccine symptom of cough was found to be significant in our study (p=0.015). Our study reported cough to be a side effect in 2.1% Sinopharm recipients. Other symptoms included lethargy in 9.2% of participants, while 4.07% reported with back pain, abdominal pain, and diarrhea in 0.74% of individuals.[20] El Gendy et.al reported very conflicting results as compared to our study where all the post-vaccine symptoms were found to be less frequent.[21] Some participants reported pain, redness, or swelling at the injection site (12.5%), while 45% experienced fatigue and lethargy.[21] Headaches were reported by 10% of participants, with smaller proportions experiencing runny noses (7.5%), sore throats, allergies, and rashes (5% each).[21] Muscle pain was reported by 12.5% of participants, while dizziness and fever were noted in 2.5%.[21]

According to our study a large number of volunteers received the Sinovac vaccination; of them, 67.4% reported feeling exhausted and worn out, and 47.8% felt discomfort and swelling at the injection site. Fever and headache were reported as additional symptoms in 30.4% and 34.8% of the subjects, respectively. According to a study done in Indonesia, the most common side effects after receiving Sinovac vaccination was injection-related itchy pain, which affected up to 54.6% of participants[22] which is comparable but higher as compared to our study. It was also reported that a much lower percentage of individuals reported feeling fatigued and exhausted (46.9%)[22] as compared to our study. A comparable study conducted in Turkey found that 44.6% of subjects experienced injection site pain[23] after being vaccinated by Sinovac which is comparable to our study. A much lower percentage of individuals reported that they experienced fever (2.2%,) and headache (4.3%)[23] as compared to our study. On the other hand, 3.3% of participants reported experiencing nausea. Other associated symptoms people presented with were sleepiness, itching, excessive sweating and weakness.[23]

CONCLUSION

Among individuals who received Sinopharm vaccine pain at the injection site was the most prevalent side effect followed by tiredness and headache respectively. The individuals who received Sinovac reported tiredness and fatigue as the most prevalent post-vaccination followed by pain at injection site and then headache respectively. Fever and cough were the two post vaccination symptoms significant associations with vaccination type both of which were more prevalent in individuals who received Sinovac.

RECOMMENDATION

Vaccines can lower susceptibility in both diseased and uninfected people. This study, which focuses on the effectiveness of various vaccination types, will undoubtedly be useful to the medical community and the general public. People will be able to discover which vaccine is least likely to result in post-vaccination side effects. This study will aid in the choice of vaccines and the regulation of those with more severe adverse effects. As per our study due to the scarcity of individuals who received other vaccines, we had to limit our analysis to Sinopharm and Sinovac vaccines and it was found that most of the symptoms were more prevalent in Sinovac recipients as compared to Sinopharm. Post-vaccination COVID 19 infection was also more prevalent in those who received Sinovac.

LIMITATION

Due to scarcity of sample who received Pfizer, Moderna and Cansino, the analysis had to be limited to Sinopharm and Sinovac. A multicenter study with a larger sample size would have helped unravel more findings.

CONFLICT OF INTEREST

None

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHOR CONTRIBUTION

Sarah Mansoor: Conceptualization of study manuscript writing, data collection, data analysis, data interpretation, revision

Muhammad Hashim Nawaz: Manuscript drafting, data collection, data analysis

Hira Butt: Conceptualization of study manuscript writing, data collection, data analysis, data interpretation, revision

Ayesha Chheena: Concept and design, data interpretations, critical review

Amina Zahid, Maham Nasir: Manuscript drafting, concept and design, data collection,

REFERENCES

1. Kim Y, Bae S, Chang HH, Kim SW. Characteristics of long COVID and the impact of COVID-19 vaccination on long COVID 2 years following COVID-19 infection: Prospective cohort study. Sci Rep. 2024; 14(1): 854. DOI: https://doi.org/10.1038/s41598-023-50024-4
2. Wang J, Jing R, Lai X, Zhang H, Lyu Y, Knoll MD, *et al*. Acceptance of COVID-19 vaccination during the COVID-19 pandemic in China. Vaccines. 2020; 8(3): 482. DOI: https://doi.org/10.3390/vaccines8030482
3. Le TT, Andreadakis Z, Kumar A, Román RG, Tollefsen S, Saville M, *et al*. The COVID-19 vaccine development landscape. Nat Rev Drug Discov. 2020; 19(5): 305-6. DOI: https://doi.org/10.1038/d41573-020-00073-5
4. Bick A, Blandin A, Mertens K. Work from home before and after the COVID-19 outbreak. AEJ: Macroeconomics. 2023; 15(4): 1-39.
5. Bennett BW, Phillips LS, Gazmararian JA. The Association of vaccination for common adult infectious diseases and uptake of COVID-19 vaccines among 5,006,851 veterans, 20 December 2020–31 October 2021. Vaccines. 2024; 12(2): 145. DOI: https://doi.org/10.3390/vaccines12020145
6. Shekhar R, Garg I, Pal S, Kottewar S, Sheikh AB. COVID-19 vaccine booster: To boost or not to boost. Infect Dis Rep. 2021; 13(4): 924-9. DOI: https://doi.org/10.3390/idr13040084
7. Alhazmi A, Alamer E, Daws D, Hakami M, Darraj M, Abdelwahab S, *et al*. Evaluation of side effects associated with COVID-19 vaccines in Saudi Arabia. Vaccines. 2021; 9(6): 674. DOI: https://doi.org/10.3390/vaccines9060674
8. Abdollahi A, Naseh I, Kalroozi F, Kazemi-Galougahi MH, Nezamzadeh M, Billandi SS, *et al*. Comparison of side effects of COVID-19 vaccines: Sinopharm, astraZeneca, sputnik V, and covaxin in women in terms of menstruation disturbances, hirsutism, and metrorrhagia: a descriptive-analytical cross-sectional study. Int J Fertil Steril. 2022; 16(3): 237. DOI: https://doi.org/10.22074/ijfs.2022.544706.1236
9. Mohebbi A, Eterafi M, Fouladi N, Golizadeh M, Panahizadeh R, Habibzadeh S, *et al*. Adverse effects reported and insights following Sinopharm COVID-19 vaccination. Curr Microbiol. 2023; 80(12): 377. DOI: https://doi.org/10.1007/s00284-023-03432-8
10. Halim M, Halim A, Tjhin Y. COVID-19 vaccination efficacy and safety literature review. J Clin Med Res. 2021; 3(1): 1-0. DOI: https://doi.org/10.4103%2Fijmr.IJMR_474_21

11. Zhao H, Li Y, Wang Z. Adverse event of Sinovac coronavirus vaccine: Deafness. Vaccine. 2022; 40(3): 521-3. DOI: https://doi.org/10.1016/j.vaccine.2021.11.091
12. Khani E, Khiali S, Entezari-Maleki T. Potential COVID-19 therapeutic agents and vaccines: An evidence-based review. J Clin Pharmacol. 2021; 61(4):429-60. DOI: https://doi.org/10.1002/jcph.1822
13. Qureshi A, Sulaiman SSA, Rehman W, Mehmood A, Idrees S, Kumar N. Prevalence of post-vaccine side effects among COVID-19 immunized community of Southern Pakistan. Plos One. 2023; 18(5): e0285736. DOI: https://doi.org/10.1371/journal.pone.0285736
14. Daniel WW, Cross CL. Determination of sample size for estimating proportions. Biostatistics A foundation for analysis in the health sciences. 1999; 8: 189-90.
15. Hatmal MM, Al-Hatamleh MA, Olaimat AN, Hatmal M, Alhaj-Qasem DM, Olaimat TM, *et al.* Side effects and perceptions following COVID-19 vaccination in Jordan: A randomized, cross-sectional study implementing machine learning for predicting severity of side effects. Vaccines. 2021; 9(6): 556. DOI: https://doi.org/10.3390/vaccines9060556
16. Remmel A. COVID vaccines and safety: What the research says. Nature. 2021; 590(7847): 538-40. DOI: https://doi.org/10.1038/d41586-021-00290-x
17. Anvari E, Talepoor AG, Vakili ME, Karimi N, Ataollahi M, Najafi G, *et al*. Comparison of the antibody responses following vaccination with Astra Zeneca and Sinopharm. Iran J Immunol. 2022; 19(3): 321. DOI: https://doi.org/10.22034/iji.2022.94298.2300
18. Syed SSM, Ranjha AA, Ghazanfer S, Sultan HO, Sattar A, Miran SM, Hussain M. Prevalence and predictors for adverse effects of Sinopharm and Sinovac COVID-19 Vaccines. Professional Med J. 2024. 2022; 29(11): 1701-7. DOI: https://doi.org/10.29309/TPMJ/2022.29.11.7116
19. Haider T, Abidi S, Fatima M, *et al*. (April 26, 2023) The Prevalence of side effects of Sinopharm COVID-19 vaccine: An experience from Pakistan. Cureus. 2023 Apr 26; 15(4): e38180. DOI: https://doi.org/10.7759/cureus.38180
20. Al Kaabi N, Oulhaj A, Ganesan S, Al Hosani FI, Najim O, Ibrahim H, *et al*. Effectiveness of BBIBP-CorV vaccine against severe outcomes of COVID-19 in Abu Dhabi, United Arab Emirates. Nat Commun. 2022; 13(1): 3215. DOI: https://doi.org/10.1038/s41467-022-30835-1
21. Elgendy MO, El-Gendy AO, Alzarea AI, Mahmoud S, Alqahtani SS, Fahmy AM, *et al*. SARS-CoV-2 post vaccinated adverse effects and efficacy in the Egyptian Population. Vaccines. 2021; 10(1): 18. DOI: https://doi.org/10.3390/vaccines10010018
22. Nurzak AN, Iqbal M, Yunus A, Wahyuni DF. The evaluation of adverse effects of Sinovac® COVID-19 vaccine after receiving the first dose–maros health center. J Pharm Negat Results. 2022: 13(1): 78-83. DOI: https://doi.org/10.47750/pnr.2022.13.S01.10
23. Yilmaz S, Çetin P, Khorshid L, Bayraktar D. Comparison of adverse effects of BioNTech mRNA and Sinovac vaccines in adults in Turkey. İKÇÜSBFD. 2023; 8(2): 395-400.

ORIGINAL ARTICLE

CD 4 count stratification and its accuracy in predicting the HIV-Tuberculosis co-infection

Hina Sadiq[1], Nasim Akhtar[1], Sana Tahir Virk[1], Kazim Abbas Virk[2], Abeer Zafar[1], Lubna Meraj[3]

[1]Pakistan Institute of Medica Sciences, Islamabad Pakistan
[2]HBS Medical and Dental College, Islamabad Pakistan
[3]Benazir Bhutto Hospital, Rawalpindi Pakistan

ABSTRACT

Background: Co-infection of HIV and TB is a significant public health concern. The relationship between increased HIV replication and low CD4+-TLC in HIV-positive patients with treatment interruptions is well documented. Moreover, TB preventive therapy is highly effective in reducing TB incidence and mortality among HIV-positive patients. The objective of this study was to stratify in terms of different ranges and see the association of CD4+ T-lymphocyte count with different presentations of TB in HIV-positive patients.

Material and Methods: This observational cross-sectional study was conducted from October 2022 to March 2023. A total of seventy-four outdoor and indoor patients were enrolled. Patient data were collected using a structured questionnaire. The MTB gene Xpert, screening for HIV, and CD4+ T-lymphocyte count testing was performed. All the patients aged> 18 years who were found to have positive HIV rapid tests and microbiologically confirmed tuberculosis were included in the study. The CD 4 count was stratified in terms of ranges. The data was analyzed using SPSS 29. The association was established by Spearman's coefficient and odd ratios keeping the significance level <0.05.

Results: 74 patients were enrolled in the study, out of which 67 (90.5%) were males and 7 (9.5%) were females. The mean age of study participants was 38.33 ± 11.43 years (21-78 years) and the mean CD4 count was 85.7 ± 59.48. Most frequent was pulmonary TB; 43 (44.5%) followed by disseminated TB; 11 (24.3%), pleural TB; 9 (9.4%), and TB meningitis 6 (8.1%). There was no association between CD4+ T-lymphocyte count and the site of involvement of TB (p>0.05). Pulmonary TB, miliary TB, TB brain abscess, tuberculomas, and disseminated TB were found more at CD 4 count <100 as signified by the Odd Ratios (1.1, 1.3, 1.3, 1.3, 1.01 within 95% CI)). On the other hand, Pleural TB, Spinal TB, TB lymph adenitis, and TB meningitis were found at CD4 count >100 (4.5, 2.3, 1.51 respectively within 95%CI.

Conclusion: Among HIV-TB co-infected individuals, the frequency of pulmonary TB was found to be highest followed by disseminated TB and pleural TB. No association was found between CD4+ Lymphocyte count and different presentations of TB in this study. The CD4 count is a poor predictor of HIV/TB co-infection unless it is <100. Mostly tuberculosis occurred at count <100 as depicted by odd ratios.

Keywords: CD4+ T-lymphocyte count, Extrapulmonary tuberculosis, HIV-TB co-infection, Pulmonary tuberculosis

BACKGROUND

Human Immunodeficiency Virus (HIV) is a global health issue, affecting millions of people worldwide. HIV is a retrovirus that attacks the immune system and targets CD4+ T-lymphocytes (CD4+) which weakens the body's ability to fight against infections and diseases. This eventually leads to "acquired immunodeficiency syndrome" (AIDS), a condition in which the immune system is severely compromised, leaving individuals susceptible to various opportunistic infections and certain types of cancer. Globally 36.7 million cases of HIV/AIDS have been recorded. Every year 2.1 million people are infected with HIV leading to mortality in 1.1 million cases.[1] In Pakistan, HIV infections were first described in 1987, and from that point onward, the number of positive cases has expanded to 0.18 million.[2] In 2013, the number of HIV-positive patients in Pakistan was only 4,500. With 20,000 new HIV infections in 2017, Pakistan has the second fastest-growing HIV epidemic in the Asia Pacific.[2] By June 2020, the National AIDS Control Program (NACP) had registered 42,563 HIV-positive patients. Tuberculosis (TB) is a major global public health problem with an estimated 10.4 million newly emerging active TB cases worldwide in 2016.[3] Previously due to a lack of diagnostic options, diagnosis

Correspondence: Dr. Hinda Sadiq, Fellow Infectious Diseases, Pakistan Institute of Medica Sciences, Islamabad Pakistan

Email: hinasadiq1290@gmail.com

This article can be cited as Sadiq H, Akhtar N, Virk ST, Virk KA, Zafar A, Meraj L. CD 4 count stratification and its accuracy in predicting the HIV/ tuberculosis co-infection. Infect Dis J Pak. 2024; 33(2): 63-68. DOI: https://doi.org/10.61529/idjp.v33i2.296

Receiving date: **20 Feb 2023** Acceptance Date: **01 Apr 2024**
Revision date: **26 Mar 2024** Publication Date: **28 Jun 2024**

of TB was missed in many instances. These days due to advancements in molecular and microbiological techniques, diagnosis of TB has been made relatively easy leading to a significant increase in the number of diagnosed TB patients.[4] The co-infection of TB and HIV is particularly problematic because each condition can worsen the other. TB can accelerate the progression of HIV to AIDS, and HIV can increase the risk of developing active TB disease in individuals with latent TB infection. This co-infection can lead to more severe symptoms, increased mortality rates, and more challenges in managing both diseases. In Pakistan, the prevalence of HIV-TB co-infection might vary based on several factors, including the prevalence of HIV and TB in the general population. Around 33% of 39.5 million HIV-positive patients were infected with TB and up to half of people living with HIV are supposed to develop TB.[5] The presence of other infections including TB tends to increase the rate of HIV replication in HIV-positive people. This may result in high replication of HIV along with a more rapid progression to develop AIDS. Of the 8.8 million TB cases around the world, an expected 1.1 million (13%) were also co-infected with HIV. Treatment with antiretroviral therapy (ART) suppresses HIV replication, this allows the immune system to recover and reduces the chances of death and illness associated with TB. The low CD4+-TLC remains an important risk factor for TB mortality in HIV-positive patients receiving first-line ART.[6] The relationship between increased HIV replication and low CD4+-TLC in HIV-positive patients with treatment interruptions is well documented.[7] HIV-positive patients with TB and low CD4+-TLC (<100 cells/mm3), often have atypical chest X-rays and negative acid-fast bacilli (AFB) sputum smears, compared to the HIV-negative patients.[8] TB preventive therapy is highly effective in reducing TB incidence and mortality among HIV-positive patients. Although it is well established that tuberculosis can occur at any CD4 count value, CD4+-TLC stratification has not been evaluated previously as a strategy to guide the testing and treatment of active TB infection for HIV-positive patients.[9] In a resource-limited country like ours where already prevalence of tuberculosis in the general population is very high, screening patients of HIV for TB co-infection below a specific cut-off of CD 4 count will have a positive impact on reducing morbidity and mortality. The CD 4 count is easily performed, rapid, and cost-effective for the initial assessment of patients living with HIV. CD 4 count assessment is though provided at almost all the centers of national and provisional Aids Control programs free of cost, but its excessive ordering will of course create a burden on HIV care continuum services. This idea also supports the rationale of the study i.e. defining cut-off and stratifying CD 4 count into ranges.

MATERIAL AND METHODS

This cross-sectional observational study was conducted over 6 months from May 2023 to October 2023 after approval from the Ethical Review Board (F.1-1/2015/ERB/SZABMU/1117 dated 17-04-2023). The convenience sampling technique was followed. Sample size of seventy-four was calculated by using the World Health Organization (WHO) calculator, keeping the significance level at 5%, power of test (1-β) at 90 %, anticipated value of population proportion of 0.78[10] and specified relative precision at 0.12. Patients from the inpatient and outpatient facilities of the Department of Infectious Diseases of Pakistan Institute of Medical Sciences (PIMS), Islamabad, also registered with the National Aids Control Program (NACP) were enrolled. All adult (>18 years) HIV-positive patients with confirmed tuberculosis (either by AFB smear, Xpert MTB, or AFB Culture) irrespective of the site of involvement, and regardless of CD4+-TLC were included. The data was collected using a structured questionnaire that included demographic details, CD4 count and status of AFB smear, Gene Xpert MTB, and AFB Culture positivity. Informed consent was taken from the patient or attendant before collecting the information with assurance to maintain confidentiality and anonymity. The patients who were found to be HIV-positive at the regional ART center of PIMS and had any symptom or sign that directed the workup of TB were identified. The AFB smear, MTB gene Xpert (PCR-based test for mycobacterium tuberculosis) and AFB Culture were sent to the laboratory. The MTB gene Xpert test was performed with the device “Gene Xpert GXIV-4”. Moreover, those patients who were already on anti-TB therapy for TB involving any site and presented to the departmental clinic or emergency were also screened for HIV and included in the study if found positive. The screening for HIV was done using the WHO three test protocol (Bio Alere HIV 1/2, Ag/Ab Combo test followed by Uni- Gold HIV kit of Trinity

Biotech & Abott and SD bio line kit as a third test). The baseline CD4+-TLC of every patient was noted at the time of diagnosis of co-infection. The CD4+-TLC was performed on analyzer by "Alere Pima" at the ART Centre of PIMS. The data was analyzed by IBM SPSS, Statistics 29.0 software. The demographic details of patients like gender, marital status, education, occupation, residential area, mode of transmission, history of foreign travel and types of TB in HIV-positive patients were presented as percentages. The association of different ranges of CD4+-TLC with different sites of TB was assessed.

A CD4 count cut-off of 100 was kept for analysis assuming that most TB cases occur at the CD4 count of <100. A 2×2 contingency table was constructed for each site of TB involvement. The Spearman coefficient (r) and ODD ratios were calculated as a measure of association and a level of significance was identified keeping p=value < 0.05 as significant. Fischer's Exact probability test was used to calculate odd ratios.

RESULTS

The demographic characteristics of 74 patients are summarized in Table-I. Pulmonary involvement was found to be highest in all co-infected patients followed by TB Meningitis, Pleural TB, and TB Lymphadenitis. There was only one case of Bone Marrow tuberculosis, one case of TB pericarditis, and two cases of tuberculous arthritis in whom TB was also diagnosed from other sites and was counted as disseminated tuberculosis. The frequency and proportions of sites of tuberculosis involvement are given in Table-II.

The CD 4 count of each patient was recorded and then categorized into different ranges for ease of analysis. There was no association found between CD4 count ranges and the site of TB involvement in HIV-TB Co-infection (p>0.05), but it was interesting to note that multiple sites (disseminated disease) were involved at CD4 counts at or below 100. Pulmonary tuberculosis occurred at any CD4 count ranging from 1-350 as depicted in Figure-I.

The Spearman rank-order coefficient and odd ratios were also calculated for each TB site as given in Table III. Most values of the Spearman coefficient were either slightly above zero on the positive side or below zero on the negative side signifying no or negative correlation of site of involvement of TB with CD 4 count.

Pulmonary TB, miliary TB, TB brain abscess, tuberculomas, and disseminated TB were found to occur at CD 4 count <100 as signified by the odd ratios. On the other hand, Pleural TB, Spinal TB, TB lymph adenitis, and TB meningitis developed at CD4 count >100 (Table-III).

Receiver Operating Curve analysis was performed to find sensitivity and specificity and appropriate cut-off CD 4 count keeping Xpert MTB Rif as the gold standard for diagnosis. The sensitivity and specificity of CD 4 count was found to be 68.4% and 41.2% respectively. The appropriate cut-off CD 4 count was found to be 53.5 at a point where maximum sensitivity and specificity (accuracy) was identified. This was calculated using the Youden Index. The Curve with different coordinates is shown in Figure-II.

Table-I: Demographic characteristics of the patients.

Demographic Details		
Age	38±11.24 years	
CD4 Count	86±59.48	
Gender	Males	67 (90.54%)
	Females	7 (9.45%)
Residence	Punjab	34 (45.94%)
	KPK	12 (16.21%)
	AJK & GB	11 (14.86)%
	Islamabad	17 (22.97%)
Source of transmission	Unknown	46 (62.16%)
	Sexual	15 (20.27) %
	IVDU	13 (17.56%)
Foreign Travel	Yes	11 (14.86%)
	No	63 (85.13%)
Compliance to ART	Yes	72 (97.29%)
	No	2 (2.70%)

Table-II: Different sites of involvement of tuberculosis (n=74).

Tuberculous Site	Frequency (%)
Miliary TB	3 (4.1%)
Pulmonary TB	33 (44.5%)
Pleural TB	7 (9.4%)
TB Lymphadenitis	4 (5.4%)
TB Brain Abscess	1 (1.4%)
TB meningitis	6 (8.1%)
Tuberculoma	1 (1.4%)
Spinal tuberculosis	1 (1.4%)
Disseminated tuberculosis	18 (24.3%)

Table-III: Odds Ratios and Spearman Coefficient values along with level of significance.

TB Site	Spearman coefficient (r)	Odd Ratios (OR) for CD4 count		P Value
		<100	>100	
Miliary TB	-0.11	1.3	-	0.34
Pulmonary TB	-0.10	1.1	0.67	0.38
Pleural TB	0.04	0.92	1.27	0.71
TB Lymphadenitis	0.15	0.6	2.3	0.19
Spinal TB	0.21	-	4.5	0.06
TB brain Abscess	-0.06	1.3	-	0.58
TB Meningitis	0.07	0.85	1.51	0.53
Tuberculomas	-0.06	1.3	-	0.58
Disseminated TB	-0.01	1.01	0.95	0.93

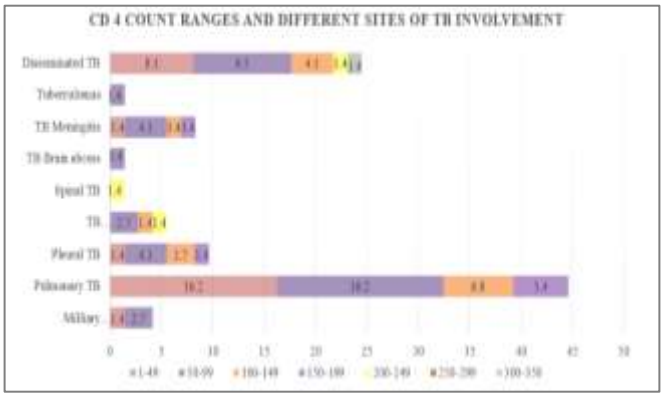


Figure-I: Percentage of involvement of various sites at different CD 4 count ranges.

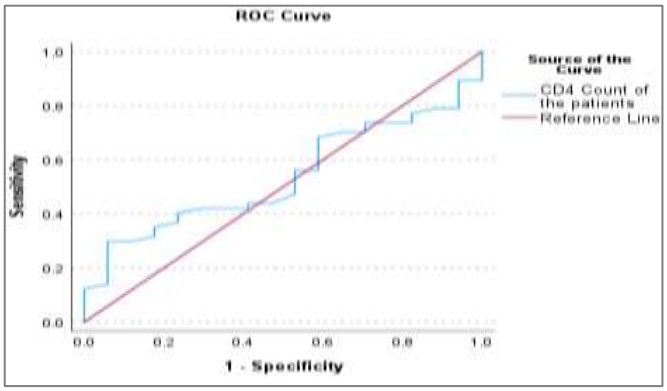


Figure-II: ROC curve for CD 4 count keeping Xpert MTB Rif as Gold Standard.

DISCUSSION

TB is one of the most common opportunistic infections in HIV-positive patients. The co-infection of HIV and TB is spreading across the globe, especially in developing countries.[11] HIV-positive patients are estimated to have a 20-30 times higher risk of developing active TB than HIV-negative individuals.[12] Globally, 1.2 million people are diagnosed with HIV-TB coinfection[13] and the overall prevalence of TB in HIV-positive patients is 16%.[14] In our study, we found that TB coinfection was more prevalent among males, compared to females which may be due to a higher number of male patients with HIV infection enrolled. The main source of HIV transmission was unclear in this study followed by sexual (20.2%) and then IVDU (17.5%).[15] It seems that lack of awareness about routes of transmission is a major risk factor for both HIV and TB infections in Pakistan.[16]

CD4+-TLC has an important role in HIV-TB coinfection, and a low CD4+-TLC has been implicated as a strong predictor of TB in HIV-positive patients in a few studies.[17] The pulmonary was the most common site of TB involvement when calculated frequencies were split at each site.

These results are consistent with other studies[18]. TB infection is normally fought by cell-mediated immunity in the host. Cell-mediated immunity is a limb that is often depressed in HIV-positive patients. Impaired mechanisms to control TB infection among HIV patients lead to unusual presentations of TB. Consequently, TB has become a major contributing factor in the mortality of AIDS patients.[19] This is well-documented that HIV-positive patients are more likely to develop EPTB.[20] However, Click and coworkers, demonstrated that pulmonary TB was more prevalent in HIV-TB coinfection patients in their study.[21] Some of the studies identified that EPTB along with pulmonary involvement was more common than EPTB alone in co-infection patients.[22]

In the Pacific and Asia, India, Myanmar, Thailand, and Indonesia, are among the 41 countries that have the highest burden of HIV-TB co-infection.[23] In a study from South Asia, 4% of HIV-positive TB patients had EPTB, particularly in those with significant immune suppression.[24] Leeds and researchers showed that TB lymphadenitis (28%) was the most widely recognized EPTB, followed by disseminated TB (23%), and cerebrospinal TB (22 %).[16] TB Lymphadenitis and pleural TB were the most common locations of EPTB in the other studies.[25,26] Reports from the US also showed that pleural TB is the second most pervasive sort of EPTB.[27]

A study published in India in 2019 found that the prevalence of all types of tuberculosis was higher when the CD4 cell count was <300 cells in contrast to our study where active TB cases were more common below a CD4 count of 100. They have also studied the impact of ART on the association of CD 4 count with the type of tuberculosis, but the results were insignificant.[28]

In a Chinese study CD 4+ T-cell count was significantly associated with TB at a cut-off of <200 and the odds of developing active Tuberculosis were high (P = 0.002, OR = 3.714, 95% CI: 1.612–8.577) which signifies that CD 4+ T-cell count is a predictor of active TB by lowering cut-offs to < 200. These findings match our study, in which the sensitivity and specificity of CD 4 count is found to be 68.4% and 41.2%, and OR >1 for most forms of TB at CD4 count < 100 (Pulmonary TB, miliary TB, TB brain abscess, tuberculomas, and disseminated TB).[29] Conversely, pleural TB, Spinal TB, TB lymph adenitis, and TB meningitis occurred more at CD4 count >100. Among this group overall TB, prevalence was very low indicating that these results might not have depicted the true association of CD4 count with the site of TB involvement.

CONCLUSION

TB is a common opportunistic infection among HIV-positive patients. Most forms of TB occurred at CD4+-TLC of between 50-99 with a cut-off at 53.3 regardless of the site of involvement. The overall prevalence of tuberculosis was highest below CD 4 count of 100 and there was no significant correlation between sites of involvement and CD4 count.

CONFLICT OF INTEREST

None

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHOR CONTRIBUTION

Hina Sadiq: Conceived the research idea, data collection

Nasim Akhtar: Critical review

Sana Tahir Virk: Data analysis and drafting of manuscript

Kazim Abbas Virk: Literature review and referencing

Abeer Zafar: Data collection.

Lubna Meraj: Data Interpretation and review.

REFERENCES

1. Yoshimura K. Status of HIV/AIDS in the ART era. J Infect Chemother. 2017; 23(1): 12-16. DOI: https://doi.org/10.1016/j.jiac.2016.10.002
2. Malik M, Jamil MS, Johnson CC, Pasha MS, Hermez J, Qureshi SUH. Integrating assisted partner notification within HIV prevention service package for people who inject drugs in Pakistan. J Int AIDS Soc. 2019; 22 Suppl 3(Suppl Suppl 3): e25317. DOI: https://doi.org/10.1002%2Fjia2.25317
3. Glaziou P, Floyd K, Raviglione MC. Global epidemiology of tuberculosis. Semin Respir Crit Care Med. 2018; 39(3): 271-85. DOI: https://doi.org/10.1055/s-0038-1651492
4. Scott L, da Silva P, Boehme CC, Stevens W, Gilpin CM. Diagnosis of opportunistic infections: HIV co-infections - tuberculosis. Curr Opin HIV AIDS. 2017; 12(2): 129-38. DOI: https://doi.org/10.1097/coh.0000000000000345
5. Glaziou P, Falzon D, Floyd K, Raviglione M. Global epidemiology of tuberculosis. Semin Respir Crit Care Med. 2013; 34(1): 3-16. DOI: https://doi.org/10.1055/s-0038-1651492
6. Bayabil S, Seyoum A. Joint modeling in detecting predictors of CD4 cell count and status of tuberculosis among people living with HIV/AIDS under HAART at Felege Hiwot teaching and specialized hospital, North-West Ethiopia. HIV AIDS (Auckl). 2021; 13:527-37. DOI: https://doi.org/10.2147%2FHIV.S307069

7. Kiros T, Dejen E, Tiruneh M, Tiruneh T, Eyayu T, Damtie S, *et al*. Magnitude and associated factors of pulmonary tuberculosis among HIV/AIDS patients attending antiretroviral therapy clinic at Debre Tabor specialized hospital, Northwest Ethiopia, 2019. HIV AIDS (Auckl). 2020; 12: 849-58. DOI: https://doi.org/10.2147/hiv.s282616
8. Henry RT, Jiamsakul A, Law M, Losso M, Kamarulzaman A, Phanuphak P, *et al.* SECOND-LINE Study Group. Factors associated with and characteristic of HIV/Tuberculosis Co-Infection: A retrospective analysis of SECOND-LINE clinical trial participants. J Acquir Immune Defic Syndr. 2021; 87(1): 720-9. DOI: https://doi.org/10.1097/qai.0000000000002619
9. Maimaiti R, Zhang Y, Pan K, Mijiti P, Wubili M, Musa M, *et al*. High prevalence and low cure rate of tuberculosis among patients with HIV in Xinjiang, China. BMC Infect Dis. 2017; 17(1): 15. DOI: https://doi.org/10.1186/s12879-016-2152-4
10. Chaisson LH, Saraceni V, Cohn S, Seabrook D, Cavalcante SC, Chaisson RE, et al. CD4+ cell count stratification to guide tuberculosis preventive therapy for people living with HIV. AIDS. 2020; 34(1): 139-47. DOI: https://doi.org/10.1097/qad.0000000000002398
11. Zerdali E, Nakir İY, Sürme S, Sayılı U, Yıldırım M. Predictors for tuberculosis co-infection in people living with HIV/AIDs. Afr Health Sci. 2021; 21(3): 995-1002. DOI: https://doi.org/10.4314%2Fahs.v21i3.6
12. Meintjes G, Brust JCM, Nuttall J, Maartens G. Management of active tuberculosis in adults with HIV. Lancet HIV. 2019; 6(7): e463-74. DOI: https://doi.org/10.1016/s2352-3018(19)30154-7
13. Kwan CK, Ernst JD. HIV and tuberculosis: A deadly human syndemic. Clin Microbiol Rev. 2011; 24(2): 351-76. DOI: https://doi.org/10.1128%2FCMR.00042-10
14. Leeds IL, Magee MJ, Kurbatova EV, del Rio C, Blumberg HM, Leonard MK, *et al*. The site of extrapulmonary tuberculosis is associated with HIV infection. Clin Infect Dis. 2012; 55(1): 75-81. DOI: https://doi.org/10.1093/cid/cis303
15. Ahmed A, Hashmi FK, Khan GM. HIV outbreaks in Pakistan. Lancet HIV. 2019;6(7): e418. DOI: https://doi.org/10.1016/S2352-3018(19)30179-1
16. Folasayo AT, Oluwasegun AJ, Samsudin S, Saudi SN, Osman M, Hamat RA. Assessing the knowledge level, attitudes, risky behaviors, and preventive practices on sexually transmitted diseases among university students as future healthcare providers in the central zone of Malaysia: A cross-sectional study. Int J Environ Res Public Health. 2017; 14(2): 159. DOI: https://doi.org/10.3390/ijerph14020159
17. Nouchi A, Caby F, Palich R, Monsel G, Caumes AE. Travel-associated STI amongst HIV and non-HIV infected travelers. J Travel Med. 2019; 26(8): taz090. DOI: https://doi.org/10.1093/jtm/taz090
18. Miyahara R, Piyaworawong S, Naranbhai V, Prachamat P, Kriengwatanapong P, Tsuchiya N, *et al*. Predicting the risk of pulmonary tuberculosis based on the neutrophil-to-lymphocyte ratio at TB screening in HIV-infected individuals. BMC Infect Dis. 2019; 19(1): 667. DOI: https://doi.org/10.1186%2Fs12879-019-4292-9
19. Álvaro-Meca A, Díaz A, de Miguel Díez J, Resino R, Resino S. Environmental factors related to pulmonary tuberculosis in HIV-Infected patients in the combined antiretroviral therapy (cART) era. PLoS One. 2016; 11(11): e0165944. DOI: https://doi.org/10.1371/journal.pone.0165944
20. Isomiddin U, Umrzok S. Features of the clinical course, the state of diagnosis and treatment of HIV-associated pulmonary tuberculosis in modern conditions literature review. Annals RSCB [Internet]. 2021; 25 (4): 1809-28.
21. Hasnain J, Memon GN, Memon A, Channa AA, Creswell J, Shah SA. Screening for HIV among tuberculosis patients: a cross-sectional study in Sindh, Pakistan. BMJ Open. 2012; 2(5): e001677. DOI: https://doi.org/10.1136/bmjopen-2012-001677
22. Click ES, Moonan PK, Winston CA, Cowan LS, Oeltmann JE. Relationship between Mycobacterium tuberculosis phylogenetic lineage and clinical site of tuberculosis. Clin Infect Dis. 2012; 54(2): 211-9. DOI: https://doi.org/10.1093/cid/cir788
23. Nair N, Wares F, Sahu S. Tuberculosis in the WHO South-East Asia Region. Bull World Health Organ. 2010; 88(3): 164. DOI: https://doi.org/10.2471%2FBLT.09.073874
24. Qian X, Nguyen DT, Lyu J, Albers AE, Bi X, Graviss EA. Risk factors for extrapulmonary dissemination of tuberculosis and associated mortality during treatment for extrapulmonary tuberculosis. Emerg Microbes Infect. 2018; 7(1): 102. DOI: https://doi.org/10.1038/s41426-018-0106-1
25. Kingkaew N, Sangtong B, Amnuaiphon W, Jongpaibulpatana J, Mankatittham W, Akksilp S, *et al*. HIV-associated extrapulmonary tuberculosis in Thailand: epidemiology and risk factors for death. Int J Infect Dis. 2009; 13(6): 722-9. DOI: https://doi.org/10.1016/j.ijid.2008.11.013
26. Kipp AM, Stout JE, Hamilton CD, Van Rie A. Extrapulmonary tuberculosis, human immunodeficiency virus, and foreign birth in North Carolina, 1993 - 2006. BMC Public Health. 2008; 8: 107. DOI: https://doi.org/10.1186/1471-2458-8-107
27. Jeon D. Tuberculous pleurisy: an update. Tuberc Respir Dis (Seoul). 2014; 76(4): 153-9. DOI https://doi.org/10.4046%2Ftrd.2014.76.4.153
28. Kesari SP, Basnett B, Chettri A. Spectrum of Tuberculous Infection in Patients Suffering from HIV/AIDS, and Its Correlation with CD-4 Counts: A Retrospective Study from Sikkim. Indian J Otolaryngol Head Neck Surg. 2019; 71(2): 167-171. DOI: https://doi.org/10.4046/trd.2014.76.4.153
29. Cui Z, Lin M, Nie S, Lan R. Risk factors associated with tuberculosis among people living with HIV/AIDS: A pair-matched case-control study in Guangxi, China. PLoS One. 2017; 12(3): e0173976. DOI: https://doi.org/10.1371%2Fjournal.pone.0173976

ORIGINAL ARTICLE

Antibiogram of chronic suppurative otitis media in relation to the current bacteriological profile in a tertiary care hospital

Naila Iqbal[1], Saba Anwar[2], Saira Salim[3], Tahira Tehseen[2], Saleha Ali[4], Asif Raza[1]

[1]Izzat Ali Shah Hospital Wah Cantt Taxila, Rawalpindi Pakistan
[2]Wah Medical College Wah Cantt Taxila, Rawalpindi Pakistan
[3]Islamabad Diagnostic Center Wah Cantt Taxila, Rawalpindi Pakistan
[4]HITEC Medical College Taxila, Rawalpindi Pakistan

ABSTRACT

Background: Every year, about 31 million cases of chronic suppurative otitis media are reported. Due to a lack of access to healthcare, inadequate sanitation and hygiene standards, a lack of knowledge about ear care, underlying socioeconomic issues, and a lack of infrastructure for diagnosis and treatment, it is a common illness in low-income countries. The objective of this study is to determine the bacterial profile and susceptibility pattern of microorganisms isolated from chronic suppurative otitis media patients.

Material and Methods: This Descriptive cross-sectional study was conducted at the Department of Microbiology, Izzat Ali Shah Hospital and POF Hospital Wah Cantt from December 2022 to January 2024. A total of 105 ear swabs were received for culture and sensitivity. Growth was observed in 70 ear swab cultures after 48 hours of incubation at 35±2ºC at ambient air. Isolates were identified as *Staphylococcus aureus, Enterococcus spp, Enterobacterales including E. coli, Klebsiella pneumoniae, Proteus mirabilis,* and *Pseudomonas aeruginosa*. Kirby Bauer disk diffusion method was used to assess susceptibility profiles of positive cultures using Muller Hinton agar incubated at a temperature of 35±2°C for 24 hours in ambient air.

Results: Out of all 70 positive cultures, 40 isolates were gram-positive cocci while 30 isolates were gram-negative rods. In gram-positive isolates, *Staphylococcus aureus* was 90% susceptible to ampicillin while Enterococcus spp. was found to be 99% susceptible. Ampicillin was not tested for gram-negative organisms because of its proven inactivity against gram-negative bacteria. Susceptibility of *Staphylococcus aureus* was also tested against Penicillin and cefoxitin, where it was found to be 95% and 90% sensitive, respectively. Vancomycin was tested by minimum inhibitory concentrations using E-strip method. No resistance was observed in *Staphylococcus aureus against vancomycin however,* 0.5% vancomycin-resistant enterococcus spp were observed. *Pseudomonas aeruginosa* and Enterobacterales were found to have variable susceptibility to antimicrobials tested including Ceftazidime, Gentamicin, Imipenem, Ceftriaxone, cefepime, and Ciprofloxacin.

Conclusion: Bacterial profile and susceptibility pattern of microorganisms isolated from chronic suppurative otitis media should be carried out routinely which can help establish an antibiogram as well as guide empirical therapies.

Keywords: Antibiogram, Antibiotic stewardship, CSOM, Ear swab culture

BACKGROUND

Chronic suppurative otitis media (CSOM) manifests as persistent irritation of the middle ear, accompanied by tympanic membrane perforation and continuous ear discharge. These symptoms can persist for up to six weeks, highlighting the chronic nature of the condition.[1] Chronic otitis media with effusion (OME) is characterized by the presence of middle ear effusion without acute signs of infection. While OME may originate from acute otitis media, it differs in its chronicity and lacks the acute inflammatory manifestations. This condition is often associated with conductive hearing loss due to the accumulation of fluid in the middle ear space.[2]

Approximately, 31 million CSOM cases are encountered per year. It's a prevalent illness in nations with little resources due to limited access to healthcare, poor hygiene and sanitation practices, lack of education on ear care, underlying socioeconomic factors, and inadequate infrastructure for diagnosis and treatment.[3,4] Children under the age of two are most susceptible to

Correspondence: Dr. Saira Salim, Consultant Pathologist, Islamabad Diagnostic Center, Wah Cantt Taxila, Rawalpindi Pakistan

Email: sairasalim2010@hotmail.com

This article can be cited as: Iqbal N, Anwar S, Salim S, Tehseen T, Ali S, Raza A. Antibiogram of chronic suppurative otitis media in relation to the current bacteriological profile in a tertiary care hospital. Infect Dis J Pak. 2024; 33(2): 69-73. DOI: ----

Receiving date: **25 Feb 2024** Acceptance Date: **14 May 2024**
Revision date: **04 Apr 2024** Publication Date: **28 Jun 2024**

CSOM due to a short ear canal, however, older children and adults have been found to have higher rates of the condition.[5,6]

There are two variants of CSOM. Attico-antral type and Tubo-tympanic type.[7] Microorganisms such as *Staphylococcus aureus,* CONS, *Pseudomonas aeruginosa, Proteus mirabilis, Enterococcus spp.,* and others are frequently isolated.[8] The symptoms of CSOM include middle ear discharge that usually lasts longer than six weeks and hearing loss brought on by tympanic membrane perforation.[9] CSOM is considered the most common cause of preventable hearing loss. Extracranial and intracranial issues might result in severe, sometimes fatal side effects from insufficiently treated CSOM.

Analyzing the bacteriological profile of microbes causing CSOM and their susceptibility to existing antibiotics is crucial for guiding effective treatment strategies. By identifying the specific pathogens responsible for CSOM and understanding their antibiotic susceptibility patterns, clinicians can tailor treatment plans to target the causative organisms more effectively. This approach not only improves treatment outcomes but also helps mitigate the risk of antibiotic resistance and the development of complications associated with inadequately treated CSOM. Through our analysis, we aim to provide valuable insights into the microbial landscape of CSOM and inform evidence-based antibiotic prescribing practices for better patient care.

MATERIAL AND METHODS

This descriptive cross-sectional study was carried out at the Department of Microbiology, Izzat Ali Shah Hospital Wah Cantt, from December 2022 to January 2024. We determined a sample size of 105 by keeping the prevalence of CSOM is 275.[4] After approval of the IRB, informed consent was obtained from all the patients included in the study.

Patients who had not received any systemic or local antibiotics for seven days before sample collection and who experienced unilateral, bilateral, chronic, or intermittent ear discharge through a perforated tympanic membrane for more than 12 weeks duration were included in the study. Duplicate samples, patients taking antibiotics before sample collection and patients with acute suppurative otitis media, chronic otitis media with effusion, and otitis externa were excluded from the study. Contaminated samples yielding mixed growths were also excluded from the study. With a sterile cotton-tipped swab stick, two aseptic aural swabs were taken from each patient's discharging ear, one for microscopy and the second for bacterial culture. After the auditory canal was cleared with 70% ethanol, a sterile aural speculum was used to direct insertion of a swab stick into the middle ear to collect pus without coming into contact with the surrounding skin. The sterile tubes containing swabs were transported to microbiology lab as soon as possible and processed according to lab protocols.

Microscopy: A uniformly thin smear was prepared on a sterile glass slide from each swab. It was lightly flame-fixed and then air-dried. Gram staining was performed and the smear was then examined under oil immersion (x100) lens for presence of any pus cells and identifiable microorganisms.

Culture and Identification of Bacteria: The swab was inoculated onto Blood and MacConkey agar plates (Oxoid, UK) and incubated in ambient air at 37°C for 24-48 hours. Any bacterial growth was identified using morphological characteristics of the growth and microscopy (gram reaction, shape, organization). Gram-positive isolates were subjected to catalase and coagulase tests to differentiate between different Staphylococcus and Enterococcus spp. Gram-negative isolates were further identified by lactose fermentation properties, oxidase, citrate utilization, motility, indole, urease, and triple sugar iron tests. Antibiotic susceptibility of isolated bacterial pathogens was evaluated using the modified Kirby Bauer disc diffusion method, in compliance with the guidelines provided by the Clinical and Laboratory Standard Institute (CLSI)2024.[10] For each isolate, a 0.5 McFarland standard solution was prepared in sterile normal saline by mixing a loopful of well-isolated colonies, taken from blood agar plate after a growth of at least 18-24 hours. Mueller-Hinton agar (Oxoid, UK) was lawned by dipping a sterile swab in a prepared solution. A variety of commercially available antibiotic discs (Oxoid, UK) were placed on the media and incubated at 37°C in ambient air for 24 hours. According to CLSI criteria, the results were recorded as resistant and sensitive after observing zone diameter formed around all the tested antibiotic discs. Gram-positive organisms were tested for susceptibility against the following antibiotics: amoxicillin/clavulanate (20/10 µg), cefoxitin (30 µg), ampicillin (30 µg), ceftriaxone (30 µg), clindamycin (02

µg), ciprofloxacin (05 µg), gentamicin (10 µg), imipenem (10 µg), linezolid (30 µg), and vancomycin (30 µg). Gram-negative isolates were tested for susceptibility against amoxicillin/clavulanate (20/10 µg), Ceftazidime (10 µg), Gentamicin (10 µg), Imipenem (10 µg), Ceftriaxone (30 µg), cefepime (30 µg), and Ciprofloxacin (5 µg). *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were used as QC strains for agar medias as well as for antimicrobials.

Data was analyzed using statistical package for social sciences, SPSS version 23. Frequencies and percentages were calculated for categorical variables. Chi-square test used as the test of significance keeping a significant p-value of <0.05.

RESULTS

A total of 105 ear swab samples were included in the study. Gender distribution of all included patients is shown in Table-I.

Out of 105 cultured samples, 70 yielded pure growth of various microorganisms. 25 samples did not yield any growth after 48 hours of incubation while 10 samples yielded contaminated growth. *Pseudomonas aeruginosa* and *S. aureus* were the predominant isolates (30% and 20% respectively). Other isolated organisms included Enterococcus spp, Coagulase-negative staphylococcus spp. (CONS) and Enterobacterales including Klebsiella spp., Proteus spp., and *E. coli*. Frequency and percentages of all organisms isolated is shown in Table-II. The Antibiotic sensitivity profile of microorganisms is shown in Table-III. *P. aeruginosa* was found to be most sensitive to Tazobactam-piperacillin (TZP) i.e. > 90%. Amikacin was the most effective antibiotic observed after carbapenems. However, Gentamicin was not found to be as susceptible as Amikacin. Sensitivity to Ciprofloxacin and Ceftazidime was also observed to be low. *S. aureus* demonstrated ≥90% sensitivity to both Vancomycin and linezolid where Vancomycin was tested by E-strip while Linezolid was tested by disc diffusion method. Cefoxitin disc diffusion method was used to differentiate between Methicillin-sensitive (MSSA) and Methicillin-resistant *Staphylococcus aureus* (MRSA). About 95% of isolates were MSSA.

Table-I: Gender distribution of patients (n=105).

Gender	n (%)
Females	34 (32.39%)
Males	71 (67.61%)
Total	**105**

Table-II: Percentages and frequencies of isolated organisms (n=70).

CSOM EAR SWABS	Microbiota n (%)
Staphylococcus aureus	30(42.85%)
***Enterococcus* spp.**	5(7.1%)
Enterobacterales (***E. coli, K. pneumoniae, Enterobacter cloacae, Proteus mirabilis***)	10(14.28%)
Pseudomonas aeruginosa	20(28.57%)
CONS	5(7.1%)
Total	**70(100.0%)**

Table-III: Antibiogram of CSOM (% susceptible).

Antimicrobials	*Staphylococcus aureus* & CONS	*Enterococcus spp*	Enterobacterales	*Pseudomonas aeruginosa*
Ampicillin	40%	98%	Not tested	Not tested
Cefoxitin	95%	-	Not tested	Not tested
vancomycin	100%	99%	Not tested	Not tested
linezolid	100%	100%	Not tested	Not tested
Erythromycin	90%	-	Not tested	Not tested
Gentamycin	90%	Not tested	80%	87%
ciprofloxacin	100%	100%	80%	60%
amikacin	Not tested	100%	100%	100%
Piperacillin-tazobactam	-	-	-	100%
Meropenem	-	-	100%	100%
ceftriaxone	-	-	100%	Not tested
Trimethoprin-sufamethoxazole	100%	-	100%	Not tested

DISCUSSION

The majority of studies on similar topics regard *S. aureus* to be the most prevalent bacterium. According to our research, *S. aureus* is also the most prevalent isolate. The second-most prevalent isolate in this study was *P. aeruginosa*. *P. aeruginosa* colonizes the auditory canal more frequently as compared to other bacteria due to several advantageous features, including a low nutritional need for life and relative resistance to antibiotics and their byproducts, and the ability to form biofilm. The organism is shielded from the typical host defense systems and antibiotic drugs by the devitalized tissue, disrupted circulation, and damaged epithelium that make up the niche. The organism also thrives in the external auditory canal, functions as an opportunistic pathogen, and may result in suppurative illness in adjacent areas[11] Enterobacterales including *Citrobacter spp., Enterobacter spp., Proteus spp., E. coli, and Klebsiella spp*. were among the other bacterial isolates. Gram-positive isolates other than *Staphylococcus aureus* included CONS and Enterococcus spp. Similar growth patterns were observed in several other studies conducted in Bangladesh and China.[14,15] Growth of polymicrobial organisms was also frequent.[12, 13]

Because of bacterial, environmental, and host variables, CSOM is most likely a complex illness. Bacteria from the external auditory canal that enter through a ruptured tympanic membrane can induce CSOM.[16] Use of contaminated water for bathing/showering may also play a role in pathogenesis of CSOM.[16-18] Pathogenic factors leading to CSOM may include bacterial biofilm formation.[12] Additional variables include increased proinflammatory cytokines, reduced middle ear ciliary activity, and overproduction of mucin.[18,19]

The antibiogram analysis of chronic suppurative otitis media (CSOM) in our research study revealed Staphylococcus aureus susceptibility to Ampicillin and Penicillin at 90%, and to cefoxitin at 95%, with 100% sensitivity to all other antimicrobials tested. For Enterococcus, susceptibility to ampicillin and penicillin was 99%, while vancomycin exhibited 99.5% sensitivity, with all other antibiotics showing complete sensitivity. Enterobacteriaceae and Pseudomonas demonstrated susceptibility to all antimicrobials in the antibiogram. This study's findings were compared with a similar investigation conducted in Pakistan and another in Malaysia.[20,21]

Timely and appropriate treatment of upper respiratory tract infections (URTI) is paramount to prevent the progression to community-acquired severe otitis media (OSOM). Incomplete courses of therapy contribute to the emergence of resistant organisms, underscoring the importance of completing prescribed treatments. Physicians should prescribe antibiotics with broad-spectrum coverage, targeting both Gram-positive and Gram-negative bacteria, along with anaerobic organisms. Accessible culture results are essential for accurate antibiotic de-escalation. In regions where first-line antibiotic resistance is prevalent due to irrational and excessive antibiotic use, last-resort medications may be the only viable treatment options. Therefore, promoting awareness and adherence to appropriate antibiotic stewardship practices is crucial in combating antimicrobial resistance and improving patient outcomes in URTI and associated conditions like OSOM.

CONCLUSION

The significant predominance of microbiota in the bacteriological profile of chronic suppurative otitis media (CSOM) underscores the challenge of antibiotic susceptibility. To address this, it's crucial to stay abreast of recent shifts in bacteriological profiles and tailor antibiotic prescriptions based on sensitivity patterns. Utilizing local antibiograms for antibiotic selection is paramount, with a focus on judicious antibiotic use. Regular assessments of microbiological profiles and antibiotic sensitivities are necessary to develop and update local antibiograms, providing valuable empirical guidance in clinical practice.

CONFLICT OF INTEREST

None

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHOR CONTRIBUTION

Naila Iqbal: Main idea
Saba Anwar: Data collection
Saira Salim: Data analysis
Tahira Tehseen: Result writing
Saleha Ali: Discussion writing
Asif Raza: Proof reading

REFERENCES

1. van den Broek M. In vitro and in vivo probiotic potential of Lactobacillus spp. for otitis media: University of Antwerp; 2018.
2. Roland PS. Chronic suppurative otitis media: a clinical overview. Ear Nose Throat J. 2002 Aug;81(8 Suppl 1):8-10. PMID: 12199189.
3. Khan SA, Khan N, Iqbal M, Khan S, Hussain G. Bacteriological study of discharging ear in patients of active mucosal chronic otitis media attending a tertiary are hospital. J Saidu Medical College, Swat. 2019; 9(1): 38-41.
4. Master A, Wilkinson E, Wagner R. Management of chronic suppurative otitis media and otosclerosis in developing countries. Otolaryngol Clin North Am. 2018; 51(3): 593-605. DOI: https://doi.org/10.1016/j.otc.2018.01.017
5. Chauhan J, Nautiyal S. Bacterial and fungal profile in chronic suppurative otitis media in a tertiary care hospital in Uttarakhand. IOSR J Pharmacy Biological Sci. 2019;14(1):38-44.
6. Yadav K, Kaushik S, Rani K, Tyagi AK. Bacterial profile and antimicrobial susceptibility pattern of chronic suppurative otitis media from a Tertiary Care Hospital in Kannauj, Uttar Pradesh, India. J Clin Diag Res. 2021;15(4): DC05-DC08. DOI: https://doi.org/10.7860/JCDR/2021/48334.14770
7. Taoussi AA, Malloum MSM, Ali YA. Prevalence and clinico-bacteriological aspects of chronic suppurative otitis media at the Renaissance University Hospital in N'Djamena, Chad. Egypt J Otolaryngol. 2023; 39(1): 72. DOI: https://doi.org/10.1186/s43163-023-00437-9
8. Lathi OP, Sharma A, Maheshwari M. Surveillance of antimicrobial susceptibility pattern of chronic suppurative otitis media in Tertiary Care Hospital of Southern Rajasthan. Indian J Otolaryngol Head Neck Surg. 2023;75(Suppl 1):588-95. DOI: https://doi.org/10.1007/s12070-023-03648-x
9. Mohamed IA, Mohamed ZA, Ning F, Xin W. The Prevalence and Risk factors associated with otitis media in children under five years of age in Mogadishu, Somalia: A hospital-based cross-sectional study. Int J Otolaryngol Head Neck Surg. 2023;12(6):426-43. DOI: https://doi.org/10.4236/ijohns.2023.126046
10. Veeraraghavan B, Bakthavatchalam YD, Sahni RD. Lessons learned from an external quality assurance program in applying CLSI interpretive criteria for reporting piperacillin/tazobactam susceptibility. Indian J Med Microbiol. 2024; 48: 100552. DOI: https://doi.org/10.1016/j.ijmmb.2024.100552
11. Sharma M, Ray B, Sahu RK, Raman S, Bagga RV. A study of prescription pattern in the drug therapy of CSOM at a tertiary care hospital in eastern part of India. Int J Otorhinolaryngol Head Neck Surg. 2017;3:188-91.
12. Reiss M, Reiss G. [Suppurative chronic otitis media: etiology, diagnosis and therapy]. Med Monatsschr Pharm. 2010; 33(1): 11-6; quiz 7-8.
13. Morris P. Chronic suppurative otitis media. BMJ Clin Evid. 2012; 2012.
14. Khatun MR, Alam KMF, Naznin M, Salam MA. Microbiology of chronic suppurative otitis media: An update from a Tertiary Care Hospital in Bangladesh. Pak J Med Sci. 2021; 37(3): 821-6. DOI: https://doi.org/10.12669%2Fpjms.37.3.3942
15. Xu J, Du Q, Shu Y, Ji J, Dai C. Bacteriological profile of chronic suppurative otitis media and antibiotic susceptibility in a Tertiary Care Hospital in Shanghai, China. Ear Nose Throat J. 2021; 100(9): Np391-np6. DOI: https://doi.org/10.1177/0145561320923823
16. Brennan-Jones CG, Head K, Chong LY, Burton MJ, Schilder AG, Bhutta MF. Topical antibiotics for chronic suppurative otitis media. Cochrane Database Syst Rev. 2020; 1(1): Cd013051. DOI: https://doi.org/10.1002%2F14651858.CD013051.pub2
17. Restuti RD, Sriyana AA, Priyono H, Saleh-Saleh RR, Airlangga TJ, Zizlavsky S, *et al.* Chronic suppurative otitis media and immunocompromised status in paediatric patients. Med J Malaysia. 2022;77(5):619-21.
18. Xia A, Thai A, Cao Z, Chen X, Chen J, Bacacao B, et al. Chronic suppurative otitis media causes macrophage-associated sensorineural hearing loss. J Neuroinflammation. 2022;19(1): 224. DOI: https://doi.org/10.1186/s12974-022-02585-w
19. Acuin J, Smith A, Mackenzie I. Interventions for chronic suppurative otitis media. Cochrane Database Syst Rev. 2000(2): Cd000473. DOI: https://doi.org/10.1002/14651858.cd000473
20. Wan Draman WNA, Md Daud MK, Mohamad H, Hassan SA, Abd Rahman N. Evaluation of the current bacteriological profile and antibiotic sensitivity pattern in chronic suppurative otitis media. Laryngoscope Investig Otolaryngol. 2021;6(6):1300-6. DOI: https://doi.org/10.1002%2Flio2.682
21. Jamil R, Sajjad A, Arshad F, Khawaja A, Siddiqui GM, Ullah R. Bacterial etiology of chronic suppurative otitis media of patients attending a tertiary care teaching hospital. Med Forum. 2020; 31 (9): 63-66.

ORIGINAL ARTICLE

To assess the diagnostic accuracy of LRINEC Score for prediction of necrotizing fasciitis in patients presenting with clinical skin and subcutaneous tissue infections

Muhammad Riaz Shahid[1], Usman Ghani[1], Muhammad Ayub Ashraf[2], Fahd Rashid[1], Muhammad Shahid[1], Fahim Khattak[1]

[1]Combined Military Hospital, Kohat Pakistan
[2]Combined Military Hospital, Lahore Pakistan

ABSTRACT

Background: Necrotizing fasciitis is an inflammatory disease that causes the skin, soft tissues, and fascia to break down. A strain of Streptococcus pyogenes bacteria is frequently the source of it, however mixed infections involving coliforms, anaerobes, and Gram-negative bacteria can also be to blame. Even with contemporary medical care, necrotizing fasciitis is associated with a significant fatality rate. The objective of this study is to determine the diagnostic accuracy of the Laboratory Risk Indicator for Necrotizing Fasciitis (LRINEC) score for predicting necrotizing fasciitis in patients with cutaneous and subcutaneous tissue infections.

Material and Methods: This Cross-sectional study was conducted at Combined Military Hospital, Rawalpindi from May 2021 to October 2022. Hundred cases of probable necrotizing fasciitis coupled with clinical cutaneous or subcutaneous infections were included. They underwent clinical examinations and blood investigation (Sodium, Hemoglobin, total white cell count, Glucose, Creatinine, C-reactive protein (CRP), and biopsy for histopathology). Patients under the age of 15 or older than 75, with soft-tissue infections, undergone surgical debridement and without the result of CRP in the initial 48 hours of retention were excluded. LRINEC score ≥6 was used for labelling a case with necrotizing fasciitis. LRINEC score was then compared with results of histopathology and sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated using SPSS version 23.

Results: 35 (35%) out of total belonged to the age group between 46-55 years. Male and female ratio were 64 (64%) and 36 (36%). Diabetes mellitus was the most common etiological cause occurring in 56 (56%) patients. The sensitivity of the LRINEC scoring system was 79.7% and the specificity noted was 87.8%. The PPV was 90.4% and the NPV came out to be 75%**.**

Conclusions: LRINEC scoring system has a significant sensitivity and reasonable specificity in diagnosing cases of Necrotizing fasciitis among patients with severe soft-tissue infection. LRINEC score can be used for predicting the outcome of such cases.

Keywords: Laboratory risk indicator for necrotizing fasciitis (LRINEC), Necrotizing fasciitis, Subcutaneous tissue infections

BACKGROUND

An inflammatory condition characterized by the destruction of fascia, skin, and soft tissues is known as necrotizing fasciitis. It is often caused by a strain of Streptococcus pyogenes bacteria[1] but may also be caused by mixed infections including anaerobes, coliforms, and Gram-negative bacteria.[2] There is a high mortality rate associated with necrotizing fasciitis, even with modern medical care.[3]

People with advanced age, diabetes, obesity, immunosuppression, smoking, malnutrition, and steroid therapy are more likely to have the disease.[4] 52.1% to 70.8% of patients with necrotizing fasciitis are caused or influenced by diabetes.[5] Intoxicated individuals often develop painful, red, and gangrenous skin due to diminished blood circulation, which can facilitate the spread of infection along the fascial plane. The infectious process can rapidly spread, causing infection of the fascia and perifascial planes as well as secondary infection of the overlying and underlying skin, soft tissue, and muscle.[6]

Necrotizing fasciitis is difficult to diagnose because of its overlapping clinical presentation of certain soft tissue diseases e.g., cellulitis. Physicians have had difficulty diagnosing it for decades. Although advances in

Correspondence: Dr. Muhammad Riaz Shahid, Registrar Surgery, Combined Military Hospital, Kohat Pakistan

Email: ali_riaz2515@yahoo.com

This article can be cited as: Shahid MR, Ghani U, Ashraf MA, Rashid F, Shahid M, Khattak F. To assess the diagnostic accuracy of LRINEC Score for prediction of necrotizing fasciitis in patients presenting with clinical skin and subcutaneous tissue infections. Infect Dis J Pak. 2024; 33(2): 74-78

DOI: https://doi.org/10.61529/idjp.v33i2.313

Receiving date: **09 Apr 2024** Acceptance Date: **03 Jun 2024**
Revision date: **14 May 2024** Publication Date: **28 Jun 2024**

treatment have greatly reduced necrotizing fasciitis mortality, still, it is high at 20 to 30%.[7]

It is possible to reduce morbidity and mortality rates of necrotizing fasciitis by early diagnosis and surgical treatment. In simple terms, the longer the delay, the more tissue is lost, and sepsis occurs, resulting in a higher death rate. Due to the lack of clinical symptoms of necrotizing fasciitis on the onset, patients with necrotizing fasciitis today have a high mortality rate which is increased by a lack of early diagnosis and treatment. Reducing the interval between presentation and definitive treatment can help in improved patient outcomes and this can be achieved by diagnosing the case early with accurate tests.[8]

An easy-to-follow standardized scoring system with high positive and negative predictive values is needed for early diagnosis of necrotizing fasciitis that can help us to treat the cases timely. According to Wong *et al.*[9], of A Laboratory Risk Indicator for Necrotizing Fasciitis (LRINEC) scoring system in 2004, it demonstrated a 92% positive predictive value and a 96% negative predictive value for diagnosis necrotizing fasciitis. In their original proposal, Wall *et al.*[10] suggested Lrinec scoring system in 2000, which was then modified by Wong *et al.*[9] in 2004.

LRINEC scores are determined by the following few hematological and biochemical laboratory tests routinely performed in a laboratory. These include total white cell count, creatinine, hemoglobin, serum sodium, plasma glucose level, and C reactive protein (CRP).

We planned our study keeping in mind that our country Pakistan is a developing country with a high disease mortality rate (70%).[3] Our resources are scarce. So, we are in dire need of early diagnosis of necrotizing fasciitis that can help us to treat the cases timely, therefore, help us to reduce mortality and morbidity. So, we designed this study to determine the diagnostic accuracy of the LRINEC score in predicting necrotizing fasciitis in cutaneous and subcutaneous infections.

MATERIAL AND METHODS

A total of 100 patients during a period of 18 months (May 2021 to October 2022) were recruited in this cross-sectional study after taking approval from the institutional review board (IRB) via reference number 291 at the surgical department of Combined Military Hospital Rawalpindi. After a thorough literature search and by using the WHO calculator a sample size of 23 was obtained, keeping a 5% margin of error, 95% confidence level, prevalence of 0.015%, and power of the test of 80%.[11] A maximum number of available participants during the study period were recruited because a large number of cases of Necrotizing Fasciitis with Clinical Skin and Subcutaneous Tissue Infections were reported.

Patients who were suspected of having necrotizing fasciitis with clinical skin and subcutaneous tissue infections and had symptoms that suggested soft tissue infections underwent clinical examination and blood investigations. Patients with ages> 15 years and < 75 years belonging to either gender were included.

Patients under the age of 15 or older than 75. Patients who took a minimum of three antibiotic doses before a presentation or were on antibiotic therapy for the last 48 hours were also excluded. Cases with soft-tissue infections who have undergone surgical debridement were excluded too. Patients with burns or furuncles that do not appear to have cellulitis and patients without CRP results within the first 48 hours of reporting to hospital were excluded from the study.

Informed consent was obtained from all study participants and research was explained to them. Then after a thorough history taking, all consenting necrotizing fasciitis patients underwent investigations: A 5ml venous blood was obtained for analysis of Sodium, Total white cell count, Hemoglobin, Creatinine, C-reactive protein (CRP). Biopsy was obtained for histopathology. All laboratory investigations were conducted as per documented standard operating procedures of the institute. LRINEC ≥ 6 was used for establishing a diagnosis of necrotizing fasciitis. By using a 2×2 table data was entered in SPSS version 23 and was analyzed. Positive and negative predictive value (PPV, NPV) was determined. The gold standard test used for labeling patients with necrotizing fasciitis irrespective of the result of the LRINEC scoring system as a result of biopsy for histopathology. LRINEC scoring system used is shown in Table-I as below.

RESULTS

Out of 100 patients suspected of having necrotizing fasciitis, 35 (35%) belonged to the age group between 46-55 years indicating that soft tissue infections occur more with advancing age. 64 (64%) patients were male and 36 (36%) were females. Diabetes mellitus was the most common etiological cause occurring in 56 (56%)

patients followed by trauma which was reported in 29 (29%) patients. Details are given in Table-II.

In LRINEC scoring high Random blood glucose levels was the most common abnormality associated with Necrotizing fasciitis having been reported in 75 (75%) patients. The second most common abnormality was Hb <13 g/dl, which was present in 68 (68%) of the total 100 (100%) patients, followed by Sodium – 61 (61%), TLC-53 (53%), CRP-52 (52%) and Creatinine in 44 (44%) patients was reported as abnormal. Out of 100, 42 (42%) patients LRINEC score was ≤5, and 58 cases (68%) had LRINEC scores≥6 as shown in Figure-1-A and 1-B below.

In a comparison of the result of histopathology reports with the LRINEC scoring system, 47 (47%) cases were noted as true positive (TP) whereas 36 (36%) patients were True negatives (TN). Only 12 (12%) patients were reported to be false positives and 5 (5%) as false negatives in comparison with histopathology. In our study sensitivity was calculated to be 79.7% and specificity as 87.8%. PPV was 90.4% and NPV came out to be 75% as shown in Table-III.

Table-I: LRINEC scoring scale used.

Laboratory Parameters	LRINEC Score
CRP	
< 150 mg/l	0
≥ 150	4
Total white cell count (microliter)	
<15	0
15-25	1
>25	2
Hemoglobin (g/dl)	
>13.6	0
11-13.5	1
<10.9	2
Sodium (mmol/l)	
≥135	0
<135	2
Serum creatinine (mg/dl)	
<1.6 or = 1.6	0
>1.6	2
Glucose (mg/dl)	
<180 or = 180	0
>180	1

Table-II: Etiological classification of necrotizing fasciitis.

Etiology	Frequency	Percentage
Unknown	3	3.0%
Diabetes	56	56.0%
Trauma	29	29.0%
Bites	6	6.0%
CKD	6	6.0%
Total	**100**	**100.%**

Table-III: Comparison of histopathology results with LRINEC scoring system for diagnosing necrotizing fasciitis.

Histopathology	LRINEC scoring system		p-Value
	Positive	Negative	
Positive	47 (47.0%) TP	5 (5.0%) FN	<0.0001
Negative	12 (12.0%) FP	36 (36.0%) TN	

Sensitivity= TP/(TP+FN)= 47/(47+5)*100=79.7%
Specificity= TN/(TN+FP)= 36/(36+12)*100=87.8%
Positive Predictive Value= TP/(TP+FP)*100= 47/(47+12)= 90.4%
Negative Predictive Value= TN/(TN+FN)*100=36/(36+5)= 75.0%

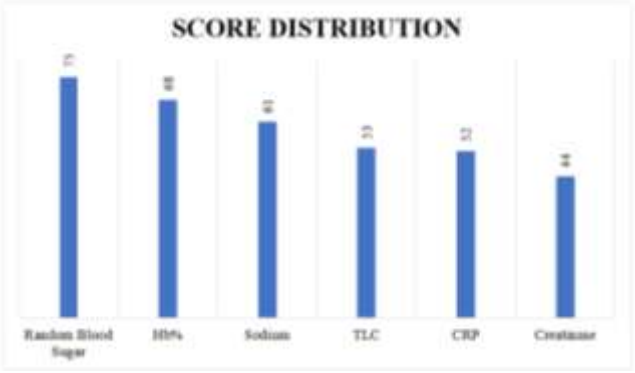


Figure-IA: Showing LRINEC Score abnormalities in patients

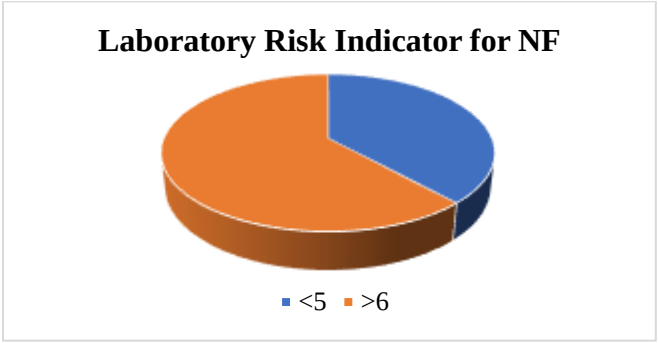


Figure-1B: Showing Risk Indicator for necrotizing fasciitis.

DISCUSSION

Necrotizing fasciitis is a rapidly progressive inflammatory infection of the deep fascia associated with a high morbidity and mortality. The infection may follow trauma or occur around foreign bodies in surgical wounds. Diabetes, trauma, and immunodeficiency states are the most common risk factors for necrotizing fasciitis. Early diagnosis and prompt treatment with antibiotics can significantly reduce patient grievances. Necrotizing fasciitis still has significant morbidity and mortality, despite the latest advances in laboratory diagnosis and pharmaceutical therapies. The application

of a biochemical-based scoring system may lead to an improved diagnostic process.[12]

The clinical importance of the LRINEC score for early detection of necrotizing fasciitis has been evaluated by many studies However, few studies are available in which the LRINEC score has been evaluated for diagnosing and predicting outcomes in necrotizing fasciitis. If the diagnosis of necrotizing fasciitis is based solely on LRINEC scoring it can cause a bias in the study because LRINEC scoring does not include a clinical picture of patients. Our study demonstrated that patients with Diabetes Mellitus and other comorbidities have abnormal baseline investigations. Chao *et al.* **[13]** demonstrated that an LRINEC score of 2% or greater than 2 was adaptive in 71% of cases with 11.9-fold increased risk for the presence of necrotizing fasciitis, 83% precise with 85% PPV. that the average LRINEC scoring of 2 or greater was 85% positive predictive, 83% precise, and 71% adaptive (P<0.0001). They also documented that LRINEC score and hemorrhagic bullous or blistering lesion are predictors of necrotizing fasciitis.

Su *et al.* [14] in a multicentered retrospective observational cohort study determined the relationship between LRINEC score and duration of hospital stay ultimately leading to morbidity or mortality. They included a total of 100 cases with LRINEC scores≥ 6 and compared them with 109 cases having LRINEC scores <6. They noted gross differences in amputation (p 0.02) and mortality (p 0.04) between the two groups. Corbin *et al.* [15] demonstrated that complication rate was high in patients with LRINEC score above 6 as compared to cases with LRINEC score < 6. The overall mortality rate of 15 patients was three in a study conducted by Swain et al. The overall LRINEC score determined by them in all death cases was 9 (range 6-13).**[16]** Another study was done by Colak *et al* **[17]** in the year 2015 in Turkey on 25 patients. There was a statistically significant increase in diabetic complications (52%), followed by peripheral circulatory disorders (24%). A similar frequency of Diabetes was noted in our study (56%) but in ours, trauma was the second most frequent etiological cause of necrotizing fasciitis. They also documented that LRINEC scores and mean the number of debridement were higher in the non-surviving group and concluded that the LRINEC score can be used to predict mortality in necrotizing fasciitis.

A literature review was conducted by Wong and Wang to highlight recent advances available for diagnosing necrotizing fasciitis. They discussed the diagnostic use of magnetic resonance imaging (MRI), ultrasound (USG), LRINEC score, tissue oxygen saturation, and frozen section. They documented a high PPV and NPV of LRINEC score and suggested using it frequently for diagnosing necrotizing fasciitis. On the whole, the quality of available evidence in favor of LRINEC may be low but none the less it has been shown that the LRINEC score is a useful tool in the diagnosis of necrotizing fasciitis and it is recommended that to evaluate its diagnostic accuracy further.[18]

LRINEC score comprises only laboratory data which is one of the weak points of this scoring system, where clinicians argue that it should include vital signs as well. Another point of discussion is that this score has two cut-off values, which has always been a source of confusion among clinicians.**[19]** It is important to note that the LRINEC score is an important diagnostic tool, but it requires more research and experimentation before it can be widely utilized as a screening tool. Until a standardized guideline is developed, clinicians should use other diagnostic modalities if necrotizing fasciitis is suspected until a scoring system with adequate accuracy can be utilized in clinical practice.

The aim of our study was to establish the validity of the LRINEC scoring system in diagnosing cases of necrotizing fasciitis by comparing it with the gold standard of histopathology reports. With considerable sensitivity and fairly high specificity, our study has shown that LRINEC scoring is an adequate, easy, and fast tool to assess the risk of developing necrotizing fasciitis in patients presenting with skin and subcutaneous tissue infections.

LIMITATION OF THE STUDY

This was a small, single-centered study with limitations on time and resources. We used only laboratory parameters and determined their validity in assessing the LRINEC score. Further multicentered studies with the inclusion of vital signs in addition to laboratory scores are suggested for a better diagnosis of necrotizing fasciitis.

CONCLUSION

LRINEC scoring system has a significant sensitivity and reasonable specificity. It can be used as an effective

diagnostic tool in labeling cases with necrotizing fasciitis. By using the LRINEC scoring system we can not only diagnose necrotizing fasciitis cases early but also manage and treat them promptly which can help us to reduce the overall morbidity and mortality of this disease. However, it is important to consider the clinical aspects of patients alongside the scoring system for effective management and improved outcomes in patients with necrotizing fasciitis.

CONFLICT OF INTEREST

None

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHOR CONTRIBUTION

Muhammad Riaz Shahid: Idea conception, study design, acquisition of data, Manuscript drafting

Usman Ghani: Data collection

Muhammad Ayub Ashraf: Substantial contributions to analysis and interpretation of data

Fahd Rashid: Substantial contributions to concept, study design, Critical review

Muhammad Shahid: Critical review, revisions

Fahim Khattak Manuscript drafting

REFERENCES

1. Tessier JM, Sanders J, Sartelli M, Ulrych J, De Simone B, Grabowski J, *et al*. Necrotizing soft tissue infections: A focused review of pathophysiology, diagnosis, operative management, antimicrobial therapy, and pediatrics. Surg Infect. 2020; 21(2): 81-93. DOI: https://doi.org/10.1089/sur.2019.219
2. Desta R, Yee J. Necrotizing fasciitis. JETem. 2020; 5(2): 1-25. DOI: https://doi.org/10.21980/J84M1D
3. Fernando SM, Tran A, Cheng W, Rochwerg B, Kyeremanteng K, Seely AJ, *et al*. Necrotizing soft tissue infection: diagnostic accuracy of physical examination, imaging, and LRINEC score: A systematic review and meta-analysis. Ann Surg. 2019; 269(1): 58-65. DOI: https://doi.org/10.1097/sla.0000000000002774
4. Sophy FJ, Devi RL, Anubhav MP. Validation of laboratory risk indicator for necrotizing fasciitis scoring system for diagnosis of necrotizing fasciitis in patients presenting with soft-tissue infections. Int J Sci Study. 2021; 8(10): 166-9.
5. Chen PC, Tsai SH, Wang JC, Tzeng YS, Wang YC, Chu CM, *et al*. An elevated glycemic gap predicts adverse outcomes in diabetic patients with necrotizing fasciitis. PLoS One. 2019; 14(10): e0223126. DOI: https://doi.org/10.1371/journal.pone.0223126
6. Harasawa T, Kawai-Kowase K, Tamura JI, Nakamura M. Accurate and quick predictor of necrotizing soft tissue infection: usefulness of the LRINEC score and NSTI assessment score. J Infect Chemother. 2020; 26(4): 331-4. Doi: https://doi.org/10.1016/j.jiac.2019.10.007
7. Kumar M, Lal M. Predictors of mortality in necrotizing fasciitis. Formosan J Surg. 2021; 54(2): 52-60. DOI: http://dx.doi.org/10.4103/fjs.fjs_43_20
8. Wei XK, Huo JY, Yang Q, Li J. Early diagnosis of necrotizing fasciitis: Imaging techniques and their combined application. Int Wound J. 2024; 21(1): e14379. DOI: https://doi.org/10.1111/iwj.14379
9. Wong CH, Khin LW, Heng KS, Tan KC, Low CO. The LRINEC (Laboratory Risk Indicator for Necrotizing Fasciitis) score: A tool for distinguishing necrotizing fasciitis from other soft tissue infections. Crit Care Med. 2004; 32(7): 1535-41. DOI: https://doi.org/10.1097/01.ccm.0000129486.35458.7d
10. Wall DB, de Virgilio C, Black S, Klein SR. Objective criteria may assist in distinguishing necrotizing fasciitis from non-necrotizing soft tissue infection. The Am J Surg. 2000; 179(1): 17-20. DOI: https://doi.org/10.1016/S0002-9610(99)00259-7
11. Choi HK, Seo GH, Han E. The incidence and seasonal variation of necrotizing fasciitis in Korea: A nationwide cross-sectional study. Clin Microbiol Infect. 2020; 26(8): 1090-e1. DOI: https://doi.org/10.1016/j.cmi.2020.01.002
12. Stevens DL, Bryant AE, Goldstein EJ. Necrotizing soft tissue infections. Infect Dis Clin North Am. 2021; 35(1):135-55. DOI: https://doi.org/10.1016/j.idc.2020.10.004
13. Chao WN, Tsai SJ, Tsai CF, Su CH, Chan KS, Lee YT, *et al*. The laboratory risk indicator for necrotizing fasciitis score for discernment of necrotizing fasciitis originated from Vibrio vulnificus infections. J Trauma Acute Care Surg. 2012; 73(6): 1576-82. DOI: https://doi.org/10.1097/ta.0b013e318270d761
14. Su YC, Chen HW, Hong YC, Chen CT, Hsiao CT, Chen IC. Laboratory risk indicator for necrotizing fasciitis score and the outcomes. ANZ J Surg. 2008; 78(11): 968-72. DOI: https://doi.org/10.1111/j.1445-2197.2008.04713.x
15. Corbin V, Vidal M, Beytout J, Laurichesse H, D'Incan M, Souteyrand P, *et al* . Prognostic value of the LRINEC score (Laboratory Risk Indicator for Necrotizing Fasciitis) in soft tissue infections: a prospective study at Clermont-Ferrand University hospital. Ann Dermatol Venereol 2009; 137 (1): 5-11. DOI: https://doi.org/10.1016/j.annder.2009.12.002
16. Swain RA, Hatcher JC, Azadian BS, Soni N, De Souza B. A five-year review of necrotising fasciitis in a tertiary referral unit. Ann R Coll Surg Engl. 2013; 95(1): 57-60. DOI: https://doi.org/10.1308/003588413X13511609956093
17. Colak E, Ozlem N, Kucuk GO, Aktimur R, Kesmer S. Laboratory risk indicators for necrotizing fasciitis and associations with mortality. Turk J Emerg Med. 2014; 14(1): 15-9. DOI: https://doi.org/10.5505/1304.7361.2014.55476
18. Wong C, Wang Y. The diagnosis of necrotizing fasciitis. Curr Opin Infect Dis. 2005; 18: 101–6. DOI: https://doi.org/10.1097/01.qco.0000160896.74492.ea
19. Harasawa T, Kawai-Kowase K, Tamura JI, Nakamura M. Accurate and quick predictor of necrotizing soft tissue infection: usefulness of the LRINEC score and NSTI assessment score. J Infect Chemother. 2020; 26(4): 331-4. DOI: https://doi.org/10.1016/j.jiac.2019.10.007

ORIGINAL ARTICLE

Susceptibility pattern of bacteria isolated from blood cultures of neonates admitted with sepsis at a tertiary care hospital

Saqib Munir, Mudassar Hussain, Shahid Rashid, Salman Arshad, Sidra Ijaz, Nadia Qamar, Abdul Sattar

Allama Iqbal Memorial Teaching Hospital (Khawaja Muhammad Safdar Medical College), Sialkot Pakistan

ABSTRACT

Background: Neonatal sepsis is one of the leading causes of neonatal mortality rate (NMR) in developing countries like Pakistan, where epidemiologic surveillance of organisms and their antimicrobial sensitivity patterns remains poor. The objective of this study is to identify common organisms implicated in neonatal sepsis and their susceptibility patterns as well as the emergence of antibiotic resistance at a tertiary care hospital.

Material and Methods: This Retrospective descriptive study, Blood culture reports of neonates admitted to the Nursery of Allama Iqbal Memorial Teaching Hospital (Khawaja Muhammad Safdar Medical College) with suspected sepsis from January 2020 to December 2022 were taken.

Results: Blood culture reports were positive in 303 (12.8%) out of 2368 neonates. The proportion of gram-negative and gram-positive micro-organisms were 278 (91.7%) and 25 (8.3%) respectively. *Acinetobacter baumannii* was the most predominant microorganism isolated (32.3%), followed by *E coli* (21.4%) and *Citrobacter freundii* (13.5%). Among the gram-negative microorganisms high level of resistance was seen with first-line agents, such as ampicillin (97.3%), third generation cephalosporins i:e cefotaxime (89.8%), while the majority of these organisms were sensitive to carbapenems (56.0%) and cefoperazone-sulbactam (53%). Methicillin resistant *Staphylococcus aureus* rate was 47.6% and no resistance to vancomycin and linezolid was detected.

Conclusion: This study shows the emergence of unusual gram-negative microorganisms causing neonatal sepsis and a high level of resistance to first-line empirical antibiotic therapy. This highlights the urgent need for the implementation of effective infections control program and antibiotics stewardship in neonatal ICU.

Keywords: Antibiotic susceptibility, Blood culture, Neonatal sepsis

BACKGROUND

Neonatal sepsis is a clinical syndrome in infants younger than 28 days of life manifested by nonspecific signs and symptoms due to invasion of microorganisms in the bloodstream.[2] Globally it is a common cause of morbidity and mortality among neonates with an estimated annual incidence of 22 per 1000 live births and an associated mortality rate of 11 to 19 percent.[2] It is accounted for an estimated 430,000 deaths worldwide which is approximately 15 percent of all-cause neonatal deaths in the year 2013[15], and 26 percent of neonatal deaths in resource-poor countries.[13] In Pakistan neonatal sepsis is one of the major causes of neonatal mortality rate accounting for 17.2 percent of total deaths.[4]

Microorganism causing neonatal sepsis are different among various regions of the world. In developed countries Group B *streptococcus* (a gram-positive organism) and *E. coli* are the most common organisms causing neonatal sepsis, [15,16,17,18] however literature from developing countries suggest that gram-negative organisms *including E. coli, Pseudomonas, Klebsiella pneumoniae, Acinetobacter species, Enterobacter species,* and *Neisseria meningitides predominate.*[3,4,7]

Early administration of broad-spectrum antibiotics is essential to decrease the morbidity and mortality associated with neonatal sepsis because blood culture and sensitivity takes time.[14] World Health Organization guidelines for suspected neonatal sepsis recommends empirical treatment with ampicillin combined with gentamicin as the first line therapy.[3,4,14] However antimicrobial resistance (AMR) has emerged globally rendering these first line regimens ineffective and is responsible for increase in morbidity and mortality.

Correspondence: Dr. Saqib Munir, Assistant Professor, Allama Iqbal Medical Teaching Hospital, Sialkot Pakistan

Email: saqibmunir13086@gmail.com

This article can be cited as: Munir S, Hussain M, Rashid S, Arshad S, Ijaz S, Qamar N, Sattar A. Susceptibility pattern of bacteria isolated from blood cultures of neonates admitted with sepsis at a tertiary care hospital. Infect Dis J Pak. 2024; 33(2): 79-85.
DOI: https://doi.org/10.61529/idjp.v33i2.237

Receiving date: **21 Jul 2023** Acceptance Date: **25 Jun 2024**
Revision date: **21 May 2024** Publication Date: **30 Jun 2024**

According to a study an estimated 31 percent of deaths in neonatal sepsis are attributed to this rising antimicrobial resistance.[12] Globally around 214,000 neonates die each year due to this antimicrobial resistance.[40] Injudicious use of antibiotics and lack of local antibiograms are among the causes of this rising trend of antimicrobial resistance.[4,12,14] Data from Pakistan shows that an estimated 25,692 neonates succumb to resistant sepsis each year.[4]

Predominant organisms and their sensitivity pattern differ among different regions and also in the same region over time.[1,14] Therefore, it is important to know the bacterial isolates and their sensitivity patterns not only at country level but also at local levels. Because neonatal mortality rate is very high in Pakistan (42/1000 live births)[4] and sepsis is one of the major causes, such evidence-based data will help clinicians to formulate effective empirical antibiotic regimen. Present study was conducted to describe the microbial pattern of neonatal sepsis as well as build the current antibiogram that will help the local practioners to use effective empirical antimicrobial treatment

MATERIAL AND METHODS

This was a retrospective descriptive study conducted at Allama Iqbal Memorial Teaching Hospital (Khawaja Muhammad Safdar Medical College) from January 2020 to December 2022, after taking the approval of ethical review board via reference number 113/REC/KMSMC, that analyzed data of blood culture reports of neonates admitted in NICU with suspected sepsis. Culture and sensitivity reports were obtained from hospital record system. A total record of 2368 neonates with suspected sepsis was collected during the study period. All positive blood culture were included in the study however in the case of a new blood culture taken after 5 days from the initial culture, it was classified as a new episode of suspected sepsis and if a neonate medical record showed a positive blood culture for a different pathogen within 48 hours after the initial positive culture, and contamination has been ruled out, it was considered a new episode of Neonatal sepsis case. The initial control blood cultures reports taken within 5 days of the first positive blood culture to verify the effectiveness of treatment were excluded. Additionally, confirmatory blood cultures were excluded if taken to rule out or confirm contamination. Duplicate medical records of blood cultures for the same neonate at the same time of specimen collection, were also excluded. The total excluded medical records were 5950; Blood culture samples were positive in 303 (12.8%) neonates while remaining 2065 (87.2%) were culture negative.

Blood samples were inoculated in manual blood culture bottles and incubated at 37 degrees Celsius aerobically for 24 hours. Periodic subcultures were done on blood and MacConkey agar on day two, three and four followed by incubation at 37 degrees Celsius. The growth obtained was identified by colony morphology, gram staining and biochemical testing was done as per standard protocols. Antibiotic susceptibility testing of isolated microorganisms was performed by modified Kirby-Bauer disc diffusion method on Mueller Hinton agar plates as recommended by clinical Laboratory Standard Institute (CLSI) recommendations.

Data were presented as descriptive statistics (frequency tables, charts and percentages). Data analysis was carried out using Microsoft Excel 2023 version 16.72.

RESULTS

A total of 2368 neonates with suspected sepsis had their blood samples collected during the study period. Blood culture samples were positive in 303 (12.8%) neonates while remaining 2065 (87.2%) were culture negative (Figure-I). The number of isolated microorganisms were 278 (91.7%) gram negative, 25 (8.3%) gram positive and 6 (2.0%) fungal.

Among the gram-negative isolates *Acinetobacter baumannii* was the predominant organism (32.3% of the total isolates) followed by *E coli* (21.4%), *Citrobacter freundii* (13.5%), *Burkholderia Capacia* (8.3%), and *Enterobacter cloacae (*7.6%). Among gram positive isolates *Coagulase negative Staph.(CoNS)* was predominant which constituted 6.9% of the total isolates followed by *Staph aureus* (1.3*%). Candida Albicans* was detected in 6 cultures which constitutes 2.0% of total isolates (Figure-II).

Antimicrobial susceptibility pattern of gram-positive isolates revealed that rate of *Coagulase negative staph* was 47.6% whereas all four *Staph aureus* isolated were methicillin resistant. No resistance to vancomycin and linezolid was detected in gram positive isolates however all the isolates were resistant to ampicillin. Sensitivity of gram-positive organisms to chloramphenicol, clindamycin and Amoxiclav was 83.3%, 63.7 % and 26.7 % respectively (Table-II). Sensitivity to tetracyclines was 92.9 percent but these antibiotics are not routinely used in neonatal sepsis.

Most of the gram-negative microorganisms were resistant to first line antimicrobial therapy (Table-II).

Overall sensitivity to fluoroquinolones was 42.3 % but it was very low for the most commonly isolated organism that is *Acinetobacter baumannii* (7.1%) however it was quiet sensitive for *Pseudomonas* (77.0%). Sensitivity to tetracycline group was between 22.0 to 25.0% which is better than third generation cephalosporins. Overall sensitivity to carbapenems was 56.0%, however it was very low for the most commonly isolated organism (*Acinetobacter*) which was 32.6%. The susceptibility to piperacillin-tazobactam was 49.0 % and to cefoperazone-sulbactam was 53.0%.

The most commonly isolated organism is *Acinetobacter baumannii* which has highest susceptibility to cefoperazone-sulbactam (45.0%) and carbapenems (32.6%), but it was least susceptible to third generation cephalosporins (1-3.0%). *E coli* was most Susceptible to carbapenems (83.0-87.0%) while its susceptibility to Cefoperazone-sulbactam, piperacillin-tazobactam and amikacin was 66.0%,65.0% and 64.6% respectively and it was least susceptible to third generation cephalosporins (cefotaxime 19.0%, ceftriaxone 29.2%). *Pseudomonas* and *klebsiella* were less commonly isolated bacteria. *Klebsiella* is most susceptible to carbapenems (66.0%) and aminoglycosides (45.0%) whereas susceptibility to Cefoperazone-sulbactam and piperacillin-tazobactam was low (22.0%). *Pseudomonas* is most susceptible to Cefoperazone-sulbactam (88.0%), piperacillin-tazobactam (88.0%) and amikacin (66.0%) whereas it is relatively low to carbapenems (56.0%). Carbepenem resistant *Enterobacter cloacae* were 35.0%.

Table-I: Antimicrobial susceptibility pattern among gram positive micro-organisms.

Antibiotics	*Staph. aureus (n=4)*	*Coagulase negative Staphylococci (n=21)*
Methicillin	0%	47.6%
Linzolid	100%	100%
Vancomycin	100%	100%
Chloramphenicol	100%	66.6%
Clindamycin	75.0%	52.3%
Amikacin	75.0%	85.7%
Minocycline	100%	85.7%
Doxycycline	100%	85.7%
Ciprofloxacin	50.0%	19.0%
Septran	25.0%	23.8%
Amoxyclav	25.0%	28.5%
Cephradine	25.0%	47.0%
Meropenem	25.0%	19.0%
Imipenem	25.0%	19.0%
Ampicillin/amoxicillin	0%	0%
Fusidic acid	100%	76.0%
Piperacillin-Tazobactam		23.8%

Table-II: Antimicrobial susceptibility pattern among gram negative organisms.

Antibiotics	*Acinetobacter baumannii*	*E.coli*	*Citrobacter freundii*	*Burkholderia cepacia*	*Enterobacter cloacae*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	Overall sensitivity
Meropenem	32.6%	87.0%	85.3%	0%	65.0%	66.0%	56.0%	56.0%
Imipenem	32.6%	83.0%	85.3%	4.0%	65.0%	66.0%	56.0%	56.0%
Piperacillin-Tazobactam	29.6%	65.0%	88.0%	44.0%	8.6%	22.0%	88.0%	49.0%
Cefoperazone sulbactam	45.0%	66.0%	0%	84.0%	13.0%	22.0%	88.0%	53.0%
Doxycycline	32.6%	47.6%	48.7%	12.0%	21.7%	11.0%	0%	24.8%
Minocycline	29.5%	44.6%	48.7%	12.0%	13.0%	11.0%	0%	22.6%
Ampicillin	0%	9.2%	7.3%	0%	0%	0%	0%	2.3%
Moxifloxacin	7.1%	49.2%	56.0%	76.0%	8.6%	22.0%	77.0%	42.3%
Amikacin	15.3%	64.6%	46.3%	56.0%	52.0%	45.0%	66.0%	49.3%
Ciprofloxacin/ Levofloxacin	7.1%	49.2%	56.0%	76.0%	8.6%	22.0%	77.0%	42.3%
Cotrimoxazole	17.0%	52.0%	66.0%	84.0%	13.0%	11.0%	0%	34.7%

Ceftriaxone	1.0%	29.2%	29.2%	4.0%	4.5%	11.0%	0%	11.3%
Gentamicin	14.2%	43.0%	36.5%	44.0%	52.0%	45.0%	55.0%	41.3%
Co-amoxiclav	8.1%	27.6%	14.6%	0%	8.6%	11.0%	0%	10.0%
Cefoperazone	1.0%	23.0%	7.3%	16.0%	4.5%	11.0%	22.0%	12.0%
Cefotaxime	3.0%	19.0%	19.0%	4.0%	4.5%	11.0%	11.0%	10.2%
Cefixime	1.0%	18.5%	7.3%	0%	4.5%	11.0%	0%	6.0%
Cefepime	3.0%	32.3%	14.6%	4.0%	4.5%	11.0%	22.0%	13.0%
Ceftazidime	2.0%	21.5%	9.7%	4.0%	4.5%	11.0%	22.0%	10.6%

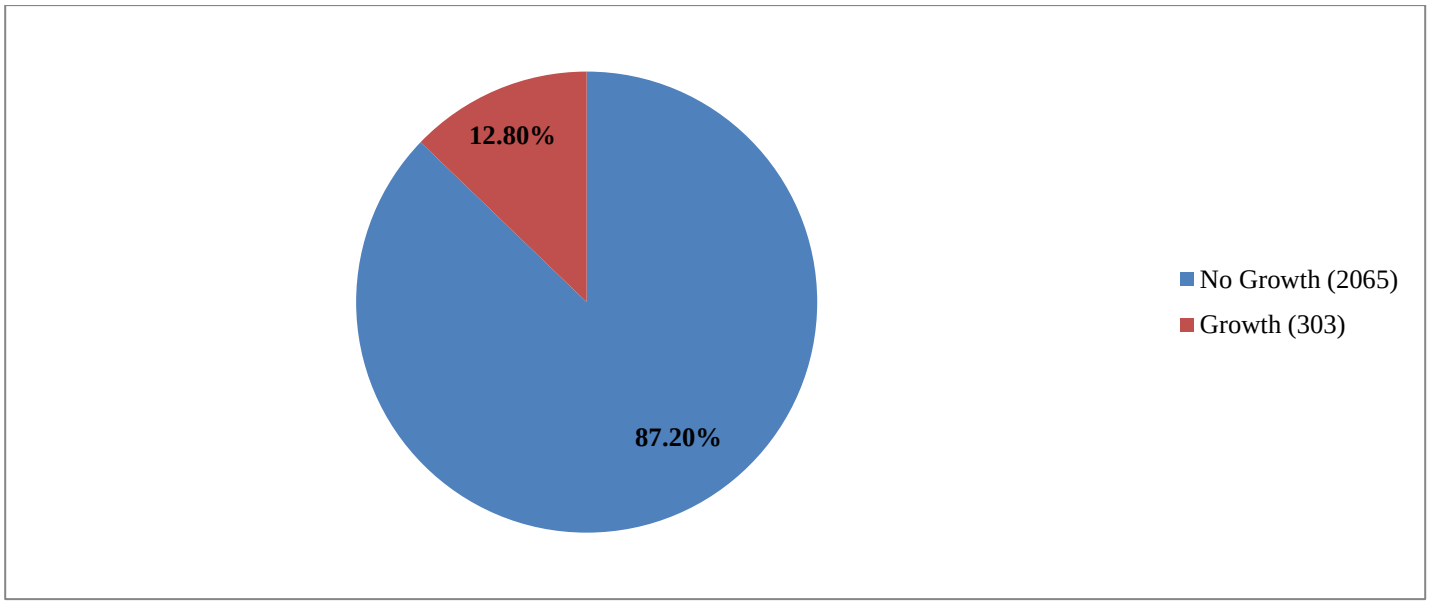


Figure-I: Growth pattern.

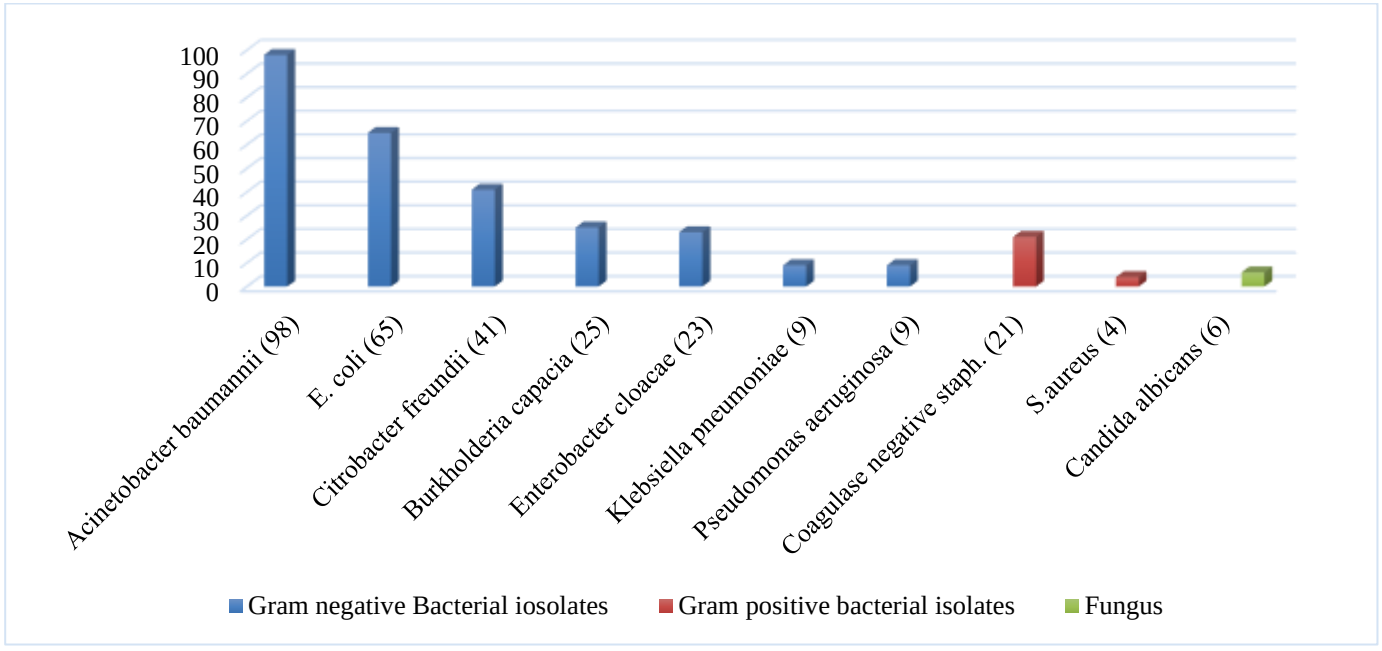


Figure-II: Frequency of bacteria isolated in blood cultures.

DISCUSSION

Blood culture yield varies among different laboratories depending upon the method used. In our study the blood culture positivity rate was 12.8 % which is comparable with the study conducted earlier at our hospital (13.7%),[1] Muhammad Almas *et alET ALF* (7.4%),[6] and Muhammad Atif *et al*. (8.9%).[4] This yield is slightly lower than that reported by studies conducted at Ghana (21%),[3] Nepal (20%),[11] and Nigeria (25%).[13] Some studies in India, and Nepal reported even higher

positivity rates of 46.7%,[10] 57%[5] and 42%.[8] The low blood culture positivity rate in our study could be due to low sample volume collected, technique of culture used and antibiotic therapy before referral to our hospital.

In our study, bacterial isolates were 98% while 2% were candida species. Isolation of gram-negative microorganisms was higher than gram positive in our study which is in accordance with that reported by Obaid *et al*[5], Dharshni *et al*[12], Shreshtha *et al*[10], and Bhishma *et al*. But studies from north India,[8] Germany, [14] Ghana[3] and east Nigeria[13] reported predominant growth of gram-positive microorganisms.

Among the gram-negative *Acinetobacter baumannii* emerged as the most common isolate (32.3%) in our study. Although a few recent studies from India (23.0%),[9] (3.0%),[8] and Pakistan (6%)[7], (17.7%)[6] did report it as one of the isolates, none has so far reported such a degree of dominance and resistance to commonly used antimicrobials. It was followed by *E. coli* as the second most common pathogen (21.4%). *Coagulase negative Staphylococcus (CoNS)* was the most common pathogen among the gram positives followed by S. aureus which is reported by Mudassar *et al*[1], Dharshni *et al*[9], Bhishma *et al*[11] and Belay *et al*[14] but other studies reported S. aureus[8,13,10,6] as the predominant one. Group *B. streptococcus* was not isolated in our study which along with Listeria monocytogenes and *E. coli* is the common pathogen implicated in neonatal sepsis in western countries[1,9]. This organism was also not reported by other studies in this region[4,5,6,7,8,10] *Acinetobacter species, coagulase negative staphylococci, Klebsiella and Pseudomonas species,* usually recognized as nosocomial pathogens were among the dominant pathogens in our study. This is possibly due to horizontal transmission from delivery rooms and NICUs or vertical transmission from maternal genital tracts colonized with these microorganisms after unhygienic personal and obstetric practices. [9]

Ampicillin and third generation cephalosporins are mostly used as empirical treatment in neonatal sepsis at our hospital but they were found to be least susceptible to pathogens isolated in this study. All gram-positive organisms isolated were resistant to ampicillin while only 2.3% gram-negative pathogens were sensitive to it. All gram-negative pathogens showed sensitivity of 13% or lower to all cephalosporins tested. This is in comparison to earlier study in this institute (14%)[1] and other studies done in this region (5.0%)[6], (9.5%) [11], (37.0%),[3] (23.0%),[8] (13.0%).[10] Although third generation cephalosporins have broad coverage, their injudicious use has made them less effective. Fortunately, amikacin which is also used as first line has very good sensitivity against gram-negative pathogens (49.3%). This is lower than that reported by Edna *et al* (79%),[3] Obaid *et al* (61.5%)[5] and Muhammad Atif *et al* (61.0%).[4] Methicillin resistance (MRSA) rate was showed at 47.6 percent against Coagulase negative Staphylococci (CoNS) (n=21) while all Staphylococci (n=4) isolated were resistant to it.

Linezolid and Vancomycin both were found to be most effective against gram-positive pathogens with sensitivity of 100%, followed by minocycline/ doxycycline (92.9%), fusidic acid (88.0%), and chloramphenicol (83.3%). This trend is similar to that reported earlier in our setup[1], by Kenechi *et al*[13] and multiple other authors.[6,8,10,12,9] This is because these antibiotics are not in routine use for the management of neonatal sepsis. Carbapenems showed susceptibility of only 56% against gram negative bacteria, followed by cefoperazone-sulbactam (53.0%), piperacillin-tazobactam (49.0%), and amikacin (49.0%). This is even lower than that reported by multiple other authors in this region[4,9,10,8,11] and other regions of the world [3,12,13,14].

As mentioned earlier *Acinetobacter* emerged as the most common pathogen implicated in neonatal sepsis in present study, is found to be highly resistant to all the antibiotics tested, with highest sensitivity to cefoperazone-sulbactam of only 45.0%. It has resistant rate of 100% against ampicillin, 97% against third generation cephalosporins while 84.3% against amikacin. Isolation of this unusual pathogen and such a high level of resistance to first line antibiotics and even to highly reserved antibiotics is an alarming situation for us and if steps are not taken to curb this resistance pattern, then we will not be left with any choice, and morbidity and mortality from neonatal sepsis will be even worse.

Our study carries a few limitations. First, this study was conducted at only one tertiary care hospital of the province Punjab, Pakistan. Therefore, the findings cannot be generalized for whole of Pakistan as type of pathogens and antibiotic use may vary across the country. Second, it was a retrospective study due to which we were unable to identify some of the

confounding variables, for example prior use and duration of antibiotics by the neonates, maternal variables and risk factors etc. which may have affected the results. Third, small sample and low culture yield rate at our setup was an important limitation of the study. Hence multi center prospective studies with larger sample size and latest blood culture isolation techniques are required to validate our findings.

CONCLUSION

The most commonly isolated organisms were Acinetobacter baumannii followed by E. coli and Citrobacter species. Among the gram-positive organism's resistance to commonly prescribed first and second-line agents was very high however no resistance was documented to vancomycin and linezolid. Among the gram-negative organism's resistance to commonly used antibiotics like ampicillin, third generation cephalosporins and cefepime was alarmingly high. The overall sensitivity to aminoglycosides is high however resistance to commonly isolated organism is still high. Carbapenems, piperacillin-tazobactam, and cefoperazone-sulbactam has highest susceptibility in our study but resistance documented to these drugs is alarming.

To curb the high resistance to antimicrobials found in our study comprehensive approach consisting of improvement of laboratory techniques to increase culture yield, evaluation of antibiotic consumption, rational use of empirical treatment, de-escalation of therapy when suitable along with continuous monitoring and surveillance of local epidemiology is need of the hour.

CONFLICT OF INTEREST

None

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHOR CONTRIBUTION

Saqib Munir: Idea conception, manuscript writing, revisions

Mudassar Hussain, Shahid Rashid: Data analysis, data interpretations

Salman Arshad: Literature review, critical review

Sidra Ijaz, Nadia Qamar: Data collection

Abdul Sattar: Overall supervision

REFERENCES

1. Mudassar H, Sattar A. Bacteriological profile and antibiogram of neonatal sepsis at a tertiary care hospital. Pak Pediatr J. 2019; 43(2): 109-14
2. Fleischmann-Struzek C, Goldfarb DM, Schlattmann P, Schlapbach LJ, Reinhart K, Kissoon N. The global burden of paediatric and neonatal sepsis: A systematic review. Lancet Respir Med. 2018; 6(3): 223-30. DOI: https://doi.org/10.1016/s2213-2600(18)30063-8
3. Edna N, Joseph A. Neonatal sepsis in a resource limited setting: Causative microorganisms and antimicrobial susceptibility profile. Hindawi Interdisciplinary Perspectives Infect Dis. 2022; 7905727: 1-7. DOI: https://doi.org/10.1155/2022/7905727
4. Atif M, Zia R, Malik I, Ahmad N, Sarwar S. Treatment outcomes, antibiotic use and its resistance pattern among neonatal sepsis patients attending Bahawal Victoria Hospital, Pakistan. PLoS One. 2021 16(1): e0244866. DOI: https://doi.org/10.1371/journal.pone.0244866
5. Ullah O, Khan A, Ambreen A, Ahmad I, Akhtar T, Gandapor AJ, *et al*. Antibiotic sensitivity pattern of bacterial isolates of neonatal septicemia in Peshawar, Pakistan. Arch Iran Med. 2016; 19(12): 866–9.
6. Hashmi MA, Lodhi MA, Toor KM, Tahir A, Khan HS, Aziz R. Emerging antimicrobial resistance in neonatal sepsis. J Coll Physicians Surg Pak 2020; 30(12):1312-15. DOI: https://doi.org/10.29271/jcpsp.2020.12.1312
7. Ahmed A, Sarwar N. Pattern of clinical drug resistance and occurrence of gram negative bacterial neonatal sepsis at a tertiary care hospital. Pak J Pharm Sci. 2021; 34 (5(Suppl)): 1873-78.
8. Thakur S, Thakur K, Sood A, Chaudhary S. Bacteriological profile and antibiotic sensitivity pattern of neonatal septicaemia in a rural tertiary care hospital in North India. Indian J Med Microbiol. 2016; 34: 67-71. DOI: https://doi.org/10.4103/0255-0857.174108
9. Investigators of the Delhi Neonatal Infection Study (DeNIS) collaboration. Characterization and antimicrobial resistance of sepsis pathogens in neonates born in tertiary care centers in Delhi, India: a cohort study. Lancet Glob Health. 2016; 4: e752–60. DOI: https://doi.org/10.1016/s2214-109x(16)30148-6
10. Shrestha S, Shrestha NC, Singh SD, Shrestha RPB, Kayestha S, Shrestha M, *et al*. Bacterial isolates and its antibiotic susceptibility pattern in NICU. Kathmandu Univ Med J 2013; 41(1): 66-70. DOI: https://doi.org/10.3126/kumj.v11i1.11030
11. Pokhrel B, Koirala T, Shah G, Joshi S, Baral P. Bacteriological profile and antibiotic susceptibility of neonatal sepsis in neonatal intensive care unit of a tertiary hospital in Nepal. BMC Pediatr. 2018; 18: 208. DOI: https://doi.org/10.1186/s12887-018-1176-x
12. Pillay D, Naidoo L, Swe-Han KS, Mahabeer Y. Neonatal sepsis in a tertiary unit in South Africa. BMC Infect Dis. 2021; 21: 225. DOI: https://doi.org/10.1186/s12879-021-05869-3
13. Kenechi O, Chioma P. Bacterial isolates, antibiogram and outcomes of blood culture proven sepsis in neonates at a tertiary institution in South East Nigeria: A cross-sectional study. Ther Adv Infect Dis. 2022; 9: 1–11. DOI: https://doi.org/10.1177%2F20499361221122479
14. Tessema B, Lippmann N, Knüpfer M, Sack U, König B. Antibiotic resistance patterns of bacterial isolates from neonatal sepsis patients at University Hospital of Leipzig, Germany. Antibiotics. 2021; 10: 323. DOI: https://doi.org/10.3390/ antibiotics10030323
15. Bizzarro MJ, Raskind C, Baltimore RS, Gallagher PG. Seventy-five years of neonatal sepsis at Yale: 1928-2003. Pediatrics. 2005; 116(3): 595-602.

DOI: https://doi.org/10.1542/peds.2005-0552
16. Schrag SJ, Farley MM, Petit S, Reingold A, Weston EJ, Pondo T, *et al*. Epidemiology of invasive early-onset neonatal sepsis, 2005 to 2014. Pediatrics. 2016; 138(6): e20162013. DOI: https://doi.org/10.1542/peds.2016-2013
17. Kuhn P, Dheu C, Bolender C, Chognot D, Keller L, Demil H, *et al*. Incidence and distribution of pathogens in early-onset neonatal sepsis in the era of antenatal antibiotics. Paediatr Perinat Epidemiol. 2010; 24(5): 479-87. DOI: https://doi.org/10.1111/j.1365-3016.2010.01132.x
18. Stoll BJ, Puopolo KM, Hansen NI, Sánchez PJ, Bell EF, Carlo WA, *et al.* Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. Early-Onset Neonatal Sepsis 2015 to 2017, the Rise of Escherichia coli, and the Need for Novel Prevention Strategies. JAMA Pediatr. 2020; 174(7): e200593. DOI: https://doi.org/10.1001/jamapediatrics.2020.0593
19. Laxminarayan R, Matsoso P, Pant S, Brower C, Røttingen JA, Klugman K, *et al*. Access to effective antimicrobials: a worldwide challenge. The Lancet. 2016; 387(10014): 168-75. DOI: https://doi.org/10.1016/S0140-6736(15)00474-2

ORIGINAL ARTICLE

Impact of COVID-19 on a cohort of hemodialysis patient: A nested case-control study in a tertiary care Hospital in Northern Pakistan

Farhan Zaid[1], Malik Nadeem Azam Khan[2], Aakash Aslam[3], Ahsan Tanvir[1], Fahad Javed Awan[4], Amna Butt[4]

[1]Combined Military Hospital, Malir Karachi Pakistan
[2]Emirates Military Hospital (National University of Medical Sciences), Rawalpindi Pakistan
[3]Combined Military Hospital, Silakot Pakistan
[4]Combined Military Hospital, Kohat Pakistan

ABSTRACT

Background: The outbreak of the highly contagious coronavirus has significantly threatened human health, particularly for individuals with underlying diseases. Despite this, the effects of the epidemic on hemodialysis (HD) patients have not been thoroughly assessed.

Material and Methods: An observational prospective nested case control study was designed that covered the epidemic period from May 2020 to June 2020 at the Hemodialysis (HD) Center in Rawalpindi. Study was devised to investigate the differences in outcomes and complications between hemodialysis patients testing positive and negative for the coronavirus. The study included a total of 294 registered hemodialysis patients, with a case group of 29 individuals testing positive for coronavirus compared to 29 COVID-negative counterparts from the same cohort. Various data, encompassing epidemiological, clinical, laboratory, and radiological characteristics, were systematically collected and analyzed.

Results: Of the 294 hemodialysis patients, 29 (9.86%) were diagnosed with COVID-19. Most COVID-19-diagnosed patients presented with mild-moderate respiratory symptoms. Only 3(10.3%) patients with a positive COVID test were admitted to the Intensive Care Unit (ICU) for severe conditions while only two deaths were recorded among COVID-19-positive HD patients, and none of them was linked to COVID-19 or its complications.

Conclusions: This study highlights the susceptibility of HD patients during the COVID-19 epidemic and underscores HD centers as high-risk areas. Patients with COVID-19 and undergoing hemodialysis typically exhibit mild clinical symptoms and are less likely to progress to severe pneumonia, attributed to compromised cellular immune function and an inability to mount cytokine storms.

Keywords: Coronavirus, Hemodialysis, Patients

BACKGROUND

Since January 2020, the epidemic of highly contagious and rapidly fatal droplet infection tilted COVID 19 has ascended to the status of pandemic and occupied center stage at global and national health care systems.[1-6]

Initially, COVID-19 was feared for its indiscriminate impact across age groups, with distressing social media videos showing both young and elderly individuals succumbing to the virus.[7,8] However, subsequent epidemiological studies reiterated that COVID-19 patients with pre-existing conditions like diabetes, hypertension, cardiovascular disease, or those who are elderly, are not only susceptible but also more likely to experience complications, contributing to the overall fatality statistics.[9]

Out of the various chronic co-morbidities noted in COVID 19 patients, the case of chronic kidney disease was considered a special case as it not only contributed to most chronic conditions but had a natural history of its own with important implications on natural immune system.[10] Considering the rising size of chronic kidney disease patients on hemodialysis, there was a need to study their susceptibility and response to COVID 19 as a special group. With around 294 registered patients receiving HD treatment in 2 centers in two medical set ups in Rawalpindi city, there is often high concentration of patients in HD centers, and the compromised immune function of uremic patients is a dreaded risk factor.[11]

Correspondence: Dr. Farhan Zaid, Combined Military Hospital, Malir Karachi Pakistan

Email: fzaid@gmail.com

This article can be cited as: Zaid F, Khan MNA, Aslam A, Tanvir A, Awan FJ, Butt A. Impact of COVID-19 on a cohort of hemodialysis patient: A nested case-control study in a tertiary care Hospital in Northern Pakistan. Infect Dis J Pak. 2024; 33(2): --. DOI: https://doi.org/10.61529/idjp.v33i2.286

Receiving date: **28 Jan 2024** Acceptance Date: **04 Apr 2024**
Revision date: **15 Mar 2024** Publication Date: **30 Jun 2024**

It has been postulated that patients with compromised immunity might not develop severe COVID disease owing to their impaired immune response or inability to initiate a cytokine response. But results from different studies have been contradicting where few studies have shown severe COVID in immunocompromised patients while others have done the opposite.[10-11]

The aim of the study is to follow the outbreak of Covid19 epidemic in hemodialysis centers and to provide insights into the effective management and outcome of COVID positive patients on hemodialysis.

MATERIAL AND METHODS

An observational prospective Nested Case Control study was designed. Epidemic course from the first laboratory-confirmed case of COVID-19 infections from May 5th to June 30th 2020 in the HD Center was reviewed and total 294 HD registered patients were included in this study.

The diagnosis of COVID-19 related pneumonia adheres to the New Coronavirus Pneumonia Prevention and Control Program's 5th, 6th, and 7th editions - valid from February 4 to March 4, 2020. This program is governed by a CC-BY-NC-ND 4.0 International license [15]. Within its guidelines in the fifth edition, it defines a suspected case presenting with symptoms correlating with an epidemiological history or clinical evidence such as decreased white blood cell count or lymphocyte count alongside fever and respiratory symptoms. A clinically diagnosed case is established when these indicated cases exhibit image-based features corresponding to that of pneumonia. We followed the same guidelines to identify our positive cases.

Following the pathway laid by the guidelines, a confirmed diagnosis was made if individuals designated as either suspected or clinically diagnosed patients were verified positive via pathology blood tests according to protocol procedures. It remains essential though for valid suspect cases presentation aligns precisely with criteria set forth pertaining their epidemiological histories alongside aforementioned clinical symptoms indicators. If pathogen evidence materialized - particularly through Polymerase Chain Reaction (PCR) positivity, the considered potential patient received an official confirmed diagnosis for COVID-19 infection.

All the patients were followed up and related clinical data was collected. A cohort of 29 COVID positive confirmed cases were included as incident cases among the population of 294 hemodialysis patients. A parallel cohort of 29 matched patients who tested negative for COVID among the remaining 265 hemodialysis cases were identified for comparison as control.

The research protocol received approval from the hospital's Ethics Committee, ensuring adherence to ethical standards. Given the epidemic status of infectious diseases, written informed consent was not required, likely due to the urgency and potential risks to patients.

The research team meticulously examined the medical records of all participants, compiling a wide range of data including epidemiological, clinical, laboratory, and radiological information. The collected data encompassed demographic specifics, medical backgrounds, existing health conditions, symptoms, physical indicators, blood test results, and chest CT scans, thereby offering a thorough insight into the health profiles of the participants. During the follow-up period, instances of mortality were recorded and meticulously evaluated to ascertain the presumed cause of death based on clinical manifestations, time, and location. SARS-CoV-2 detection was performed using real-time PCR (RT-PCR) on nasopharyngeal swab samples collected from participants, a widely accepted method for diagnosing COVID-19 due to its high sensitivity and specificity. Blood tests were conducted to explore the impact of SARS-CoV-2 infection on host immune responses, with comparisons made between COVID-19 HD patients and non-COVID-19 HD patients to identify potential differences in immune function.

Statistical analysis was conducted using SPSS version 22. Parametric tests were applied for normally distributed continuous data while categorical data was expressed as frequencies (%). The independent group t-test was applied for comparisons, with significance set at $p < 0.05$.

RESULTS

29 out of 294 registered patients were diagnosed with Covid19 at HD centers. Figure-I provides a schematic representation of the evolving progression of the COVID-19 epidemic, from its initial emergence to its subsequent development. The first case of COVID-19 was diagnosed on May 5th, followed by a second diagnosis on May 8th.

Between 8th to 15th May, another 4 new patients were further confirmed with COVID-19. Realizing this quick rise in infection among HD patients reporting for their

scheduled dialysis at Nephrology department, a plan was approved by hospital authorities to screen all patients with chest CT and optional blood test. On June 5, there were total 11 HD cases being diagnosed with COVID-19. To find out the infected cases post Eid ul Fitr leave, the second round of screening was initiated from June 5, 2020 to June 30, 2020 and another 18 HD patients were diagnosed with COVID 19.

Throughout the screening period, all patients found to be infected were categorized, isolated, or moved to specific hospital units according to established protocols. The data revealed that total 29 patients, constituting 9.86% of the total, were diagnosed with COVID-19, and 2 patients passed away since the onset of the epidemic. Our research team closely monitored and reviewed both fatalities. Of the infected individuals, 3 were admitted to the Intensive Care Unit (ICU) due to severe conditions. Remarkably, the two deceased patients did not experience the respiratory complications that were expected in such cases.

First fatal case of HD unit had his primary cause of death labeled as Hypertensive Encephalopathy while the second case primary cause of death was being worked up for Vasculitis. During the two rounds of screening conducted to identify infected patients in the HD center, COVID-19 diagnosis relied on the detection of positive SARS-CoV-2 nucleic acid tests from nasopharyngeal swabs. All the HD patients testing positive for COVID 19 had their CT scan COVID score of 8-30. HD patients are summarized in Table 1.

Out of the 29 confirmed COVID-19 patients, 20 were male and 9 were female, with a median age of 61 years. Hematological abnormalities such as lymphocytopenia and thrombocytopenia, which are frequently observed in COVID-19 patients according to previous reports [7, 8, 16, 17], were also evident in the HD patients who contracted COVID-19 (refer to Table 2).

Among HD patients diagnosed with COVID-19, typical clinical symptoms such as fever, fatigue, dry cough, chest pain, and nausea were not frequently reported. Radiological assessment through chest CT scans revealed that 2 (6.8%) confirmed diagnosed patients exhibited bilateral involvement, while 12 (41.3%) confirmed diagnosed patients and 5 (17.24%) clinically diagnosed patients showed unilateral involvement. The average score for "ground-glass opacity" lesions in the lungs ranged between 8 to 30 in both groups. These clinical presentations suggest that the majority of COVID-19 infected HD patients experienced mild conditions, a departure from previous observations in patients with comorbidities such as diabetes, hypertension, cardiovascular disease, or older age groups.[7,8,16]

The immune system plays a critical role in defending the host against invading pathogens. Upon SARS-CoV-2 infection of the host's respiratory tract, the virus replicates in airway cells, leading to significant immune activation and the release of large amounts of proinflammatory cytokines. This excessive immune response, known as a "cytokine storm," can result in severe conditions and, in some cases, lead to death in COVID-19 patients.[16]

However, the results outlined suggest that patients with COVID-19 infection experienced mild symptoms. It is speculated that this could be attributed to compromised immunity in HD patients, as evidenced by lymphopenia observed in nearly all patients. These findings imply that HD patients may have a compromised immune system, potentially limiting their ability to mount effective antiviral responses. Nevertheless, this compromised immunity may also have a beneficial aspect, as it could help mitigate tissue damage by dampening cytokine release.

Table-I: Demographic, clinical, and radiological features of HD patients.

Variables	Total n=294	Cases n=29	Controls n=29	P
Age (Years)	**55.0±12.0**	**52.0±14.0**	**54.0±6.0**	**0.08**
Male	**265**	**20**	**9**	**0.44**
Body mass Index	**24.5±4.3**	**22.2±4.5**	**23.1±3.6**	**0.53**
Comorbidity	**n (%)**	**n(%)**	**n(%)**	
Hypertension	257(87.41)	26(89.6)	24(83)	0.01
Diabetes	185(63)	18(62)	16(55)	0.31
Coronary heart disease	130(64)	17(58)	16(55)	0.48
Dyslipidemia	280(95)	22(75)	19(65)	0.39
Chronic obstructive lung disease	107(36)	18(62)	12(41)	0.45
Symptoms	**n (%)**	**n(%)**	**n(%)**	
Fever	78(26)	26(89.6)	22(75)	0.03

Cough	102(34)	28(96)	16(55)	0.02
Fatigue	256(87)	28(96)	25(86)	0.27
Diarrhea, nausea or vomiting	124(42)	24(83)	15(52)	0.05
Admission chest X-ray	**n (%)**	**n(%)**	**n(%)**	
Bilateral peripheral opacity	40(13)	26(89)	14(48)	0.03
Unilateral opacity	86(29)	3(10)	8(27)	0.33
Normal X-ray	189(64)	NIL	7(24)	0.03
HR CT	**n (%)**	**n(%)**	**n(%)**	
Multiple peripheral basal ground glass opacities	37(12)	27(93)	10(34)	
Peripheral Consolidation	18(6)	12(41)	6(20)	

Table-II: Laboratory findings at admission and 1 week after clinical onset.

Laboratory variables	Total=294	Cases=29	Controls=29	p-value[a]
1.*Lymphocytecount10*^{9},	0.79±0.47	0.83±.41	0.67	0.42
Baseline	0.66	0.760	0.38	0.04
Day 7				
2.*Hemoglobin, g/l*	10.61	10.6	10.61	0.13
Base line	9.81	9.6	10.72	0.17
Day 7				
3.*Platelet count,10*^{9}	1.64	1.74	1.37	0.13
Baseline	1.78	1.89	1.48	0.18
Day 7				
4.*Serum LDH, U/l*	235	225	274	0.27
Baseline	329	281	490	0.01
Day 7				
5.*Serum ALT, U/l*	29.25	29.55	19.21	0.72
Baseline	32.94	33.23	17.07	0.32
Day 7				
6.*Serum Ferritn, U/l*	445.64	Nil	451.04	0.24
Baseline	845.71	Nil	452.05	0.52
Day7				
9.*SerumC-reactive protein, mg/dl*	9.67	10.38	794	0.90
Baseline	10.61	8.18	674	0.08
Day7				
10.*Serum albumin, g/d*	3.70	3.70	3.44	0.18
l Baseline	3.20	3.20	3.31	0.96
Day7				

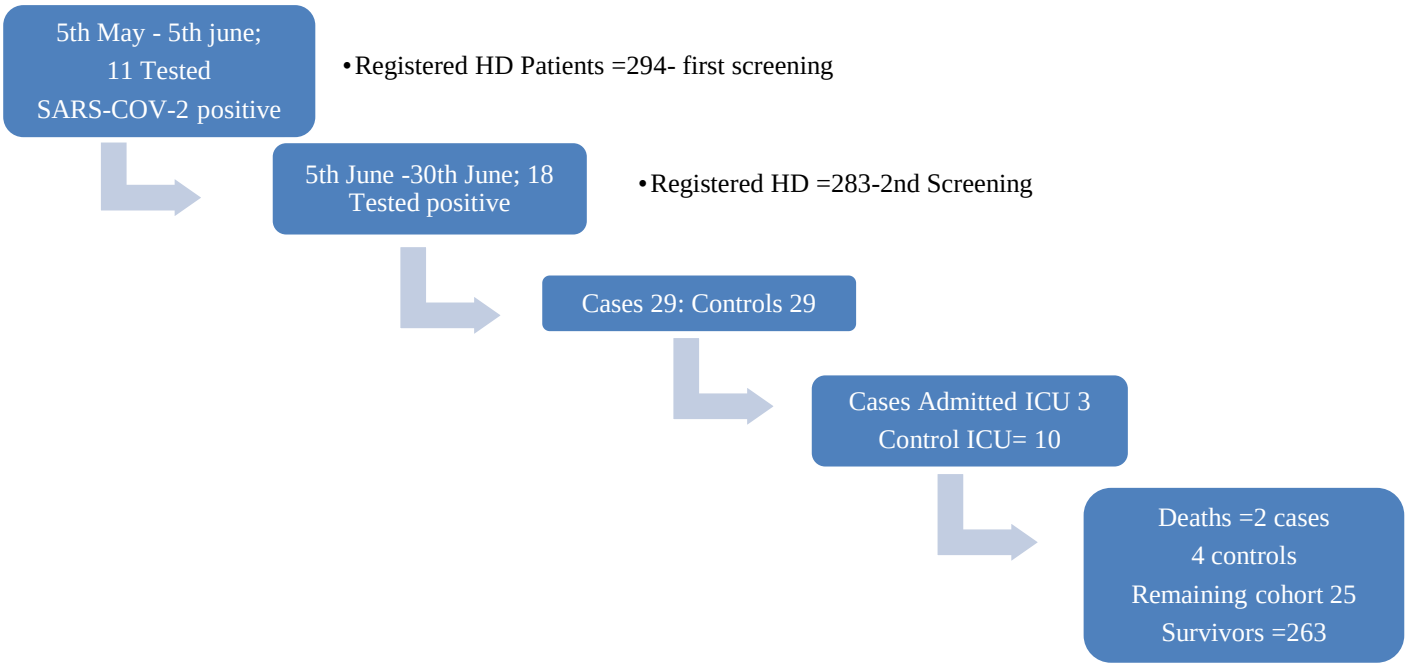


Figure-I: Follow up of outbreak of COVID among maintenance HD patients.

DISCUSSION

The COVID-19 epidemic has overwhelmed nearly all national healthcare systems worldwide and prompted the World Health Organization to declare it a pandemic, sounding a global alarm.[4] Common perception derived from epidemiological surveys indicated that the elderly or patients with comorbidities were more vulnerable to COVID-19 as well as the incidence of severe cases and the mortality risk were high.[16,18-20] However, the lack of similar reports regarding the impacts of COVID-19 epidemic on HD patients was a mystery. Patients undergoing hemodialysis (HD) constitute a unique cluster within the chronic disease population, forming a significant and specialized group that often receives concentrated dialysis treatment in spacious facilities. Additionally, their compromised immune systems raise concerns that if infected, they could potentially become "super-spreaders" of the virus.

Considering these observations, HD patients and HD centers deserve priority action for epidemic prevention and control. Wang *et al* did a systematic review on risk factors of mortality in COVID-19 patients. In their systematic review they found previous respiratory illnesses to be a greater risk factor for mortality in hemodialysis patients [21]. These findings are findings are consistent with findings of our study in which mortality was low in patients only on hemodialysis.

The COVID-19 epidemic first emerged at our center in May 2020. Despite implementing various measures such as enhancing prevention and protection, quarantine, and isolation, the most effective method we found for containing the epidemic was thorough screening to identify infected cases, primarily based on chest CT scan results. Contrary to the findings of Chen *et al*., who reported increased incidence and mortality among COVID-19 patients, our study observed no deaths directly related to COVID-19 infection. During the outbreak at our center, among 294 registered patients, two deaths were recorded, resulting in a mortality rate of 0.68%, which is higher than historical rates for the same period. However, none of the deaths were directly attributable to pneumonia; the main causes were cardiovascular and cerebrovascular complications or hyperkalemia, likely due to reduced dialysis sessions to minimize virus exposure.

While HD patients are very susceptible to COVID-19, infections in this population are less severe or fatal. Only three out of 29 infected patients required ICU admission, compared to five in the control group. Similar to the findings of Tian *et al*., cough was the most common symptom in our study, with some infected patients showing no obvious clinical symptoms.

Amid the emergence of the epidemic, implementing prevention and protection measures becomes imperative to prevent infection, while ensuring timely and sufficient dialysis remains critical for patient survival. Due to the significant biological resemblance between SARS-CoV-2 and SARS-CoV, HD patients infected with SARS-CoV-2 might require a prolonged quarantine period to contain further transmission, as they may take longer to clear the virus.

Previous studies have indicated that SARS-CoV-2 infection can reduce lymphocyte counts while significantly increasing inflammatory cytokine levels, potentially leading to cytokine storms and worsened conditions. Interestingly, the compromised immune system in HD patients may prevent the launch of an effective immune response against CoV-2 infection, thereby avoiding cytokine storms and severe organ damage which goes in parallel with our study as there was no association between individuals undergoing hemodialysis and critical Covid19 manifestation.

CONCLUSION

The findings of this investigation underscore the vulnerability of hemodialysis (HD) patients amidst the COVID-19 epidemic, emphasizing the designation of HD centers as high-risk environments. Notably, individuals undergoing hemodialysis and testing positive for COVID-19 tend to manifest mild clinical symptoms, presenting a lower likelihood of progressing to severe pneumonia. This pattern is linked to compromised cellular immune function and an inherent incapacity to incite cytokine storms.

The study illuminates the distinctive clinical trajectory of HD patients in the context of COVID-19, shedding light on factors that contribute to their relative resilience to severe respiratory complications. By elucidating the mild nature of symptoms and reduced progression to severe pneumonia, the research advocates for a nuanced understanding of the interplay between COVID-19 and the unique physiological characteristics of HD patients. These insights have implications for clinical management strategies, suggesting the necessity for tailored approaches in high-risk HD centers to enhance

patient outcomes and guide preventive measures during the ongoing pandemic.

CONFLICT OF INTEREST

None

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHOR CONTRIBUTION

Farhan Zaid: Literature search, study design, conceptualization

Malik Nadeem Azam Khan: Data analysis, data interpretation

Aakash Aslam: Data analysis

Ahsan Tanvir: Manuscript writing

Fahad Javed Awan, Amna Butt: Data collection

REFERENCES

1. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, *et al*. A novel coronavirus from patients with pneumonia in China, 2019. N Eng J Med. 2020 Feb 20;382(8):727-33. DOI: https://doi.org/10.1056/nejmoa2001017
2. Mahase E. Coronavirus: COVID-19 has killed more people than SARS and MERS combined, despite lower case fatality rate. BMJ 2020; 368: m641. DOI: https://doi.org/10.1136/bmj.m641
3. Eurosurveillance Editorial Team. Latest updates on COVID-19 from the European Centre for Disease Prevention and Control. Euro Surveill. 2020; 25(6): 2002131. DOI: https://doi.org/10.2807%2F1560-7917.ES.2020.25.6.2002131
4. WHO. WHO characterizes COVID-19 as a pandemic. Rolling Updates on Coronavirus Disease (COVID-19). 2020 Mar 11.
5. National Health Commission of the People`s Republic of China, COVID-19 epidemic situation report. 2020.
6. World Health Organization. Coronavirus disease 2019 (COVID-19): situation report, 73.
7. Chen N. Epidemiological and clinical characteristics of 99 Cases of 2019-Novel Coronavirus (2019-nCoV) Pneumonia in Wuhan, China.
8. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, *et al*. Clinical characteristics of coronavirus disease 2019 in China. N Eng J Med. 2020; 382(18): 1708-20. DOI: https://doi.org/10.1056/nejmoa2002032
9. Schwartz DA, Graham AL. Potential maternal and infant outcomes from coronavirus 2019-nCoV (SARS-CoV-2) infecting pregnant women: lessons from SARS, MERS, and other human coronavirus infections. Viruses. 2020; 12(2): 194. DOI: https://doi.org/10.3390%2Fv12020194
10. Wang J, Qi H, Bao L, Li F, Shi Y. A contingency plan for the management of the 2019 novel coronavirus outbreak in neonatal intensive care units. Lancet Child Adolesc Health. 2020; 4(4): 258-9. DOI: https://doi.org/10.1016%2FS2352-4642(20)30040-7
11. Schaier M, Leick A, Uhlmann L, Kälble F, Morath C, Eckstein V, *et al*. End-stage renal disease, dialysis, kidney transplantation and their impact on CD4+ t-cell differentiation. Immunol. 2018; 155(2): 211-24.
12. National Health Commission of China, New coronavirus pneumonia prevention and control program (5th Edition).
13. National Health Commission, New coronavirus pneumonia prevention and control program (6th Edition). 2020. Available at: https://www.partners-popdev.org/wp-content/uploads/2020/03/2-COVID-19-Prevention-and-Control-Protocol-V6.pdf
14. National Health Commission of China, New coronavirus pneumonia prevention and control program (7th Edition).
15. Wang M, Wu Q, Xu W, Qiao B, Wang J, Zheng H, *et al*. Clinical diagnosis of 8274 samples with 2019-novel coronavirus in Wuhan. MedRxiv. 2020: 2020-02. DOI: https://doi.org/10.1101/2020.02.12.20022327
16. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, *et al*. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020; 395(10223): 497-506. DOI: https://doi.org/10.1016/s0140-6736(20)30183-5
17. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, *et al*. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus–infected pneumonia in Wuhan, China. JAMA. 2020; 323(11): 1061-9. DOI: https://doi.org/10.1001/jama.2020.1585
18. Chen H, Guo J, Wang C, Luo F, Yu X, Zhang W, *et al*. Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: a retrospective review of medical records. Lancet. 2020; 395(10226): 809-15. DOI: https://doi.org/10.1016%2FS0140-6736(20)30360-3
19. Liang W, Guan W, Chen R, Wang W, Li J, Xu K, *et al*. Cancer patients in SARS-CoV-2 infection: A nationwide analysis in China. Lancet Oncol. 2020; 21(3): 335-7. DOI: https://doi.org/10.1016/s1470-2045(20)30096-6
20. Pan F, Ye T, Sun P, Gui S, Liang B, Li L, *et al*. Time course of lung changes on chest CT during recovery from 2019 novel coronavirus (COVID-19) pneumonia. Radiology. 2020; 295(3): 715-21. DOI: https://doi.org/10.1148/radiol.2020200370
21. Wang F, Ao G, Wang Y, Liu F, Bao M, Gao M, *et al*. Risk factors for mortality in hemodialysis patients with COVID-19: A systematic review and meta-analysis. Ren Fail. 2021; 43(1): 1394-407. DOI: https://doi.org/10.1080/0886022x.2021.1986408
22. Chen CY, Shao SC, Chen YT, Hsu CK, Hsu HJ, Lee CC, *et al*. Incidence and clinical impacts of COVID-19 infection in patients with hemodialysis: Systematic review and meta-analysis of 396,062 hemodialysis patients. Healthcare 2021; 9 (1): 47. DOI: https://doi.org/10.3390/healthcare9010047
23. Tian M, Li H, Yan T, Dai Y, Dong L, Wei H, *et al*. Clinical features of patients undergoing hemodialysis with COVID-19. Semin Dial. 2021; 34 (1): 57-65. DOI: https://doi.org/10.1111%2Fsdi.12928
24. Kwan BC, Leung CB, Szeto CC, Wong VW, Cheng YL, Yu AW, *et al*. Severe acute respiratory syndrome in dialysis patients. J Am Soc Nephrol. 2004; 15(7): 1883-8. DOI: https://doi.org/10.1097/01.asn.0000131522.16404.1f
25. Girndt M, Sester U, Sester M, Kaul H, Köhler H. Impaired cellular immune function in patients with end-stage renal failure. Nephrol Dial Transplant. 1999; 14(12): 2807-10. DOI: https://doi.org/10.1093/ndt/14.12.2807
26. Vaziri ND, Pahl MV, Crum A, Norris K. Effect of uremia on structure and function of immune system. J Ren Nutr. 2012; 22(1): 149-56. DOI: https://doi.org/10.1053/j.jrn.2011.10.020

ORIGINAL ARTICLE

Susceptibility pattern of pediatric uropathogens: Insights from Mirpur Azad Jammu Kashmir Pakistan

Ammara Manzoor[1], Toqeer Ahmed[1], Syeda Tahira Batool[2], Nabia Chaudhry[2], Fatima Khurshid[3]

[1]Mohtramma Benazir Bhutto Shaheed Medical College, Mirpur Azad Jammu Kashmir
[2]Divisional Headquarters Hospital, Mirpur Azad Jammu Kashmir
[3]Shifa International Hospital Limited. Islamabad Pakistan

ABSTRACT

Background: Antibiotic resistance in pediatric urinary tract infections (UTIs) is a growing concern, necessitating the assessment of antibiotic susceptibility profiles for effective treatment strategies. The study was designed to assess the clinical profile, common uropathogens causing UTI and their antimicrobial susceptibility patterns. The study was designed to assess the frequency of pediatric UTIs along with antimicrobial susceptibility pattern of the isolated uropathogens.

Material and Methods: We conducted a retrospective review of clinical records at DHQ Hospital Mirpur AJK, Pakistan from March to September 2023, focusing on urinary tract infections in pediatric patients. We identified uropathogens and their antimicrobial susceptibility pattern to guide effective treatment strategies and address drug resistance.

Results: Data was collected from the medical records of 140 pediatric patients. of which, 49% (n=69) had positive urine cultures. Majority of these patients were males (71%). The most predominant organism was *Klebsiella pneumoniae* (32%) followed by, *Staphylococcus aureus* (26%), *Escherichia coli* (22%) and *Enterococcus faecalis* (11%). Mixed growth of uropathogens was seen in 9% of the cases. Regarding Antimicrobial susceptibility pattern, Amikacin, Vancomycin, Neomycin and Tigecycline were the most susceptible (100%), followed by chloramphenicol (90.9%), Linezolid (83.4%), Rifampicin (82%), Meropenem (77.3%), Nitrofurantoin (70%), Tazobactam (68.7%) and Imipenem (68.4%). Cephalexin (66.7%), Gentamicin (63.6%) and Cefipime (50%) showed moderate susceptibility. Whereas, Sulfamethoxazole (35%), Levofloxacin (31.4%), Cefoxitin (26.3%), Ciprofloxacin (22.2%), Tetracycline (20.5%), Cefuroxime (14.2%), Ceftriaxone (9.52%) and Amoxicillin (9.52%) were the least susceptible.

Conclusion: Our research suggests that it is important to review the use of antibiotics for treating UTIs in pediatric patients due to changes in antibiotic susceptibility and the increase in resistance among bacteria. This emphasizes the significance of antimicrobial stewardship.

Keywords: Urinary tract infections. Antimicrobial susceptibility Patterns, Pediatric Population.

BACKGROUND

Urinary tract infection (UTI) is one of the commonest causes of febrile illness in pediatric population with a worldwide prevalence of 2–20%.[1] Untreated pediatric UTIs can lead to severe consequences like renal scarring, hypertension, and chronic renal failure. 50% of UTIs in children are not detected due to lack of symptoms, especially in infants. Early diagnosis and appropriate treatment can prevent complications, although antibiotic resistance is a growing concern worldwide.[2,3] Acute pyelonephritis is the most frequent dangerous bacterial illness in infancy, with many afflicted children, particularly newborns, experiencing severe symptoms. Most instances are easily treated if diagnosed promptly, however fever in pediatric population may take several days to subside. Approximately 7 to 8% of females and 2% of males have a UTI the first 8 years of life. Febrile UTIs are most common in both genders during the first year of life, but non-febrile UTIs occur mostly in females beyond the age of three. After infancy, urinary tract infections confined to the bladder are generally accompanied by localized symptoms and are easily treated. Fever, on the other hand, raises the chance of kidney involvement, as well as the likelihood of underlying nephro-urologic abnormalities and the risk of subsequent renal scarring.

Correspondence: Dr. Fatima Khurshid, Department of Radiology and Oncology, Shifa International Hospital Limited, Islamabad Pakistan

Email: fatimakhurshid61@yahoo.com

This article can be cited as: Manzoor A, Ahmed T, Batool ST, Chaudhry N, Khurshid F. Susceptibility pattern of pediatric uropathogens: Insights from Mirpur Azad Jammu Kashmir Pakistan. Infect Dis J Pak. 2024; 33(2): 92-96.
DOI: https://doi.org/10.61529/idjp.v33i2.283

Receiving date: **13 Jan 2024** Acceptance Date: **24 Jun 2024**
Revision date: **29 May 2024** Publication Date: **28 Jun 2024**

Kidney scarring caused by UTIs has been identified as a major source of long-term morbidity. Thus, children with established illnesses have been thoroughly investigated and treated, and they
have frequently had surgery or been given long-term antibiotic prophylaxis. Such strategies have been questioned. Several trials are now continuing to establish the best techniques for assessing and managing early febrile UTIs, as well as later therapies for them.[4,5]
Antibiotic treatment for children with febrile UTIs effectively reduces the risk of death, which was approximately 20% of hospitalized patients with pyelonephritis in the early 20th century. Nearly 50 years ago, two important studies highlighted the impact of UTIs had on children kidneys. In one study, renal parenchymal damage was detected in 210 out of 597 children with UTIs. Another study followed 72 children hospitalized for UTIs for 11 to 27 years and found that 18% died, 8% had kidney failure, and 22% experienced persistent or reinfection. However, these studies focused solely on kidney damage due to UTIs and disregarded the potential effects of kidney failure. The concept of reflux nephropathy emerged in the 1970s linking vesicoureteral reflux with pyelonephritis and subsequent renal scarring. Therefore, the evaluation of urinary tract abnormalities and long-term antibiotic use has become a routine treatment in children with UTIs, with surgical treatment of vesicoureteral reflux as the standard of care.[6, 7]
In the 1980s, two clinical trials compared immunotherapy with surgery alone or combined with immunosuppression. Surprisingly, both groups showed similar results. One study revealed a high rate of kidney scarring before treatment (38%), with low rates of new scarring and scar growth (2% and 9%). These findings highlight differences between pre-existing kidney damage and scarring from infections.[8,9]
The aim of our study was to evaluate uropathogens in terms of their antibiotic susceptibility patterns in pediatric patients admitted to DHQ Hospital Mirpur AJK over a period of 6 months. The purpose of this study is to provide insight into the appropriate selection of antibiotics to treat UTIs.

MATERIAL AND METHODS

A retrospective cohort study was carried out to examine the occurrence and antibiotic resistance of febrile UTIs in pediatric patients. The research took place at DHQ Hospital Mirpur AJK Pediatrics, from March to September 2023.The study included 140 pediatric patients who were selected through convenient sampling based on their symptoms suggesting a febrile UTI.
Criteria for inclusion and exclusion involved pediatric patients who had a confirmed fever of 38°C or higher, ≥5 white blood cells per high-power field in urine analysis, and a positive urine culture showing ≥ 10^5 Concentration of colony-forming units per milliliter. Patients who had identifiable urinary abnormalities or who were undergoing long-term antibiotic treatment were excluded in the study. Ethical approval was granted by the Institutional Ethical Review Board (IERB) of the DHQ hospital (IERB# REF. NO. 16).
Procedure of data collection involved reviewing medical records of both in-patients out-patients. Urine samples were collected through midstream catch technique for pediatric patients who were toilet-trained while, sterile bags were used for those who were not. Bacterial identification and testing for antibiotic susceptibility adhered to CLSI guidelines 2020, using standard methods such as disk diffusion and broth microdilution with standard culture media.[27]
The process of analyzing data was done using IBM SPSS Statistics version 20.0. The frequency of uropathogens and their susceptibility to antibiotics were categorized and reported in percentages.

RESULTS

Out of total of 140 pediatric patients, 69 (49%) had positive urine cultures suggestive of UTI. Of these 49 were females (71%) and 20 males (29%). Furthermore, 74% were 10 years old and above, and 26% were under 10 years of age (Table-I).
The most prevalent was Klebsiella pneumoniae accounting for 32% of cases followed by Staphylococcus aureus (26%). Escherichia coli was present in 22% of the cases, Enterococcus faecalis in 11%, and mixed growth of uropathogens in 9% (Figure-I).
Regarding Antimicrobial susceptibility pattern, Amikacin, Vancomycin, Neomycin and Tigecycline
were the most susceptible (100%), followed by chloramphenicol (90.9%), Linezolid (83.4%), Rifampicin (82%), Meropenem (77.3%), Nitrofurantoin (70%), Tazobactam (68.7%) and Imipenem (68.4%). Cephalexin (66.7%), Gentamicin (63.6%) and Cefipime (50%) showed moderate susceptibility. Whereas, Sulfamethoxazole (35%), Levofloxacin (31.4%),

Cefoxitin (26.3%), Ciprofloxacin (22.2%), Tetracycline (20.5%), Cefuroxime (14.2%), Ceftriaxone (9.52%) and Amoxicillin (9.52%) were the least susceptible.

Table-I: Characteristics pediatric patients with Positive urine cultures (n=69).

Characteristics	Frequency (%)
Gender	
Females	49 (71%)
Males	20 (29%)
Age	
>10	51 (74%)
<10	18 (26%)

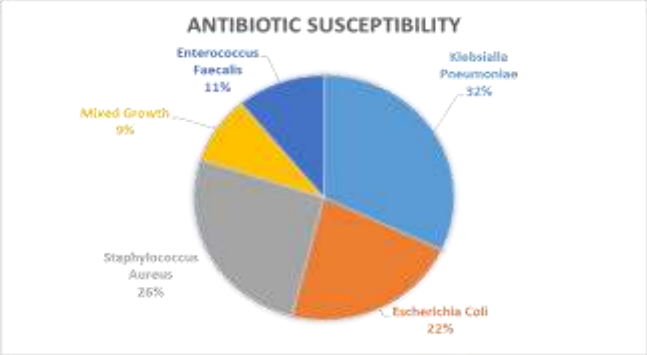


Figure-I: Pediatric uropathogens isolated among the study population (n=69).

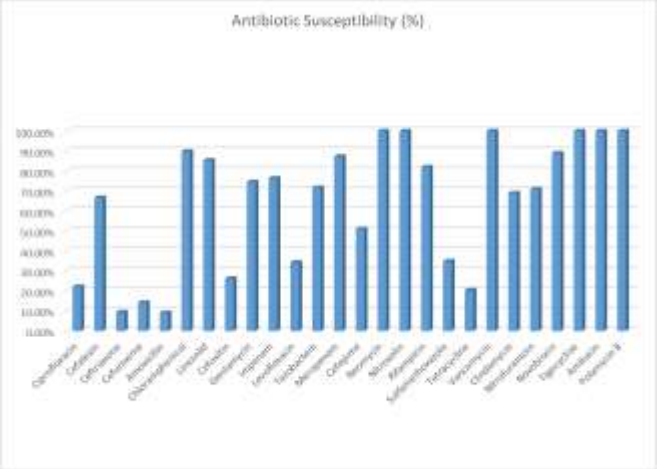


Figure-II: Antibiogram of uropathogens isolated from pediatric patients.

DISCUSSION

UTIs are a major health concern worldwide, impacting around 150 million individuals every year[49,50], with a substantial occurrence in children as well. About 3% of children in the United States are impacted annually.[10] Almost half of our study population (49%) was diagnosed with culture-proven UTI. The male-female ratio changed from 2.8-5.4:1 in the first year of life to 1:10 between ages 1-2.[11] Following early childhood, UTIs were mostly seen in females, corroborating findings of several international studies that show a higher prevalence in females.[13,14,15] *E. coli* is the leading cause of UTIs in children, accounting for 80-90%, followed by *Klebsiella pneumoniae* and *Staphylococcus aureus*. [15,16] Our results indicate a significant prevalence of *Klebsiella pneumoniae*, amounting for 32% of cases, which underscores the increasing concern regarding antibiotic-resistant bacteria. This resistance complicates the management strategies and raises the risks of morbidity and mortality in children. Urinalysis and culture are still the predominant methods for diagnosing UTIs in pediatric population with unexplained fevers.[15,16,17] Our research underscores the importance of considering local resistance patterns to steer empirical antibiotic therapy, showcasing the diverse susceptibility to different antibiotics.

Imipenem and meropenem showed relatively higher susceptibility among the isolates the most susceptible (68.4% and 77.3%).[18,19] Amikacin, an aminoglycoside, demonstrated a susceptibility rate of 100%, indicating its strong efficacy against gram-negative bacteria. Varying susceptibilities were observed with cephalosporins; cephalexin had a moderate rate of 66.7%, while ceftriaxone had lower effectiveness at 9.52%.[30] The fluoroquinolones showed decreased susceptibility, with rates of 22.2% for ciprofloxacin and 31.4% for levofloxacin.[20,21,22,23] This highlights the importance of upholding antimicrobial stewardship practices when treating pediatric UTIs. As a result, these antibiotics are excluded from the susceptibility analysis. To improve understanding, categorize the results based on types of organisms' antibiotic susceptibilities for better reader comprehension, particularly for those not well-versed in microbiology or infectious diseases.

Selecting the right antibiotic is essential for effectively treating UTIs in children, as it depends on the bacteria's susceptibility for a successful outcome. The importance of antibiotic resistance in children is a crucial factor in this situation. It should be noted that novobiocin is mainly used for diagnosis, not treatment, and CLSI recommendations advise against using polymyxin B for UTIs.

RBUS (Renal Bladder Ultrasound) and VCUG (Voiding Cystourethrogram) imaging are recommended for complex or recurrent UTIs to assist in adjusting treatments effectively. Other imaging options include DMSA (Dimercaptosuccinic Acid Scan) and NCG (Nuclear Cystogram) scans for detecting issues like vesicoureteral reflux or renal scarring.[24,25,26] Given the complexity and variability of antibiotic resistance, it is crucial to have a focused discussion on key findings.

The importance of selecting antibiotics based on specific bacteria susceptibilities is underscored by the widespread prevalence of antibiotic resistance. Enhanced management and continuous monitoring of antibiotic resistance patterns are vital for optimizing treatment efficacy and halting the spread of resistant bacteria.

The study is limited by its relatively small sample size and its regional scope, which may not accurately reflect antibiotic resistance patterns observed globally. Additionally, the retrospective design may introduce biases linked to the accuracy and completeness of medical records. Furthermore, due to the specific age range and exclusion criteria, the findings may not be applicable to all pediatric cases, such as neonates and infants, or children with ongoing antibiotic treatments. Future research should encompass larger, multi-center studies to validate these findings across broader demographics and varied geographical locations. Ongoing observation and longitudinal studies are also crucial to track the evolving patterns of resistance and assess the long-term efficacy of treatment protocols.

CONCLUSION

In conclusion, our study provides valuable insights into the antibiotic susceptibility pattern of pediatric uropathogens. To further understand the pattern of antibiotic resistance, large scale studies across diverse areas and healthcare settings are suggested. Critical is strict adherence to antimicrobial stewardship and infection control practices.

CONFLICT OF INTEREST

None

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHOR CONTRIBUTION

Ammara Manzoor: Study concept and design
Toqeer Ahmed: Data collection
Syeda Tahira Batool: Critical review
Nabia Chaudhry: Data collection and drafting of work
Fatima Khurshid: Manuscript write up and Data Analysis.

REFERENCES

1. Shrestha LB, Baral R, Poudel P, Khanal B,. Clinical, etiological and antimicrobial susceptibility profile of pediatric urinary tract infections in a tertiary care hospital of Nepal. BMC Pediatr. 2019; 19 (1): 36. DOI: https://doi.org/10.1186/s12887-019-1410-1.
2. Shaikh N, Morone NE, Bost JE, Farrell MH. Prevalence of urinary tract infection in childhood: A meta-analysis. Pediatr Infect Dis J. 2008; 27(4): 302-8. DOI: https://doi.org/10.1097/inf.0b013e31815e4122
3. Downing H, Thomas-Jones E, Gal M *et al*. The diagnosis of urinary tract infections in young children (DUTY): protocol for a diagnostic and prospective observational study to derive and validate a clinical algorithm for the diagnosis of UTI in children presenting to primary care with an acute illness. BMC Infect Dis. 2012; 158. DOI: https://doi.org/10.1186/1471-2334-12-158.
4. Desai DJ, Gilbert B, McBride CA. Paediatric urinary tract infections: Diagnosis and treatment. Australian Family Physician. 2016; 45(8): 558-564.
5. Habib S. Highlights for management of a child with a urinary tract infection. Int J Pediatr. 2012; 2012(1): 943653. DOI: https://doi.org/10.1155%2F2012%2F943653
6. Akhtar SM, Sattar A, Rizwan W, Cheema NA, Anwar A. Microbes and antibiotic susceptibility patterns of urinary tract infections in toilet-trained Children at a Tertiary Care Hospital of Sialkot, Pakistan. Professional Med J. 2021; 28(01): 22-6. DOI: https://doi.org/10.29309/TPMJ/2021.28.01.4657
7. Jamil J, Haroon M, Sultan A, Khan MA, Gul N, Kalsoom, *et al*. Prevalence, antibiotic sensitivity and phenotypic screening of ESBL/MBL producer E. coli strains isolated from urine; District Swabi, KP, Pakistan. J Pak Med Assoc. 2018; 68 (11): 1704-7.
8. Saeed CH, AL-Otraqchi KIB, Mansoor IY. Prevalence of urinary tract infections and antibiotics susceptibility pattern among infants and young children in Erbil City. Zanco J Med Sci. 2015; 19(1): 915-22. DOI: https://doi.org/10.15218/zjms.2015.0012
9. Joan LR, Finlay JC, Lang ME, Bortolussi, Canadian Paediatric Society, Infectious Diseases and Immunization Committee, Community Paediatrics Committee. Urinary tract infections in infants and children: Diagnosis and management. Paediatr Child Health. 2014 (196): 315-25. DOI: https://doi.org/10.1093%2Fpch%2F19.6.315
10. Le Saux, Nicole. Antimicrobial stewardship in daily practice: Managing an important resource. Paediatr Child Health. 2014; 19 (5): 261-70. DOI: https://doi.org/10.1093/pch/19.5.261
11. Bidell MR, Opraseuth MP, Yoon M, Mohr J, Lodise TP. Effect of prior receipt of antibiotics on the pathogen distribution and antibiotic resistance profile of key Gram-negative pathogens among patients with hospital-onset urinary tract infections. BMC Infect Dis. 2017; 17 (1): 176. DOI: https://doi.org/10.1186/s12879-017-2270-7
12. Mohammad M, Ebrahim-Saraie HS, Mansuri D, Kashei R, Hashemizadeh Z, Rajabi A, *et al*. Antimicrobial susceptibility pattern and age dependent etiology of urinary tract infections in Nemazee Hospital, Shiraz, South-West of Iran. Int J Enteric Pathog. 2015; 3(3): e26931. DOI: http://dx.doi.org/10.17795/ijep26931
13. Woo B, Jung Y, Kim HS. Antibiotic sensitivity patterns in children with urinary tract infection: Retrospective study over 8 years in a single center. Child Kidney Dis. 2019; 23(1): 22-8. DOI: https://doi.org/10.3339/jkspn.2019.23.1.22
14. Meletis, Georgios. Carbapenem resistance: Overview of the problem and future perspectives. Ther Adv Infect Dis. 2016; 3 (1): 15-21. DOI: https://doi.org/10.1177/2049936115621709
15. Cho S, Choi S, Park SE, Lee D, Choi J, Yoo J. Amikacin therapy for urinary tract infections caused by extended-

spectrum β-lactamase-producing Escherichia coli. Korean J Intern Med. 2016; 31(1): 156-61.
DOI: https://doi.org/10.3904/kjim.2016.31.1.156

16. Thy M, Timsit J, Montmollin E. Aminoglycosides for the treatment of severe infection due to resistant gram-negative pathogens. Antibiotics (Basel). 2023; 12 (5); 860.
DOI: https://doi.org/10.3390/antibiotics12050860
17. Mahony M, McMullan B, Brown J, Kennedy SE. Multidrug-resistant organisms in urinary tract infections in children. Pediatr Nephrol. 2020; 35(9): 1563-73.
DOI: https://doi.org/10.3390/antibiotics12050860
18. Cunha BA. Oral doxycycline for non-systemic urinary tract infections (UTIs) due to P. aeruginosa and other Gram negative uropathogens. Eur J Clin Microbiol Infect Dis. 2012; 31: 2865-8.
DOI: https://doi.org/10.1007/s10096-012-1680-0
19. Flores-Mireles, AL, Walker JN, Caparon M, Hultgren SJ, *et al*. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nat Rev Microbiol. 2015; 13 (5): 269-84.
DOI: https://doi.org/10.1038%2Fnrmicro3432
20. Patel S, Preuss CV, Bernice F. Vancomycin. Nih.gov. StatPearls Publishing; 2023. Available from: https://www.ncbi.nlm.nih.gov/books/NBK459263/
21. Wingler MJ, Patel NR, King ST, Wagner JL, Barber KE, Stover KR, *et al*. Linezolid for the treatment of urinary tract infections caused by Vancomycin-Resistant Enterococci. Pharmacy (Basel). 2021; 9 (4): 175.
DOI: https://doi.org/10.3390%2Fpharmacy9040175
22. Pontefract BA, Rovelsky SA, Madaras-Kelly KP. Linezolid to treat urinary tract infections caused by vancomycin-resistant *Enterococcus*. SAGE Open Med. 2020; 8: 2050312120970743.
DOI: https://doi.org/10.1177%2F2050312120970743
23. Squadrito FJ, Del Portal D. Nitrofurantoin [Internet]. Nih.gov. StatPearls Publishing; 2023. Available from: https://www.ncbi.nlm.nih.gov/books/NBK470526/
24. Hilt EE, Parnell L, Wang D, Stapleton AE, Lukacz ES. Microbial Threshold Guidelines for UTI Diagnosis: A Scoping Systematic Review. Pathol Lab Medicine Int. 2023; 15: 43–63.
DOI: https://doi.org/10.2147/plmi.s409488
25. Zeng Z, Zhan J, Zhang K, Chen H, Cheng S. Global, regional, and national burden of urinary tract infections from 1990 to 2019: An analysis of the global burden of disease study 2019. World J Urol. 2022; 40(1):
DOI: https://doi.org/10.1007/s00345-021-03913-0
26. Liang D, Wang ME, Dahlen A, Liao Y, Saunders AC, Coon ER, Schroeder AR. Incidence of pediatric urinary tract infections before and during the COVID-19 Pandemic. JAMA Network Open. 2024; 7(1): e2350061–e2350061.
DOI:https://doi.org/10.1001/jamanetworkopen.2023.50061
27. M100 Performance Standards for Antimicrobial Susceptibility Testing A CLSI supplement for global application. 30th Edition [Internet]. Available from: https://nih.org.pk/wp-content/uploads/2021/02/CLSI-2020.pdf

INSTRUCTIONS FOR AUTHORS

Infectious Disease Journal of Pakistan (IDJP) is an official journal of Medical Microbiology and Infectious Diseases Society of Pakistan (MMIDSP). IDJP is a peer-reviewed, open access journal and publishes original articles, review articles, brief reports, case reports, short communications, letter to the editor and notes and news in the fields of microbiology, infectious diseases, public health; with laboratory, clinical, or epidemiological aspects. The Journal does not publish veterinary studies and studies based on animal models alone.

SUBMISSION OF THE MANUSCRIPT

- Go to the IDJP official website: **http://ojs.idj.org.pk/index.php/Files/index**
- Click **'make a submission'** tab on IDJP official website.
- Please read checklist and make sure that no point in the checklist is missing
- Click **'register'** if you are submitting to IDJP for the first time. If you are already registered with the journal then click 'login'.
- A new page will open once you click 'register'. Enter the required information in the given fields. The journal requests all its authors **to register themselves as reviewer** by clicking 'Yes, request the reviewer role' available at the bottom of 'registration' page. After clicking on 'register' a new page will open.
- Please click **'make a new submission'** on this page
- A new page will open, with 5 tabs on the top.
    - Start,
    - Upload submission,
    - Enter metadata,
    - Confirmation,
    - Next steps. You will be in 'start' tab.
- In 'Section' dropdown list, select the appropriate option. Please make sure that all the 'submission requirements' are fulfilled. Tick the checkbox against each requirement. **Note:** if any of the requirement is not fulfilled, and you put a tick mark against it, you still will be able to proceed and complete your submission process but the submission will be declined automatically by the system. In 'comments for the editor' area you can write your comments, however, it is optional. Under 'Acknowledge the copyright statement' tick the checkboxes against both the options. Click 'save and continue'
- Now you will be in 'upload submission' tab. A dialogue box will open and here you can upload all your files **(article text, and Submission Documents e.g., Title page, ERC/IRB approval letter, undertaking)** one by one. At the moment the journal does not charge any processing fee. Click 'save and continue'.
- Now you will be in 'enter metadata' tab. You may leave 'prefix' field blank. Write title of the article in 'title' field. Write running/short title in 'subtitle' field (you may leave it blank). Copy/paste abstract in abstract area. In 'list of contributors' sections, click 'add contributor' (if you have other authors with you). A new dialogue box will open. Please fill in details of each contributor (author). Please do give **affiliation** of each contributor. Additional details about the contributor can be given in the text box below. For 'contributor's role' please click 'author'. Please tick the check box 'Principal contact for editorial correspondence' for corresponding author only. Lower checkbox 'Include this contributor in browse lists?' will remain ticked for all the authors. Click 'save' and repeat the same process for all the authors one by one. Please **add all the authors** as per the pre-decided sequence. Please fill the 'languages', 'subjects', 'discipline' and 'key words' fields appropriately. These fields are essential. Each key word **MUST be added SEPARATELY** one by one instead of 'copy/paste' all the key words together. Please click 'save and continue'.

- You are now in 'confirmation' tab. Please click 'finish submission'. Congratulations, you have successfully completed your submission to IDJP.
- You can track the status/progress of your article through editorial process any time by logging into your account.

MANUSCRIPT CATEGORIES

1. Original Articles

Articles should report original work in the fields of microbiology, infectious disease or public health. The word limit for original articles is 2000 to 2500 (excluding abstract and references).

- **Title page**: This should list the (i) title of the article: Should be concise and self-descriptive, (ii) the full names of each author with highest academic degree(s), institutional addresses, contact numbers and email addresses of all authors. (iii) The corresponding author should also be indicated with his/her name, address, telephone, fax number and e-mail address. (iv) A short running title of not more than 40 characters (count letters and spaces) placed at the foot end of the title page. (v) a conflict-of-interest statement should also be included in this section.
- **Abstract:** Abstract should be between 200-250 words and must be structured in to separate sections headed Background, Material and Methods, Results and Conclusions and Key words. Please do not use abbreviations or cite references in the abstract. A short list of three to ten key words should be provided to facilitate. Use terms from the Medical Subject Headings (MeSH) list of index medicus, if suitable MeSH terms are not yet available for recently introduced terms, present terms may be used. Key words should be arranged alphabetically.
- **Background**: The section must clearly state the background to the research and its aims. Controversies in the field should be mentioned. The key aspects of the literature should be reviewed focusing on why the study was necessary and what additional contribution will it make to the already existing knowledge in that field of study. The section should end with a very brief statement of the aims of the article.
- **Material and Methods:** Please provide details of subject selection (patients or experimental animals). Details must be sufficient to allow other workers to reproduce the results. The design of study and details of interventions used must be clearly described. Identify precisely all drugs and chemicals used, including generic name(s) and route(s) of administration All research carried out on humans must be in compliance with the Helsinki Declaration, and animal studies must follow internationally recognized guidelines. The authors are expected to include a statement to this effect in the Methods section of the manuscript. A description of the sample size calculation and statistical analysis used should be provided. ERC should bear the signature of the President of the ERC on official letter head with stamp and date of approval along with the reference number. If the president or the member of the ethical review committee is author of study, then the certificate will be signed by any senior member of the committee. Reference number of the Institution Review Committee (IRC)/ Ethical Review Board (ERB) certificate to be mentioned in the methodology section. Title of the research article should match the title on the ERC.
- **Results**: Present results in logical sequences in the text, tables and illustrations. Articles can have a maximum of 5 illustrations (in a combination of figures and tables) per article. The results should be in past tense and repetition of results presented in the tables should be avoided. Exact P-values should be reported along with reporting of OR and RR with their Confidence Intervals where applicable.
- **Discussion:** Emphasize the new and important aspects of the study and conclusions that follow from them. Do not repeat the details from the results section. Discuss and compare results of your study with national and international studies. Discuss the implications of the findings and the

strengths and limitations of the study. Link the conclusions with the goals of the study but avoid unqualified statements and conclusion not completely supported by your data.

- **Conflict of Interest:** When authors submit a manuscript, they must disclose all financial and personnel relationship that might bias their work. Authors must state explicitly whether potential conflicts do or do not exist. They should do so in the manuscript on the title page. Additional details can be provided, if necessary, in a covering letter which accompanies the manuscript. Authors of study funded by an agency with proprietary or financial interest in the outcome must sign a statement that they had full excess to all the data in the study and take complete responsibility for the integrity of the data and the accuracy of the data analysis. This statement should be submitted along with the manuscript.
- **Acknowledgments:** All contributors who do not meet the criteria for authorship should be covered in the acknowledgement section. It should include persons who provided technical help, writing assistance and departmental head that only provided general support. Financial and material support should also be acknowledged.
- **Author Contributions:** Authorship credit should be based on:

  Substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data.
  - Drafting the article or revising it critically for important intellectual content. Final approval of the version to be published.
  - Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.
  - Authors are bound to declare that the Manuscript includes the name/s of supervisor/s in authorship (Abstract of studies, thesis, dissertation or any research) (For details of authorship criteria kindly consult ICMJE recommendations for the conduct, reporting, editing and publication of scholarly work in Medical Journals.
  - Completely fill contributions of each author mentioned in the authors certificate

  Acquisition of funding, collection of data, or general supervision of the research group, alone does not justify authorship. All persons designated as authors should qualify for authorship & all those who qualify should be listed.
  - Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content.
  - In case of more than one author in a manuscript, the contributions of each person listed as author in the study should be mentioned.
  - Those who provide technical support, writing assistance, or department chair who provided just support should also be mentioned in acknowledgment
  - If there is any conflict of interest, please mention in the manuscript.

When a large, multi-center group has conducted the work, the group should identify the individuals who accept direct responsibility for the manuscript. These individuals should fully meet the criteria for authorship defined above and editors will ask these individuals to complete journal-specific author and conflict of interest disclosure forms. When submitting a group author manuscript, the corresponding author should clearly indicate the preferred citation and should clearly identify all individual authors as well as the group name. Other members of the group should be listed in the acknowledgements. Addition and deletion of authors may not be permitted after submission with authorship proforma duly signed.

2. **Review Articles:** Authoritative and state of the art review articles on topical issues are also published, with a word limit of 2000. It should consist of critical overview of existing literature along with reference to new developments in that field. These should be comprehensive and fully

referenced. Articles should contain an Abstract; Main Text divided into sections, Conclusions and References.

3. **Brief Reports:** Short clinical and laboratory observations are included as Brief Reports. The text should contain no more than 1000 words, two illustrations or tables and up to 10 references.
4. **Case Reports:** Instructive cases with a message are published as case reports. Routine syndromes or rare entities without unusual or new features are invariably rejected. The text should contain no more than 1000 words, two illustrations or tables and up to 10 references. The authorship should not exceed 3-4 persons.
5. **Letter to the Editor:** These may relate to material published in the IDJ, topic of interest pertaining to infectious diseases, and/or unusual clinical observations. A letter should not be more than 300 words, one figure and 3-5 references.
6. **News and Views:** Informative, breaking news updates in infectious diseases from around the world (approx. 200 words).
7. **Notices:** Announcements of conferences, symposia or meetings may be sent for publication at least 12 weeks in advance of the meeting date. Details of programs should not be included.

REFERENCES

Number references consecutively in the order in which they are first mentioned in the text. Reference should provide the following information: Author's name with initials, full title of cited article, name of the journal in which the article appeared (in abbreviated form), Year of Publication, Journal's Volume, Number and finally first & the last page numbers. Write Page No. like this 120-126. References appearing in a table or figure should be numbered sequentially with those in text. DOI number of those references where it is available. According to "Uniform Requirements of Manuscripts submitted to Biomedical Journals", as cited in N Engl J Med 1997; 336:309-15.

For quantitative study 18-25 references are allowed and for qualitative study 30-40 references are allowed. 50% of the references should be from the Clarivate Impact Factor Journals. Mention place /first author/ year of previous studies, mention their statistics while giving reference in the introduction and discussion. The reference should not be split and make sure uniform style (Vancouver) is followed throughout. IDJP follows Index Medicus style for references and abbreviated journal names according to the list of Journals indexed in Index Medicus.

Journals: Standard journal article. (List all authors when six or less; when seven or more, list only first six and add et al)

You CH. Lee KY, Chey WY, Manguy R. Electro gastrographic study of patients with unexplained nausea, bloating and vomiting. Gastroenterol 1980; 79: 311-314. Chapter in a book: Weinstein L, Swartz MN. Pathogenic properties of invading microorganisms. In: Sodeman WA Jr, Sodeman WA, eds. Pathologic physiology: mechanisms of disease. WB Saunders, Philadelphia 1974; 457-472.

- et al. is written after 6 authors names, list first 6 authors' names then write et al. in references.
- Write complete surname and convert first name and middle name to the initials, following each surname. e.g., Rimondini LR, Zolfanelli B, Bernardi F, Bez C.

TABLES AND FIGURES

- Data reported either in a table or in a figure should be illustrative of information reported in the text, but should not be redundant with the text. Each table must be presented on a separate sheet of paper and numbered in order of appearance in the text. Table should be numbered consecutively in Arabic numerals. Tables and Figures legends should be self-explanatory with adequate headings and footnotes. Results which can be described as short statements within the text should not be presented as figures or tables.

PLAGIARISM

- Plagiarism is the unauthorized use or close imitation of the language and thoughts of another author and representing them as one's own original work. Within the academia, a researcher is considered an academic fraud and the offenders are subjected to academic censure. Plagiarism can be unintentional or intentional reproducing academic material without appropriate citation. Similarly self-plagiarism is the re-use of significant, identical or near identical portions of one's own work without citing the original work. This is also known as ―Recycling fraud‖. Worst form of plagiarism is to steal the whole article from some journal and publish it under one's own name in another journal. Lately the use of internet has made it easier to plagiarize, by copying the electronic tests and using them as the original work. All articles are checked for plagiarism and any article found to have a similarity index of more than 33% is not processed further. It is the policy of editorial committee of IDJP to blacklist any author found to be guilty of plagiarism. The name of author(s) committing plagiarism will also be disseminated to editors of other medical journals, PMC and HEC.