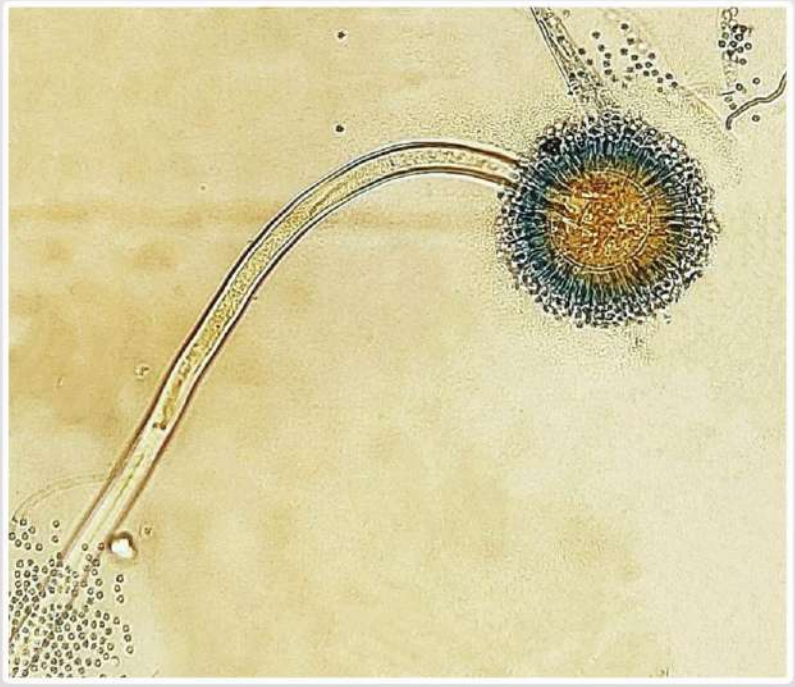
Print ISSN 1027-0299
Oline ISSN 3007-9314

INFECTIOUS DISEASES JOURNAL OF PAKISTAN | IDJP

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



Quarterly

Vol. 33, No. 3, Jul - Sep 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

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CONTENTS

ORIGINAL ARTICLE

Oral treatment options for patients with urinary tract infections caused by carbapenem-resistant *Escherichia coli*

Nasrullah Malik[1], Aqib Sultan[1], Farah Shameem[2], Summiya Nizamuddin[1]

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ABSTRACT

Background: Urinary tract infections (UTIs) are prevalent globally, with *Escherichia coli* being the predominant pathogen. Carbapenem-resistant *E. coli* strains exacerbate the clinical burden due to restricted treatment options. This study assessed the antibiotic susceptibility profiles of carbapenem-resistant *E. coli* strains associated with UTIs, aiming to identify effective oral treatment alternatives.

Material and Methods: The Cross-sectional study was conducted in the section of microbiology of the Shaukat Khanum Memorial Cancer Hospital and Research Centre, Lahore, from January 2018 to December 2022. This study was undertaken to assess the prevalence of carbapenem-resistant *E. coli* in urine samples and their susceptibility profiles against fosfomycin, nitrofurantoin, co-trimoxazole, ciprofloxacin, and tetracycline.

Results: A total of 978 carbapenem-resistant *E. coli* isolates were identified during this time period. Approximately 54% (527) of these isolates were recovered from female patients. Fosfomycin, nitrofurantoin, tetracycline, cotrimoxazole and ciprofloxacin were found to be susceptible against 82%, 67.2%, 15.2%, 9.7% and 0.1% carbapenem-resistant *E. coli* isolates, respectively. More than 80% of all *E. coli* were sensitive to fosfomycin. Ciprofloxacin exhibited the lowest susceptibility rate. 82% of carbapenem-resistant *E. coli* isolates were susceptible to fosfomycin, 67.2% to nitrofurantoin, 15.2% to tetracycline, 9.7% to cotrimoxazole, and 0.1% to ciprofloxacin.

Conclusion: The emerging carbapenem resistance among gram-negative bacteria markedly limits oral therapeutic alternatives. However, this study displays high susceptibility rates to fosfomycin and nitrofurantoin. We propose their utilization for managing uncomplicated UTIs caused by carbapenem-resistant *E. coli*.

Keywords: Carbapenem resistance, *Escherichia coli*, Urinary tract infections

BACKGROUND

Urinary tract infections (UTIs) stand out as one of the most prevalent infectious diseases, affecting individuals in both community and hospital settings, thereby contributing significantly to the healthcare burden.[1] UTIs exhibit a higher incidence in females a phenomenon attributed to the relatively shorter length of their urethras in comparison to males.[2]

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This article can be cited as: Malik N, Sultan A, Nizamuddin S, Shameem F. Oral treatment options for patients with urinary tract infections caused by carbapenem-resistant *Escherichia coli*. Infect Dis J Pak. 2024; 33(3): 102-106. DOI: https://doi.org/10.61529/idjp.v33i3.280

Receiving date: **04 Jan 2024** Acceptance Date: **04 Sep 2024**
Revision date: **18 Mar 2024** Publication Date: **30 Sep 2024**

Cystitis and other lower urinary tract infections are often managed in outpatient settings. Whereas, upper UTIs such as pyelonephritis often entail the risk of sepsis and bacteremia, necessitating the administration of intravenous antibiotics and hospitalization for effective treatment.[3]

Gram-negative bacteria constitute the predominant etiological agents of UTIs, comprising of more than 90% of reported cases. Among these, *Escherichia coli* (*E. coli*) emerges as the most prevalent gram-negative bacterium responsible for UTIs, contributing to approximately 80% of all occurrences.[4]

The escalation of antimicrobial resistance within the Enterobacterales group has presented a difficult challenge in the treatment of UTIs, primarily stemming from the restricted options of available therapeutic alternatives. The global emergence of Enterobacterales capable of producing extended spectrum beta-

lactamases (ESBL) had exacerbated this issue, resulting in the ineffectiveness of commonly prescribed oral antibiotics like trimethoprim, quinolones, cephalosporins, and penicillins for the management of UTIs.[5]

Further on, with the advent of carbapenem resistance, managing these infections with limited treatment options has become a global challenge. Carbapenem-resistant strains also present a significant public health menace, given their propensity for extensive dissemination, resulting in elevated morbidity and mortality rates within healthcare setting.[6] UTIs attributed to carbapenem-resistant Enterobacterales also correlate with prolonged hospitalization durations and escalated healthcare costs.[7]

The search for novel antibiotics is desperately needed. The revival of old antimicrobials like, fosfomycin, and nitrofurantoin could offer a valuable solution in this scenario, bridging the gap until the development of novel antimicrobials.[8]

Fosfomycin is an oral bactericidal drug, having antimicrobial properties against both gram-positive and gram-negative organisms, and has been used to treat UTIs for the last four decades. Nitrofurantoin is another oral bactericidal drug, considered as the first line therapy for acute uncomplicated UTI.[9]

Co-trimoxazole, ciprofloxacin and doxycycline are also other oral antibiotic options available for treating UTIs, however, these are usually opted for as targeted therapy choices, as opposed to being prescribed empirically.[10]

The selection of an effective oral antibiotic therapy may be aided by knowledge of the local prevalence of carbapenem-resistant *E. coli* that causes UTIs and their drug susceptibility profile. This retrospective study was therefore carried out to evaluate the antibiotic susceptibility profiles of carbapenem-resistant *E. coli* stains, in order to identify the effective oral treatment options.

MATERIAL AND METHODS

This retrospective study was conducted at Shaukat Khanum Memorial Cancer Hospital and Research Centre (SKMCH & RC), Lahore. Urine culture and susceptibility data from SKMCH & RC, Lahore and its network of laboratory collecting centers across Pakistan were analyzed. All carbapenem-resistant *E. coli* isolates recovered from urine cultures between January 2018 and December 2022 were included in this study, while carbapenem-susceptible *E. coli* isolates and duplicate isolates were excluded. *E. coli* isolates found to be resistant to imipenem, meropenem, ertapenem or doripenem according to the current Clinical and Laboratory Standards Institute (CLSI) M100, 33rd edition breakpoints (unchanged since 2011) were defined as carbapenem-resistant (11).

Using a semi-quantitative method, urine samples collected from patients either midstream or by catheterization were cultured onto Cystine Lactose Electrolyte Deficient (CLED) agar using 0.01 mL calibrated loops. Culture plates were incubated for 24 hours at 37 °C. Conventional techniques like API (BioMeurex) were utilized to identify the isolated microorganisms.

Antimicrobial susceptibility testing was done by the disc diffusion method according to CLSI M100, 33rd edition guidelines.[11]

Data on the patient's demographics, laboratory results, and susceptibility results for fosfomycin, nitrofurantoin, trimethoprim, ciprofloxacin, and tetracycline were obtained from the hospital information management system. No clinical data was gathered.

All clinical and microbiological data was compiled and analyzed using Statistical Package for Social Sciences (SPSS) version 24.0. Descriptive statistics were presented in the form of frequencies and percentages. Frequencies of susceptibility rates of fosfomycin, nitrofurantoin, co-trimoxazole, ciprofloxacin and tetracycline against carbapenem-resistant *E. coli* were represented by using percentages and graphs.

RESULTS

From 2018 to 2023, a total of 13,332 *Escherichia coli* isolates were obtained from urine cultures. Among these, 978 isolates were identified as carbapenem-resistant and were included in this study. Of these carbapenem-resistant isolates, 527 (53.9%) were from female patients and 451 (46.1%) from male patients. The mean age of patients was 50 years. Specifically, 462 (47.2%) isolates were from patients aged 19-64 years, 413 (42.2%) from patients older than 64 years, and 103 (10.5%) from patients 18 years old or younger.

The majority of 757 isolates (77.4%) originated from Punjab, followed by 181 isolates (18.5%) from Khyber

Pakhtunkhwa, 17 isolates (1.7%) from the Federally Administered Tribal Areas (FATA), 10 isolates (1%) from Balochistan, 9 isolates (0.9%) from Sindh, and 4 isolates (0.4%) from Azad Jammu and Kashmir.

Antimicrobial susceptibility testing of carbapenem-resistant *E. coli* showed that the bulk of the isolates were susceptible to fosfomycin and nitrofurantoin, with fosfomycin susceptibility at 82% (802) and nitrofurantoin susceptibility at 67.2% (657).

Simultaneously, these isolates showed poor susceptibility to tetracycline, co-trimoxazole and ciprofloxacin. Only 152 (15.5%) isolates were susceptible to tetracycline, 95 (9.7%) isolates were susceptible to co-trimoxazole, and 23 (2.4%) isolates showed susceptibility to ciprofloxacin.

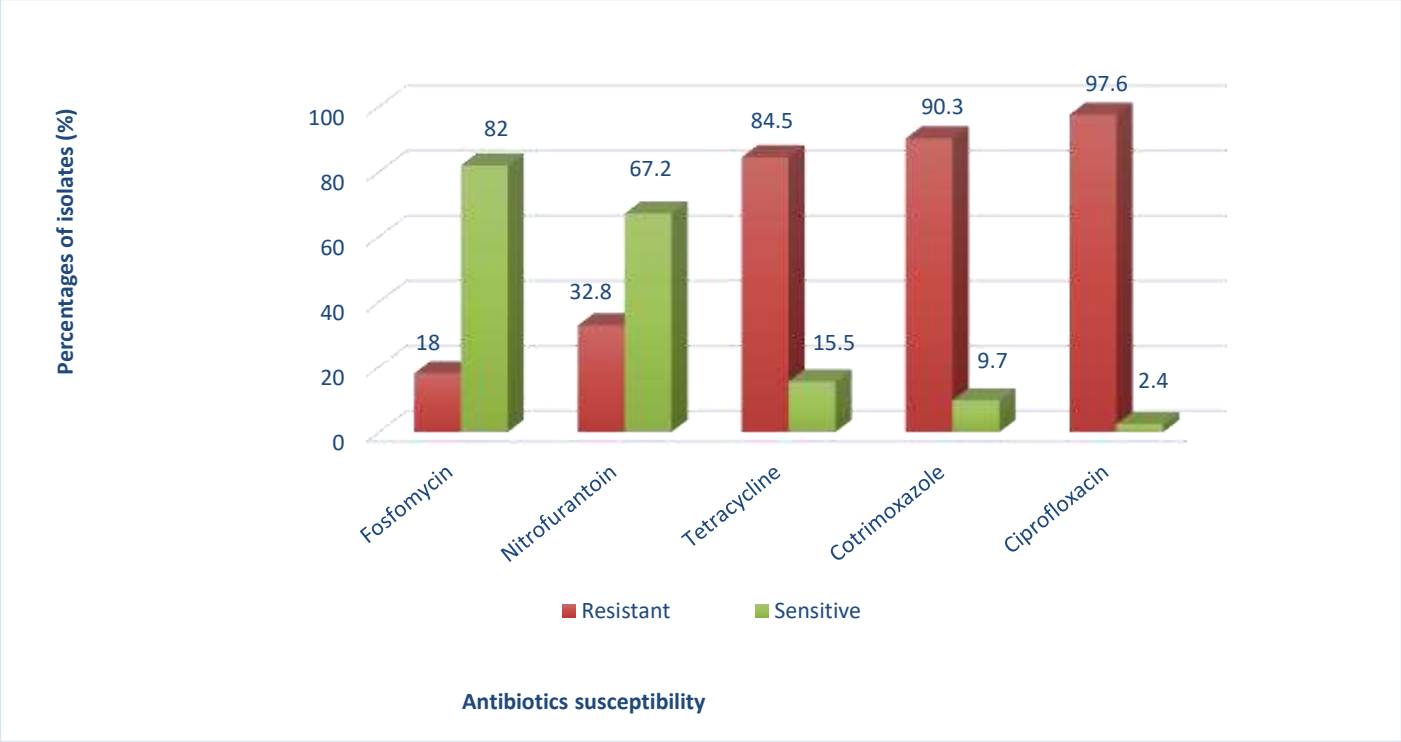

Figure-I: Susceptibility rates of oral antimicrobials against carbapenem-resistant *Escherichia coli* (n=978).

DISCUSSION

Around the world, *E. coli* is the most common cause of UTIs.[12] Recent years have seen an increase in carbapenem-resistant bacteria that cause UTIs in both hospital and community settings. This has created new and challenging obstacles for treatment decision-makers. Evolution of carbapenem resistance among Enterobacterales is posing a continuous burden on the healthcare settings due to limited treatment options.[6, 13]

The fact that carbapenem-resistant organisms frequently exhibit co-resistance to antibiotics exacerbates the already complex situation. Co-resistance in carbapenem-resistant gram-negative bacteria refers to the simultaneous resistance to multiple classes of antibiotics, apart from carbapenems. This phenomenon poses a significant challenge in the treatment of infections caused by these bacteria, as it limits the effectiveness of various antibiotic options and can lead to the use of last-resort antibiotics, further contributing to the development of antibiotic resistance.[14]

Infections caused by carbapenem-resistant Enterobacterales is associated with high morbidity and mortality among hospitalized patients. Carbapenem-resistant Enterobacterales contribute to a higher mortality rate among patients with complicated UTIs and urosepsis when compared to carbapenem-sensitive Enterobacterales.[15] Therefore, it is important and challenging to choose an appropriate empirical therapy especially in elderly patients with comorbidities.[12]

According to the present study, carbapenem-resistant *E. coli* showed the highest susceptibility to fosfomycin (82%). A study conducted in London also showed high susceptibility rates (60.5%) of fosfomycin against carbapenem-resistant Enterobacterales and 100% susceptibility against carbapenem-resistant *E. coli* isolates.[16] Additionally, according to various previous studies, over 90% of ESBL-producing *E. coli* isolates have shown susceptibility to fosfomycin. [17-20]

The present-day analysis found that 67.2% of carbapenem-resistant *E. coli* isolates were susceptible to nitrofurantoin. This finding is also consistent with various previous studies where >90% of ESBL-producing *E. coli* isolates demonstrated susceptibility to nitrofurantoin (17, 19, 20). However, another study found that nitrofurantoin inhibited only <25% of the carbapenem-resistant Enterobacterales.[16]

According to the present study, only 9.7% of carbapenem-resistant *E. coli* isolates demonstrated susceptibility to co-trimoxazole. This rate is significantly lower compared to rates reported other studies. For instance, one study found that 37.9% of ESBL producing *E. coli* isolates were susceptible to co-trimoxazole.[19] However, another study conducted in Japan found that 89% of Enterobacterales causing community-acquired UTIs were susceptible to co-trimoxazole.[21]

The current review found that only 2.4% of carbapenem-resistant *E. coli* isolates demonstrated susceptibility to ciprofloxacin. A study conducted in London also found that fewer than 25% of carbapenem-resistant Enterobacterales were susceptible to ciprofloxacin.[16] Similarly, another study conducted in Ethiopia found that only 20% of carbapenemase producing Enterobacterales were resistant to ciprofloxacin.[22]

The current study shows that nitrofurantoin and fosfomycin are effective in vitro against carbapenem-resistant *E. coli*. According to both the Infectious Diseases Society of America (IDSA) 2023 guidelines as well as the Sanford guide to antimicrobial therapy, nitrofurantoin and co-trimoxazole are the preferred antibiotics, whereas, fosfomycin is the alternative choice for treating uncomplicated cystitis.[23,24]

Considering the results of the present investigation, we recommend that fosfomycin and nitrofurantoin may be considered as empirical antibiotic choices for patients at risk of developing UTIs caused by carbapenem-resistant Enterobacterales, pending confirmation through culture results.

It is also important to note that the susceptibility rates of these antibiotics may vary between hospitals and their specific settings. Additionally, in light of the rising resistance rates, it is imperative to conduct continuous surveillance studies and generate site-specific antibiograms.

There are several limitations of the current study. It is a single-centred study. Further molecular analysis to identify the carbapenemases produced by the carbapenem-resistant *E. coli* isolates was not carried out due to budget constraints. Furthermore, the clinical response of patients on oral antimicrobials was not monitored as a part of this study.

CONCLUSION

The current study concluded that majority of carbapenem-resistant *E. coli* isolates causing urinary tract infections were susceptible to fosfomycin, followed by nitrofurantoin. Increased susceptibility to nitrofurantoin and fosfomycin suggests their potential utility as empirical treatments for urinary tract infections caused by carbapenem-resistant *E. coli*.

CONFLICT OF INTEREST

None

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHOR CONTRIBUTION

Nasrullah Malik: 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, final approval of the version to be published

Aqib Sultan: Acquisition, analysis and interpretation of data and Drafting the work or revising it critically for important intellectual content and bench work.

Summiya Nizamuddin: 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, final approval of the version to be published

Farah Shameem: Acquisition, analysis and interpretation of data

REFERENCES

1. Öztürk R, Murt A. Epidemiology of urological infections: A global burden. World J Urol. 2020; 38(11): 2669-79. DOI: https://doi.org/10.1007/s00345-019-03071-4
2. Whelan S, Lucey B, Finn K. Uropathogenic *Escherichia coli* (UPEC)-associated urinary tract infections: The molecular basis for challenges to effective treatment. Microorganisms. 2023; 11(9): 2169. DOI: https://doi.org/10.3390/microorganisms11092169
3. Johnson JR, Russo TA. Acute pyelonephritis in adults. New Eng Med J. 2018; 378(1): 48-59. DOI: https://doi.org/10.1056/NEJMcp1702758
4. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. 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
5. Tüzün T, Sayın Kutlu S, Kutlu M, Kaleli İ. Risk factors for community-onset urinary tract infections caused by extended-spectrum β-lactamase-producing *Escherichia coli*. Turkish J Med Sci. 2019; 49(4): 1206-11. DOI: https://doi.org/10.3906%2Fsag-1902-24
6. Tekele SG, Teklu DS, Legese MH, Weldehana DG, Belete MA, Tullu KD, *et al*. Multidrug-resistant and carbapenemase-producing *Enterobacteriaceae* in Addis Ababa, Ethiopia. Biomed Res Int. 2021; 2021: 9999638. DOI: https://doi.org/10.1155/2021/9999638
7. Alexander EL, Loutit J, Tumbarello M, Wunderink R, Felton T, Daikos G, *et al*. Carbapenem-resistant *Enterobacteriaceae* infections: Results from a retrospective series and implications for the design of prospective clinical trials. Open Forum Infect Dis. 2017; 4(2): ofx063. DOI: https://doi.org/10.1093/ofid/ofx063
8. Solomon SL, Oliver KB. Antibiotic resistance threats in the United States: stepping back from the brink. Am Fam Physician. 2014;89(12):938-41.
9. Tulara NK. Nitrofurantoin and Fosfomycin for Extended Spectrum Beta-lactamases Producing *Escherichia coli* and Klebsiella pneumoniae. 2018;10(1):19-21.
10. Kot B. Antibiotic resistance among uropathogenic *Escherichia coli*. Pol J Microbiol. 2019; 68(4): 403-15. DOI: https://doi.org/10.33073/pjm-2019-048
11. M100 performance standards for antimicrobial susceptibility testing. 33rd ed2023.
12. Medina M, Castillo-Pino E. An introduction to the epidemiology and burden of urinary tract infections. Ther Adv Urol. 2019; 11: 1756287219832172. DOI: https://doi.org/10.1177%2F1756287219832172
13. Vallejo-Torres L, Pujol M, Shaw E, Wiegand I, Vigo JM, Stoddart M, *et al*. Cost of hospitalised patients due to complicated urinary tract infections: A retrospective observational study in countries with high prevalence of multidrug-resistant Gram-negative bacteria: The COMBACTE-MAGNET, Rescuing study. BMJ Open. 2018; 8(4): e020251. DOI: https://doi.org/10.1136/bmjopen-2017-020251
14. Bischoff S, Walter T, Gerigk M, Ebert M, Vogelmann R. Empiric antibiotic therapy in urinary tract infection in patients with risk factors for antibiotic resistance in a German emergency department. BMC Infect Dis. 2018; 18(1): 56. DOI: https://doi.org/10.1186/s12879-018-2960-9
15. Shields RK, Zhou Y, Kanakamedala H, Cai B. Burden of illness in US hospitals due to carbapenem-resistant Gram-negative urinary tract infections in patients with or without bacteraemia. BMC Infect Dis. 2021; 21(1): 572. DOI: https://doi.org/10.1186/s12879-021-06229-x
16. Livermore DM, Warner M, Mushtaq S, Doumith M, Zhang J, Woodford N. What remains against carbapenem-resistant Enterobacteriaceae? Evaluation of chloramphenicol, ciprofloxacin, colistin, fosfomycin, minocycline, nitrofurantoin, temocillin and tigecycline. Int J Antimicrob Agents. 2011; 37(5): 415-9. DOI: https://doi.org/10.1016/j.ijantimicag.2011.01.012
17. Auer S, Wojna A, Hell M. Oral treatment options for ambulatory patients with urinary tract infections caused by extended-spectrum-beta-lactamase-producing *Escherichia coli*. Antimicrob Agents Chemother. 2010; 54(9): 4006-8. DOI: https://doi.org/10.1128/aac.01760-09
18. Falagas ME, Kastoris AC, Kapaskelis AM, Karageorgopoulos DE. Fosfomycin for the treatment of multidrug-resistant, including extended-spectrum beta-lactamase producing, Enterobacteriaceae infections: A systematic review. Lancet Infect Dis. 2010; 10(1): 43-50. DOI: https://doi.org/10.1016/s1473-3099(09)70325-1
19. Tulara NK. Nitrofurantoin and fosfomycin for extended spectrum beta-lactamases producing *Escherichia coli* and *Klebsiella pneumoniae*. J Glob Infect Dis. 2018; 10(1): 19-21. DOI: https://doi.org/10.4103%2Fjgid.jgid_72_17
20. Raja NS. Oral treatment options for patients with urinary tract infections caused by extended spectrum βeta-lactamase (ESBL) producing Enterobacteriaceae. J Infect Public Health. 2019; 12(6): 843-6. DOI: https://doi.org/10.1016/j.jiph.2019.05.012
21. Kanda N, Hashimoto H, Sonoo T, Naraba H, Takahashi Y, Nakamura K, *et al*. Gram-negative organisms from patients with community-acquired urinary tract infections and associated risk factors for antimicrobial resistance: A single-center retrospective observational study in Japan. Antibiotics (Basel, Switzerland). 2020; 9(8): 438. DOI: https://doi.org/10.3390%2Fantibiotics9080438
22. Eshetie S, Unakal C, Gelaw A, Ayelign B, Endris M, Moges F. Multidrug resistant and carbapenemase producing Enterobacteriaceae among patients with urinary tract infection at referral Hospital, Northwest Ethiopia. Antimicrob Resist Infect Control. 2015; 4(1): 12. DOI: https://doi.org/10.1186/s13756-015-0054-7
23. Tamma PD, Aitken SL, Bonomo RA, Mathers AJ, van Duin D, Clancy CJ. Infectious Diseases Society of America 2023 Guidance on the Treatment of Antimicrobial Resistant Gram-Negative Infections. Clin Infect Dis. 2023 Jul 18: ciad428. DOI: https://doi.org/10.1093/cid/ciad428
24. The Sanford Guide to Antimicrobial Therapy Dallas, TX: Antimicrobial Therapy, Inc, 1995.

ORIGINAL ARTICLE

Spectrum of hepatic dysfunction in dengue epidemic in Rawalpindi Pakistan in 2023

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ABSTRACT

Background: To study the relationship of spectrum of hepatitis dysfunction with other clinical features in Dengue fever. This research article aims to unravel the intricate spectrum of hepatic involvement in individuals afflicted by dengue fever during Dengue epidemic in year 2023.

Material and Methods: This cross-sectional study was done on adult patients with dengue fever confirmed by NS-1 antigenemia. We recorded clinical features and classified patients into different groups of severity as defined by WHO guidelines. Liver function tests were also done in all cases. Different clinical features were compared amongst patients with mild to moderate and severe hepatitis.

Results: There were 235 patients, including 184 (78%) males and 51 (22%) females, out of which 191 (81%) of patients had fever, Rigors and chills 182 (77 %), Vomiting in 115 (49%). Patients with dengue hemorrhagic fever 83 (35%), while dengue shock syndrome was present in 8 (3.40%) of patients. Our study showed statistically significance in terms of hepatic involvement based on its dysfunction and bleeding tendencies with mild / moderate hepatitis (median = 112.15) and severe hepatitis (median = 57.64), p = 0.0001, r= 0.184.

Conclusion: This study showed dengue fever has adverse impact on liver parenchyma and hepatic function resulting in a higher level of transaminases. Early intervention and supportive treatment will result in a better outcome.

Keywords: Dengue, Hepatitis, Hospital stay

BACKGROUND

The year 2023 marked a pivotal moment in the epidemiological landscape of Rawalpindi, Punjab, as the region grappled with a severe dengue epidemic. Dengue, a mosquito-borne viral infection caused by the *flavivirus*, has been a persistent public health challenge in tropical and subtropical regions, including Pakistan. While the clinical manifestations of dengue are well-documented, the focus on hepatic dysfunction during the 2023 epidemic in Rawalpindi warrants comprehensive exploration. This research article aims to unravel the intricate spectrum of hepatic involvement in individuals afflicted by dengue fever during this critical period.[1]

Dengue fever is known for its diverse clinical manifestations, ranging from mild flu-like symptoms to severe manifestations such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS).[2] Among the multiple organ systems affected, the liver plays a crucial role in dengue pathogenesis. Hepatic involvement in dengue fever has been observed in various forms, including hepatomegaly, elevated liver enzymes, and, in severe cases, hepatic failure.[3] The 2023 epidemic in Rawalpindi presented an opportunity to delve deeper into the nuances of hepatic dysfunction in the context of dengue, with a focus on understanding the variations in clinical presentation, severity, and outcomes.

This research is crucial for several reasons. Firstly, the severity of hepatic involvement in dengue can significantly impact patient management and outcomes. Timely recognition and appropriate intervention for hepatic dysfunction can be life-saving, especially in regions where resources may be limited. Secondly, the specific strains of dengue virus prevalent during the

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This article can be cited as: Zahid U, Nisar S, Saleem S, Sadiq MA, Azeem AR, Siddiqui A. Spectrum of hepatic dysfunction in dengue epidemic in Rawalpindi Pakistan in 2023. Infect Dis J Pak. 2024; 33(2): 107-111. DOI: https://doi.org/10.61529/idjp.v33i3.307

Receiving date: **28 Mar 2024** Acceptance Date: **09 Jul 2024**
Revision date: **22 May 2024** Publication Date: **30 Sep 2024**

2023 epidemic may have exhibited unique interactions with the hepatic system, necessitating a focused study on this aspect.

Furthermore, the socio-demographic profile of the affected population, environmental factors, and existing healthcare infrastructure all contribute to the complex interplay influencing the spectrum of hepatic dysfunction in dengue cases. By investigating these multifactorial dimensions, our research seeks to provide a comprehensive understanding of the hepatic manifestations of dengue.

In this article, we will present a detailed analysis of the clinical, laboratory, and radiological features of hepatic dysfunction in dengue patients. Additionally, we aim to explore the correlation between hepatic involvement and disease severity, emphasizing the importance of early detection and management strategies. Our findings aspire to contribute valuable insights to the existing body of knowledge on dengue, potentially informing future public health interventions and clinical guidelines tailored to the unique dynamics observed during the 2023 epidemic in Rawalpindi, Punjab.

MATERIAL AND METHODS

This descriptive cross-sectional study was carried out at Pak Emirates military hospital from March 2023 to October 2023. The study was conducted after due approval of ethical review committee letter no. A/28/EC/566/2023. All patients, aged greater than 12 years with history of acute fever and confirmed Dengue Non-structural protein 1 antigen positive test were included in the study. Known patient of malignancy, patients receiving chemotherapeutic agents, anti-tuberculosis treatment, massive ascites/ pleural effusion and preexisting congenital platelet or coagulation disorders were excluded from study.

The sampling technique employed was non probability consecutive sampling. Sample size was calculated using WHO sample size calculator assuming 35%, prevalence of liver dysfunction in dengue patients (4) with 95% confidence interval and p-value of less than 0.05 was considered to be clinically significant. A total of 670 suspected dengue patients were evaluated in Emergency and outdoor patient department, out of which 235 confirmed cases of Dengue infection, admitted in Dengue ward were included in the study. Data was collected by conducting one on one interviews with the participants in accordance with a structured questionnaire. The participants were counselled in detail and their verbal consent was sought. A detailed medical history and clinical examination were carried out for all enrolled subjects. A 5ml venous blood was drawn from ante cubital vein and sent for baseline investigations (CBC, Liver function tests, coagulation profile) and ultrasound abdomen and chest X-ray was carried out to confirm organomegaly, cardiomegaly and evidence of serositis. The subjects were followed up and managed indoor till discharge from hospital data was recorded.

Data included demographic details (age, gender, co-morbidities), day of Illness, clinical findings, laboratory parameters and duration of hospital stay. Mortality at 30^{th} day was also recorded. Patients developing dengue complications like bleeding and shock were monitored. Laboratory parameters recorded were serum ALT, serum creatinine. We divided patients into two groups based on ALT levels, mild to moderate hepatitis with ALT levels up till 299 IU/ L and severe acute hepatitis with ALT levels greater than 300 IU/L.[4] Patients were also classified into groups of dengue fever, dengue hemorrhagic fever and dengue shock syndrome. Statistical analysis was done using Statistical package for social sciences (SPSS) version 23.0. Descriptive statistics were used to analyze categorical variables while continuous variables were computed as mean, standard deviation and interquartile range. Categorical variables were represented as frequency and percentages. Normality of data was checked through Komogorov-Smirnov test. Variables including age, hospital stay, transaminases and bleeding disorders were found to be non-normally distributed. Mann-Whitney U test was applied to compare bleeding tendencies and transaminases derangements. Chi square test was applied to determine statistical significance among two variables and p value of <0.05 was considered statistically significant.

RESULTS

A total of 235 patients with dengue fever were enrolled in this study out of which 184 (78 %) were male and 51 (22 %) were females. Median age of sample size was 39 years (interquartile range 95 - 13 years) with 75% of the sample size less than 50 years of age. Median duration of illness while reporting at the hospital was 6 days (interquartile range 16-3 days). The clinical

manifestations in our study showed 191 (81%) of patients had fever, Rigors and chills 182 (77 %), Vomiting in 115 (49%). Patients with dengue hemorrhagic fever 83 (35%), while dengue shock syndrome was present in 8 (3.40%) of patients. Table-I shows distribution of clinical manifestations of dengue fever in sample size.

Patients below 299 IU/ L had limited disease course while above 300 IU/ L had prolonged illness and had more complications. Major Bleeding and Dengue shock was seen in 6 (100%) of the patients having ALT more than 300 IU/L. On the other hand, patients with ALT less than 299 IU/L (mild to moderate disease) had major bleeding disorder and shock in 2 (0.87%). In the group with mild/moderate hepatitis, mean hospital stay was 5 days, (IQR: 11-2 days) whereas the mean hospital stay in group with severe hepatitis was 9 days (IQR: 16-5 days). The duration of hospital stay among group with mild/moderate hepatitis and group with severe hepatitis was found statistically significant (p<0.001). Dengue shock syndrome was more prevalent in severe hepatitis group 6(100%).

Table-I: Distribution of clinical manifestations of dengue fever (n=235)

Clinical Presentation	Cases n (%)
Fever	191 (81)
Myalgias	187 (79)
Rigors & Chills	182 (77)
Headache	180 (76)
Vomiting	115 (49)
Bleeding/Epistaxis	83 (35)
Abdominal Pain	53 (22)
Hepatomegaly	52 (22)
Pleural Effusion	20 (9)
Ascites	18 (7)
Serositis	10 (4)
Sepsis/DSS	8 (3)

Table-II: Statistical analysis of various variables between patients with mild-moderate and severe hepatitis (n=235).

Variables	Hepatitis severity		p-value
	Mild to moderate (gum bleeding, mild epistasis) (ALT<299U/l) n=229	Severe (requiring transfusion) (ALT>300U/l) n=6	
Patients with minor bleed	77 (33%)	6 (100%)	0.003
Patients with major bleed	2 (0.87%)	6 (100%)	<0.001
Patients with no bleed	150 (65%)	0 (0 %)	0.64
Dengue shock syndrome	2 (0.87%)	6 (100%)	<0.001
Mean hospital stay(days)	5 (11 – 2)	9 (16 – 5)	<0.001

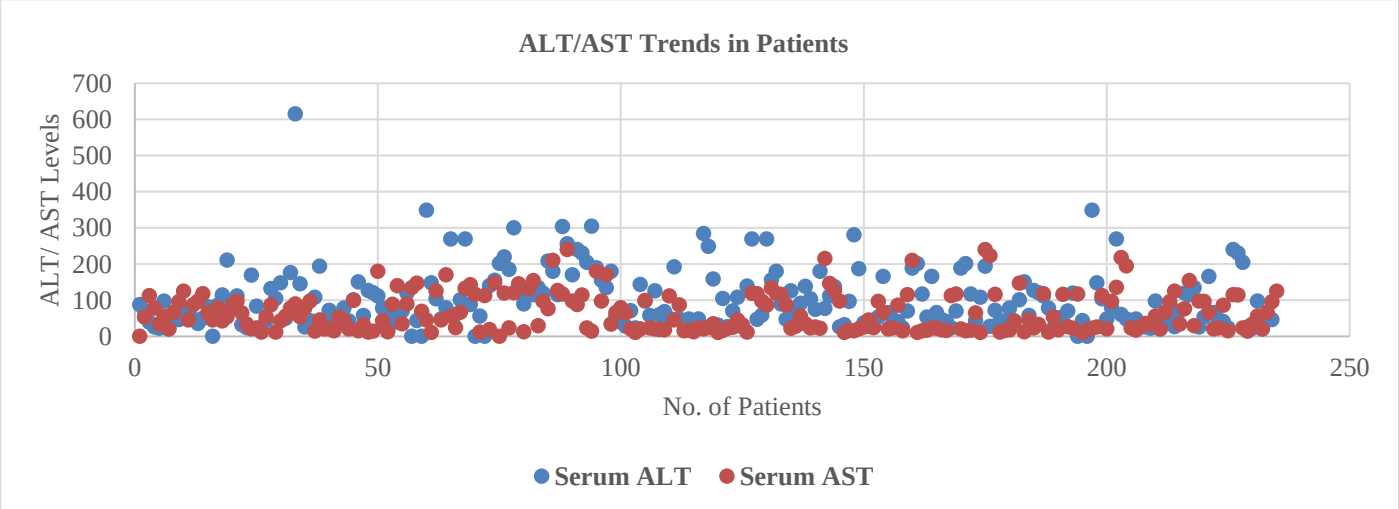

Figure-I: ALT/ AST levels in dengue patients. (n = 235)

DISCUSSION

Dengue fever illness has now been mostly reported in form of epidemics especially in South-east Asian countries.[5] First case of dengue fever was reported in Pakistan in 1994,[6] since then there have been many epidemics reported. Peak season of dengue fever in Pakistan is from July to September.[7] This study revealed males were more affected with dengue fever illness reason being clothing and other outdoor physical activities.[8]

Fever was reported as main symptom in dengue fever illness as illustrated by Khan *et al*.[9] Our study showed that 81% patients having dengue infection presented with fever associated with rigors and chills. Myalgia and arthralgia were found in 79 % of our patients which is quite comparable to study conducted by Khosavanna *et al*, where it was reported in 70% of the patients.[10] In our study, 23% patient had abdominal pain whereas Vanamali *et al* reported abdominal pain in 45% of his patients.[11] Headache was reported in 76 % of the patients in our study which is higher to study done by Fujimoto *et al* whereas it was found in 58%.[12] Vomiting occurred in 53% of our patients which is slightly higher in comparison to study done by Ren *et al* where it was reported in 46.8%.[13]

Hepatomegaly was seen in 21 % of our patients, similar trends were reported by Ahmed.[14] Our study showed 9% dengue patients had pleural effusion whereas other studies showed that 31% of dengue patients had pleural effusion.[15] Daniel *et al* reported ascites in 12% of cases while our study showed ascites in 9 % of the patients.[16] In our study DSS was reported in 3.4 % of all the dengue patients. However, Wasay *et al* reported a lower incidence of DSS (2.9%) in severe dengue fever patients in comparison to our study.[17] Mortality reported in literature ranges from 0.15 % to 7 % .[17] Mortality at 30th day in our study was none whereas in literature it has been reported 0.15 % to 7 %.[18] The likely reason of low mortality in our patients is owing to early detection with more specialist care or may be our patients are infected with different strain of dengue virus in comparison to studies conducted in patients with high mortality.

The emphasis of our study relies on hepatic involvement in dengue patients and its correlation with complicated dengue fever illness. Nearly all patients in our study had raised levels of transaminases. The liver injury caused by dengue virus is multifactorial. The leading mechanisms include cytopathic effects of virus, hepatic injury due to immune mediated mechanism and lastly liver hypoperfusion. A variety of histopathological findings are reported following postmortem of patients died of dengue. Among frequently found pattern are; hepatic micro-steatosis, necrosis, councilman bodies and hyperplasia of Kupffer cells. The hepatic hypoperfusion injury is due to microcirculatory dysfunction that leads to hepatic ischemia irrespective of patient hemodynamic status.

Innate immunity and cytokines released by dengue virus resulted in liver parenchymal injury as this was reiterated in another study done by Chaturvedi *et al* that B-Cells, monocytes and T-Lymphocytes release cytokines which in turn cause hepatocellular damage.[19] Host response plays major role in hepatic parenchymal injuries as reported by postmortem studies done on patients who died of dengue fever illness.[20]

CONCLUSION

This study showed that dengue fever has adverse impact on liver parenchyma and hepatic dysfunction resulting in higher level of transaminases. Liver involvement in dengue fever patient resulted in higher risks of complications along with longer hospital stays. Early intervention and supportive treatment results in better outcome.

LIMITATION

Study was conducted in a single tertiary care setup where patients were entitled to treatment, this study does not represent the population of a specific region.

CONFLICT OF INTEREST

None

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHOR CONTRIBUTION

Usama Zahid: Conceptualization, writing, methodology, data analysis

Shazia Nisar: Conceptualization, writing, revisions overall supervision

Salman Saleem: Conceptualization, overall supervision

Muhammad Arif Sadiq: Writing, methodology, data analysis

Abdul Rehman Azeem: Writing, methodology, data analysis

Ahreema Siddiqui: Data collection

REFERENCES

1. Samanta J, Sharma V. Dengue and its effects on liver. World J Clin Cases. 2015: 3 (2): 125-31. DOI: https://doi.org/10.12998/wjcc.v3.i2.125
2. Wang WH, Urbina AN, Chang MR, Assavalapsakul W, Lu PL, Chen YH, *et al*. Dengue hemorrhagic fever – A systemic literature review of current perspectives on pathogenesis, prevention and control. J Microbiol Immunol Infect. 2020; 53(6): 963–78. DOI: https://doi.org/10.1016/j.jmii.2020.03.007
3. Suganthan N, Sakthilingham G, Kumanan T. Dengue fever complicated with acute liver failure: A case report of expanded dengue syndrome and literature review. SAGE Open Med Case Rep. 2020; 8:2050313X20913428 DOI: https://doi.org/101177/2050313X20913428
4. Parkash O, Almas A, Jafri WS, Hamid S, Akhtar J, Alishah H. Severity of acute hepatitis and its outcome in patients with dengue fever in a tertiary care hospital Karachi, Pakistan (South Asia). BMC Gastroenterol. 2010; 10: 43. DOI: https://doi.org/10.1186/1471-230x-10-43
5. Alied M, Nguyen D, Aziz AJM, Vinh DP, Huy NT. Dengue fever on the rise in Southeast Asia. Pathog Glob Health. 2023; 117(1): 1–2. DOI: https://doi.org/10.1080/20477724.2022.2116550
6. Tabassum S, Naeem A, Nazir A, Naeem F, Gill S, Tabassum S. Year-round dengue fever in Pakistan, highlighting the surge amidst ongoing flood havoc and the COVID-19 pandemic: a comprehensive review. Ann Med Surg. 2023; 85(4): 908-12. DOI:https://doi.org/10.1097%2FMS9.0000000000000418
7. Shabbir W, Pilz J, Naeem A. A spatial-temporal study for the spread of dengue depending on climate factors in Pakistan (2006-2017). BMC Public Health. 2020; 20(1): 995. DOI: https://doi.org/10.1186/s12889-020-08846-8
8. Anker M, Arima Y. Male–female differences in the number of reported incident dengue fever cases in six Asian countries. Western Pac Surveill Response J. 2011; 2(2): 17-23. DOI: https://doi.org/10.5365/wpsar.2011.2.1.002
9. Khan J, Khan I, Ghaffar A, Khalid B. Epidemiological trends and risk factors associated with dengue disease in Pakistan (1980-2014): A systematic literature search and analysis. BMC Public Health. 2023; 18: 745. DOI: https://doi.org/10.1186%2Fs12889-018-5676-2
10. Khosavanna RR, Kareko BW, Brady AC, Booty BL, Nix CD, Lyski ZL, *et al*. Clinical symptoms of dengue infection among patients from a non-endemic area and potential for a predictive model: A multiple logistic regression analysis and decision tree. Am J Trop Med Hyg. 2021; 104(1): 121–9. DOI: https://doi.org/10.4269%2Fajtmh.20-0192
11. Vanamali DR, Venugopal L. Yeshwant P, Rampure D. A study on clinical, laboratory profile and outcome of dengue fever. J Evol Med Dent Sci. 2013; 2(50): 9739–43. DOI: http://dx.doi.org/10.14260/jemds/1686
12. Fujimoto DE, Koifman S. Clinical and laboratory characteristics of patients with dengue hemorrhagic fever manifestations and their transfusion profile. Rev Bras Hematol Hemoter. 2014; 36(2): 115–20. DOI: https://doi.org/10.5581%2F1516-8484.20140027
13. Ren ZZ, Zheng Y, Sun T, Wang GY, Chen XM, Zhou YM. A survey of clinical and laboratory characteristics of the dengue fever epidemic from 2017 to 2019 in Zhejiang, China. Medicine. 2022; 101(42): e31143. DOI: https://doi.org/10.1097%2FMD.0000000000031143
14. Ahmed F. Dengue and the liver. SM J Hepat Res Treat. 2015; 1(1): 1002.
15. Kaagaard MD, Matos LO, Evangelista MVP, Wegener A, Lima KO, Holm AE, *et al*. Prevalence of pleural effusion in dengue: systematic review and meta-analysis. Eur Respirat J. 2022; 60(suppl 66): 202. DOI:https://doi.org/10.1183/13993003.congress-2022.202
16. Daniel R, Philip ZA. A Study of clinical profile of dengue fever in kollam, Kerala, India. Dengue Bull. 2005;29.
17. Wasay M, Channa R, Jumani M, Zafar A. Changing patterns and outcome of Dengue infection; report from a tertiary care hospital in Pakistan. J Pak Med Assoc. 2008; 58(9). 488-9
18. Lien CE, Chou YJ, Shen YJ, Tsai T, Huang N. A population-based cohort study on chronic comorbidity risk factors for adverse dengue outcomes. Am J Trop Med Hyg. 2021; 105(6): 1544. DOI: https://doi.org/10.4269/ajtmh.21-0716
19. Chaturvedi UC, Agarwal R, Elbishbishi EA, Mustafa AS. Cytokine cascade in dengue hemorrhagic fever: Implications for pathogenesis. FEMS Immunol Med Microbiol. 2000; 28(3): 183–8. DOI: https://doi.org/10.1111/j.1574-695x.2000.tb01474.x
20. Kularatne SAM, Imbulpitiya IVB, Abeysekera RA, Waduge RN, Rajapakse RPVJ, Weerakoon KGAD. Extensive haemorrhagic necrosis of liver is an unpredictable fatal complication in dengue infection: A postmortem study. BMC Infect Dis. 2014; 14(1): 1-10. DOI: https://doi.org/10.1186/1471-2334-14-141

ORIGINAL ARTICLE

Neonatal sepsis by gram negative bacteria and antibiotics susceptibility pattern at a tertiary care Paediatric Hospital in Pakistan

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ABSTRACT

Background: Neonatal sepsis has become a leading contributor to mortality and morbidity among neonates in developing countries with recent surge in infections with multidrug resistant Gram-negative bacteria. Aim of this study was **t**o find out the spectrum of Gram-negative organisms and their antimicrobial susceptibility pattern in neonatal sepsis.

Material and Methods: This is a cross-sectional study conducted in the Department of Microbiology, University of Child Health Sciences, The Children's Hospital, Lahore from September to December 2023. A total of 374 blood culture samples received from neonatal unit were incubated at 37 °C for upto 7 days and sub cultured on blood and MacConkey agar after observing signs of growth. Bacterial isolates were identified by standard microbiological techniques. Antimicrobial testing was done by Kirby-Bauer disc diffusion method. Data was analyzed with descriptive statistics using SPSS 23.0.

Results: Out of 374 neonates, males were predominant (n=238, 63.6%). Eighty-seven (23.3%) cultures were positive for Gram negative bacilli which comprised of 29.9% *Klebsiella pneumoniae*, 23% *Acinetobacter baumannii*, 17.2% *Enterobacter* spp., 8.1% *Serratia marcescens*, 10.4% *Pseudomonas* spp., 4.6% *Escherichia coli*, 3.4% *Pantoea* spp., 2.3% *Burkholderia cepacia* and 1.1% *Stenotrophomonas maltophilia*. High resistance to multiple groups of antibiotics including β-lactams, β-lactam combinations, cephalosporins and aminoglycosides was observed among majority of the isolates.

Conclusion: In this study, *Klebsiella pneumoniae* were the most common isolates in neonatal sepsis. High antibiotics resistance is an alarming situation. Antimicrobial stewardship is required to develop appropriate guidelines for empiric antimicrobial use.

Keywords: Neonatal sepsis, Gram negative bacteria, Antimicrobial resistance, Antimicrobial stewardship

BACKGROUND

Neonatal sepsis has been declared as a global concern by the World Health Organization (WHO)[1]. Sepsis is a leading contributor to mortality and morbidity of neonatal age group. In developed countries, it contributes 13-15 deaths per 1000 live births while in low-middle income countries it contributes to 30-50 deaths per 1000 live births. In Pakistan, sepsis contributes to approx. 23 deaths per 1000 live births[2]. According to reports on the disease burden of neonatal sepsis in terms of mortality in Pakistan, 1-4 babies per 1000 live births die from neonatal sepsis each year.[3] Neonatal sepsis refers to a systemic infection of newborns younger than 28 days old. According to the time of presentation: Early onset sepsis (EOS) occurs at or before 72 h of life and Late onset sepsis (LOS) occurs after 72 h up to 28 days of life with possible risk factors such as compromised health care practices and overuse of maternal antibiotics[3,4]. The gold standard in the diagnosis of neonatal sepsis is blood culture, in which low positivity rates account for real management challenge[5,6]. Although the main cause of sepsis is unknown in about one- half of cases, the most common pathogens for sepsis and septic shock are Gram- positive bacteria like *Staphylococcus aureus (S. aureus)* and Coagulase-negative *Staphylococci (CONS*) followed by Gram-negatives including *Escherichia coli* (*E. coli*),

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This article can be cited as: Javed S, Mehdi N, Majeed N, Tahi A, Mir NA, Javed H. Neonatal Sepsis by gram negative bacteria and antibiotics susceptibility pattern at a tertiary Care Pediatric Hospital in Pakistan. Infect Dis J Pak. 2024; 33(3): 112-117.
DOI: https://doi.org/10.61529/idjp.v33i3.325

Receiving date: **29 May 2024** Acceptance Date: **24 Jul 2024**
Revision date: **11 Jul 2024** Publication Date: **30 Sep 2024**

Klebsiella pneumoniae (K. pneumoniae), Enterobacter spp., *Acinetobacter baumannii (A. baumannii)*, and *Pseudomonas* spp.[7,8] In recent studies, about 39-64% of the cases of neonatal sepsis were caused by Gram-negative organisms and *Klebsiella* spp., *Serratia marcescens (S. marcescens), E. coli, Enterobacter* spp., and *A. baumannii* were the most commonly isolated organisms.[9,10] *E. coli* was the most frequently isolated organism in EOS with a ratio of 2:1 in developing countries[11]. Most of the isolates show antimicrobial resistance towards first line treatment of sepsis recommended by WHO.[12] Early identification and treatment of neonatal sepsis is always challenging, so the antibiotics are given empirically to prevent the severe consequences.[6] A significant burden is placed on developing countries due to the inappropriate use of broad-spectrum antibiotics without testing, which increases the number of multidrug resistant pathogens in neonatal units. WHO has declared antibiotic resistance as a major health issue[13]. With such high rates of antibiotic-resistant organisms, less antibiotics options are available. Hence, proper institutional guidelines regarding the local prevalence of pathogens and their antimicrobial profiles are needed. The results of this study aim to provide bacterial spectrum and susceptibility pattern of pathogens causing neonatal sepsis with special reference to gram-negative bacilli. This will ultimately help us in devising appropriate management strategies regarding empiric antimicrobial treatment.

MATERIAL AND METHODS

This cross-sectional observational study was conducted at the Department of Microbiology, University of Child Health Sciences, The Children's Hospital, Lahore. After approval from Institutional Review Board (IRB letter no. 1262/SAHS dated 05/10/2023), blood culture samples from admitted neonates with suspicion of sepsis from nursery ICU were taken in compliance with the Helsinki declaration during September to December 2023. A total of 374 neonates including 160 cases with primary diagnosis of sepsis and 214 cases with other diseases as primary diagnosis and whose blood cultures had Gram negative bacteria (GNB) were included in this study. While samples from patients with history of antibiotic intake and those with yield of Gram-positive bacteria were excluded. For Blood cultures, about 1-3 ml of venous blood was collected under aseptic conditions in a Brain Heart Broth (BHI) broth of blood culture medium followed by incubation at 35–37° C $\pm 2^0$ and routine inspection for signs of microbial growth. Blood culture bottles, which showed no microbial growth after 48 hours were re-incubated upto 7 days, before labelling it as negative. Blood culture samples with signs of bacterial growth were sub cultured on commercially prepared Blood Agar and MacConkey agar (Oxoid, UK) using strict aseptic technique and incubated at 37°C in ambient air. For bacterial growth, plates were examined after 24 h of incubation. Bacterial growths were analyzed by the morphology of bacterial colonies, fermentation of lactose, odor, pattern of growth and Gram-staining. Gram negative bacterial isolates were identified by conventional biochemical tests. For the identification of *Enterobacteriaceae* and non-*Enterobacteriaceae*, Analytical profile index API-20E and API NE (Biomerieux, France) were used respectively.

Antimicrobial susceptibility of the isolates was evaluated using the Kirby-Bauer disk diffusion, following the guidelines of Clinical and Laboratory Standards Institute (CLSI) 2023[14]. Four to five isolated colonies with similar morphology were suspended in sterile saline solution adjusted to the turbidity equivalent to 0.5 McFarland standard and uniformly spread on the entire 90 mm Muller Hinton Agar (MHA) plates. Commercially available antibiotic discs from Oxoid (UK) were applied on the plates and were incubated at 35±1°C for 16-20 h. Gram- negative bacilli were tested against antibiotics including beta lactam drugs, such as amoxicillin/clavulanic acid, piperacillin/tazobactam, cefoperazone/sulbactam, cefotaxime, ceftriaxone, ceftazidime, cefepime, meropenem, aminoglycosides such as amikacin and tobramycin. Interpretation of zone of inhibitions was done according to CLSI guidelines 2023. Susceptibility results were reported as sensitive (S), Intermediate (I) or resistant (R) for clinical interpretation.

The data was analyzed by Statistical Package for the Social Sciences (SPSS V-23). Continuous variables such as age was described as mean ± SD, whereas categorical variables were analyzed with descriptive statistics and presented in frequencies and percentages. The Chi-Square test was used to determine statistically significant association among different variables. A p-

value of <0.05 was considered statistically significant between categorical variables.

RESULTS

Between September 2023 and December 2023, 374 blood cultures were received in the Microbiology laboratory from the Nursery unit. Of these, 160 (42.7%) were clinical suspects of sepsis, while 214 (57.2%) were with primary diagnosis of other diseases. Among other diseases 77(20.5%) had a primary diagnosis of bronchopneumonia, and 137 (36.9%) had other systemic infections. Out of the 374 blood cultures, 87 (23.3%) had GNB yield. Among total number of patients, male-to-female ratio was 1.73 consisting of 238 (63.6%) males and 136 (36.4%) females. The mean age of the neonates was 5 days, with an age range from 1 to 15 days. EOS accounted for 52(59.6%) cases, and LOS accounted for 35 (40.4%) of all positive cultures for GNB (Figure-I).

Of the 87 GNB, *K. pneumoniae* (26/87, 29.9%) was the most common isolate causing sepsis. Out of culture proven cases, 16(61.5%) were from EOS category and 10 (38.4%) from LOS category with a P-value of 0.7 which shows no significant correlation of isolated bacteria with onset of sepsis (Fig. 1). Other significant GNBs were *A. baumannii (*20/87, 23.0%), *Enterobacter* spp. (15/87, 17.2%), *Pseudomonas* spp. (9/87, 10.4%), *S. marcescens* (7/87, 8.1%), *E. coli* (4/87,4.6), *Pantoea* spp. (3/87, 3.4%), *Burkholderia cepacia (B. cepacia)* (2/87, 2.3%) and *Stenotrophomonas maltophilia (S. maltophilia)* (1/87, 1.1%) (Table-I**)**. *K. pneumoniae* strains exhibited resistance to all antibiotics with only 20% of the strains were sensitive to levofloxacin and 15% showed susceptibility to meropenem, amikacin, tobramycin, ciprofloxacin and piperacillin/ tazobactam.

Enterobacter spp. was 100% resistant to cephalosporins and 13.3% exhibited sensitivity to ciprofloxacin and 66.6% to levofloxacin. Approximately,55% of *A. baumannii* were susceptible to cefoperazone/sulbactam, 45% to tobramycin, 25% to ciprofloxacin and levofloxacin, 15% to meropenem and piperacillin/tazobactam, (10%) to amikacin and ceftazidime whereas (100%) resistance to cefepime was also seen. *E. coli* exhibited 100% resistance to all β- lactam antibiotics and aminoglycosides while 50% susceptibility to ciprofloxacin and moxifloxacin. *B. cepacia* were 100% sensitive to levofloxacin and chloramphenicol while 50% to meropenem and ceftazidime. *S. marcescens* were 100% resistant to all applied antibiotics. *Pseudomonas* spp. had 66% sensitivity to amikacin and tobramycin while 44% to cefepime, sulbactam/ cefoperazone, piperacillin/ tazobactam, meropenem and 55% to ceftazidime. There was one isolate of *S. maltophilia* which was sensitive to levofloxacin only. *Pantoea* spp. exhibited 77% sensitivity to meropenem and cefepime while 100% sensitivity to aminoglycosides and piperacillin/ tazobactam **(**Table-II).

Table-I: Distribution of isolated bacteria according to sepsis onset (N=87).

Isolated Organisms	n (%)	Early onset n (%)	Late onset n (%)	p-value
Klebsiella pneumoniae	26(29.9)	16(61.5)	10(38.4)	0.68
Acinetobacter baumannii	20(23.0)	14(70.0)	6(30.0)	0.68
***Enterobacter* spp.**	15(17.2)	9(60.0)	6(40.0)	1.00
Serratia marcescens	7(8.1)	2(28.6)	5(71.4)	0.10
***Pseudomonas* spp.**	9(10.4)	5(55.6)	4(44.4)	0.80
Escherichia coli	4(4.6)	2(50.0)	2(50)	0.68
***Pantoea* spp.**	3(3.4)	1(22.2)	2(77.7)	0.30
Burkholderia cepacia	2(2.3)	2(100.0)	0(0)	0.20
Stenotrophomonas maltophilia	1(1.1)	1(100.0)	0(0)	0.40
Total	87	52(59.8)	35(40.2)	0.40

* P-value < 0.05 is significant

Table-II: Antimicrobial susceptibility pattern of gram-negative bacteria isolated from blood cultures of septic neonates.

Antibiotics	*Klebsiella pneumoniae* (n=26)	*Enterobacter* spp. (n=15)	*Acinetobacter baumannii* (n=20)	*Escherichia coli* (n= 4)	*Burkholderia cepacia* (n=2)	*Stenotrophomonas maltophilia* (n=1)	*Pseudomonas* spp. (n=9)	*Serratia marcescens* (n=6)	*Pantoea* spp. (n=3)
	(frequency of antibiotic sensitive isolates(n) / %)								
Co-amoxiclav	1 (4)	*IR	-	0 (0)	-	*IR	*IR	*IR	-
Cefuroxime	1 (4)	0 (0)	-	0 (0)	-	-	*IR	*IR	-
Cefotaxime	2 (8)	0 (0)	-	0 (0)	-	-	-	0 (0)	-
Ceftazidime	2 (8)	0 (0)	2 (10)	0 (0)	1 (50)	0 (0)	5 (55)	0 (0)	0 (0)
Ceftriaxone	2 (8)	0 (0)	-	0 (0)	-	-	*IR	0 (0)	-
Cefepime	2(8)	1 (6.6)	0 (0)	0 (0)	-	-	4 (44)	0 (0)	2 (66.6)
Meropenem	4 (15.3)	2 (13.3)	3 (15)	0 (0)	1 (50)	0 (0)	4 (4)	0 (0)	2 (66.6)
Amikacin	4 (15.3)	1 (6.6)	2 (10)	0 (0)	-	-	6 (66)	0 (0)	0 (0)
Tobramycin	4 (15.3)	1 (6.6)	9 (45)	0 (0)	-	-	6 (66)	0 (0)	0 (0)
Ciprofloxacin	4 (15.3)	2 (13.3)	5 (25)	2 (50)	-	-	7 (77)	0 (0)	0 (0)
Levofloxacin	5 (20)	10 (66.6)	5 (25)	2 (50)	-	1 (100)	7 (77)	0 (0)	0 (0)
Cefoperazone/ sulbactam	1 (4)	1 (6.6)	11 (55)	0 (0)	-	-	4 (44)	0 (0)	-
Piperacillin/ tazobactam	4 (15.3)	0 (0)	3 (15)	0 (0)	-	-	4 (44)	0 (0)	0 (0)
Chloramphenicol	-	-	-	-	2 (100)	-	-	-	-

IR= Intrinsically Resistant

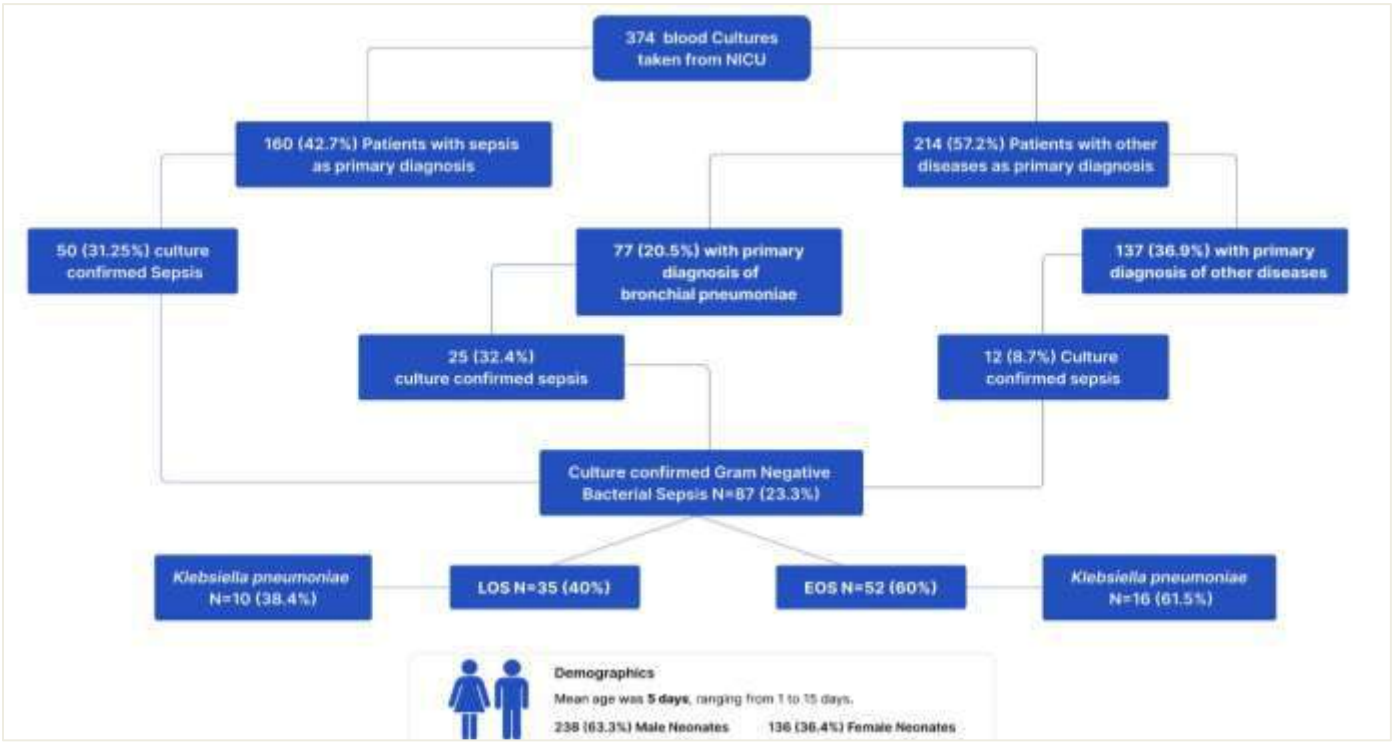

Figure-I: Frequency distribution of blood cultures according to onset of sepsis.

DISCUSSION

Neonatal sepsis has become a major concern worldwide due to increasing mortality and morbidity among neonates. In our study, 87 neonates were confirmed as having sepsis on blood culture due to Gram negative rods. There were 58 (66.6%) male babies and 29 (33.3%) female babies with ratio of 2:1 which is consistent with previous studies conducted at Kharadar hospital, Karachi and at CMH, Sialkot.[2,14]

In this study, frequency of neonatal sepsis due to Gram negative bacilli was 23.3%, which is quite

similar to 29.9% reported in NUMS, Rawalpindi.[16] In Pakistan region, high prevalence of neonatal sepsis has also been reported from Peshawar.[17] A study conducted in India reported 68% neonatal sepsis by Gram negative organisms,[18] whereas another study conducted in Cairo reported 31.7% culture positive sepsis cases caused by both Gram positive and Gram-negative bacteria, out of which more than 50% were Gram negative rods.[19] Similar to other studies, *K. pneumoniae* (29.9%) were the most common cause of sepsis in our setting.[19, 20] A previous study from this center has also reported *K. pneumoniae* to be a major pathogen of neonatal sepsis[21]. Another study carried out at a tertiary care hospital of Pakistan mentioned *E. coli* and *K. pneumoniae* as predominant organisms among GNB causing sepsis.[14] A study conducted in Rawalpindi ranked *E. coli* at the top among GNB causing sepsis in neonates.[16]

In this study, 68% were proven cases of EOS, similar to previously reported from Patan hospital, Nepal in 2017 where 78.3% cases were EOS.[22] *K. pneumoniae* has been reported the predominant organism in both EOS and LOS, as reported in the study from Cairo.[19] Like our study, *K. pneumoniae* were mentioned as the most common isolated pathogen which showed resistance only to penicllins and cephalosporins in China.[23] Other major isolates identified in our study were *A. baumannii* (23.0%), *Enterobacter* spp. (7.2%) while *E. coli* was (4.6%), *S. marcescens* (6.9%) and *Pseudomonas* spp. (5.7%). Another gram-negative isolate found in this study was *Pantoea* spp. (3.4%) which is very rare pathogen to be isolated and could have been acquired from the environment. A case series in India has also documented *Pantoea* spp. as an unusual pathogen in the etiology of neonatal sepsis.[24]

The results of our study indicate an alarming rise in Gram-negative sepsis caused by carbapenem-resistant organisms in neonates. This, combined with limited access to new antibiotics, highlights the importance of infection prevention and control measures to limit the spread of these organisms, as well as antibiotic stewardship to prevent the emergence of resistant strains.

LIMITATIONS

Not all blood cultures were drawn in automated culture bottles which have affected the yield of positive blood cultures.

CONCLUSION

Bacterial spectrum and microbial pattern are showing considerable variation with *K. pneumoniae* being the most common organism in both EOS and LOS cases. Low susceptibility was reported to antibiotics such as ß-lactams and aminoglycosides. Continuous monitoring of local data will help in determining the causative agents and antimicrobial patterns which will assist in minimizing morbidity and mortality by revising appropriate institutional guidelines.

CONFLICT OF INTEREST

None

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHORS CONTRIBUTION

Shamsa Javed: Conception of the work, manuscript writing, data acquisition, analysis, or interpretation, accountable for all aspects of the work

Naima Mehdi1: Research designing and supervision, Drafting the work, revisions, final approval of the version to be published, accountable for all aspects of the work

Nadia Majeed: Design of the work, revisions, final approval of the version to be published, final approval of the version to be published, accountable for all aspects of the work

Anum Tahir: Data acquisition, analysis, or interpretation, final approval of the version to be published, accountable for all aspects of the work

Nazia Akber Mir: Drafting the work or revising it critically for important intellectual content, final

approval of the version to be published, accountable for all aspects of the work

Humera Javed: Research designing and supervision**,** Drafting the work or revising it critically, final approval of the version to be published, accountable for all aspects of the work

REFERENCES

1. Fleischmann C, Reichert F, Cassini A, Horner R, Harder T, Markwart R, *et al*. Global incidence and mortality of neonatal sepsis: A systematic review and meta-analysis. Arch Dis Child. 2021; 106(8): 745-52. DOI:https://doi.org/10.1136%2Farchdischild-2020-320217.
2. Shaikh M, Hanif M, Gul R, Hussain W, Hemandas H, Memon A. Spectrum and antimicrobial susceptibility pattern of micro-organisms associated with neonatal sepsis in a hospital in Karachi, Pakistan. Cureus. 2020; 12(10). DOI: https://doi.org/10.7759/cureus.10924.
3. Wattal C, Kler N, Oberoi JK, Fursule A, Kumar A, Thakur A. Neonatal Sepsis: Mortality and morbidity in neonatal sepsis due to multidrug-resistant (MDR) organisms: Part 1. Indian J Pediatr. 2020; 87(2): 117-121. DOI: https://doi.org/10.1007/s12098-019-03106-z
4. Song WS, Park HW, Oh MY, *et al*. Neonatal sepsis-causing bacterial pathogens and outcome of trends of their antimicrobial susceptibility a 20-year period at a neonatal intensive care unit. Clin Exp Pediatr. 2022; 65(7): 350-7. DOI: https://doi.org/10.3345/cep.2021.00668.
5. Reyes A. Ending the culture of culture-negative sepsis in the neonatal ICU. Rev Chil Infectol. 2018; 35(2): 216-7. DOI: https://doi.org/10.4067/s0716-10182018000200216
6. Nordberg V. A decade of neonatal sepsis caused by gram-negative bacilli — a retrospective matched cohort study. Eur J Clin Microbiol Infect Dis. 20214 40(9): 1803-13. DOI: https://doi.org/10.1007/s10096-021-04211-8.
7. Virzì GM, Mattiotti M, de Cal M, Ronco C, Zanella M, De Rosa S. Endotoxin in sepsis: Methods for LPS detection and the use of omics techniques. Diagnostics. 2023;13(1):1-17. DOI: https://doi.org/10.3390/diagnostics13010079
8. Odabasi IO, Bulbul A. Review neonatal sepsis. Med Bull Sisli Etfal Hosp 2020; 54(2): 142-58 DOI: https://doi.org/10.14744/SEMB.2020.00236
9. Poggi C, Dani C. New antimicrobials for the treatment of neonatal sepsis caused by multi-drug-resistant bacteria: A systematic review. Antibiotics. 2023; 12 (6): 956. DOI: https://doi.org/10.3390/antibiotics12060956
10. Al-Matary A, Heena H, Al-Sarheed AS, Ouda W, Al Shahrani DA, Wani TA, *et al*. Characteristics of neonatal Sepsis at a tertiary care hospital in Saudi Arabia. J Infect Public Health. 2019; 12(5): 666-72. DOI: https://doi.org/10.1016/j.jiph.2019.03.007.
11. Aku FY, Akweongo P, Nyarko K, Sackey S, Wurapa F, Afari EA, *et al*. Bacteriological profile and antibiotic susceptibility pattern of common isolates of neonatal sepsis, Ho Municipality, Ghana-2016. Matern Health Neonatol Perinatol. 2018; 4: 2. DOI: https://doi.org/10.1186/s40748-017-0071-z
12. Zou H, Jia X, He X, Su Y, Zhou L, Shen Y, *et al*. Emerging threat of multidrug resistant pathogens from neonatal sepsis. Front Cell Infect Microbiol. 2021; 11(July): 1-12. DOI: https://doi.org/10.3389%2Ffcimb.2021.694093
13. Moftian N, Rezaei-Hachesu P, Arab-Zozani M, Samad-Soltani T, Esfandiari A, Tabib MS, *et al*. Prevalence of gram-negative bacteria and their antibiotic resistance in neonatal sepsis in Iran: A systematic review and meta-analysis. BMC Infect Dis. 2023; 23(1): 1-15. DOI: https://doi.org/10.1186/s12879-023-08508-1
14. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 33rd ed. CLSI supplement M100. Clinical and Laboratory Standard Institute; 2023.
15. Ghafoor T, Hussanain A, Qureshi M, Mehmood T, Ali L. Antimicrobial susceptibility pattern of blood culture isolates from patients with suspected neonatal sepsis in a tertiary care hospital in Pakistan. Sri Lankan J Infect Dis. 2020; 10(1): 30-7. DOI: https://doi.org/10.4038/sljid.v10i1.8244
16. Ahmed M, Yasrab M, Khushdil A, Qamar K, Ahmed Z, Pakistan R. Neonatal sepsis in a tertiary care hospital: Bacteriological profile and its antimicrobial sensitivity. Neonatal Sepsis Its Antimicrob Sensit. Pak Armed Forces Med J. 2018;68(6):1654-1658.
17. Rahman S, Hameed A, Roghani MT, Ullah Z. Multidrug resistant neonatal sepsis in Peshawar, Pakistan. Arch Dis Child Fetal Neonatal Ed. 2002; 87(1): 52-54. DOI: https://doi.org/10.1136/fn.87.1.f52 .
18. Viswanathan R, Singh AK, Basu S, Chatterjee S, Sardar S, Isaacs D. Multi-drug-resistant gram-negative bacilli causing early neonatal sepsis in India. Arch Dis Child Fetal Neonatal Ed. 2012;97(3). DOI: https://doi.org/10.1136/archdischild-2011-300097
19. Almohammady MN, Eltahlawy EM, Reda NM. Pattern of bacterial profile and antibiotic susceptibility among neonatal sepsis cases at Cairo University Children Hospital. J Taibah Univ Med Sci. 2020; 15(1): 39-47. DOI: https://doi.org/10.1016/j.jtumed.2019.12.005.
20. Khassawneh M, Khader Y, Abuqtaish N. Clinical features of neonatal sepsis caused by resistant Gram-negative bacteria. Pediatr Int. 2009; 51(3): 332-6. DOI: https://doi.org/10.1111/j.1442-200x.2008.02767.x
21. Salamat S, Ejaz H, Zafar A, Javed H. Detection of AmpC β-lactamase producing bacteria isolated in neonatal sepsis. Pak J Med Sci.. 2016; 32(6): 1512-6. DOI: https://doi.org/10.12669/pjms.326.10861
22. Pokherl B, Koirala T, Shah G, Joshi S, Baral P. Bacteriological profile of neonatal sepsis in neonatal intensive care unit (NICU) in a Tertiary Care Hospital in Nepal. BMC Pediatr. 2018; 18(1): 1-8. DOI: https://doi.org/10.1186/s12887-018-1176-x
23. Wang J, Zhang H, Yan J, Zhang T. Literature review on the distribution characteristics and antimicrobial resistance of bacterial pathogens in neonatal sepsis. J Matern Fetal Neonatal Med. 2022; 35(5): 861-70. DOI: https://doi.org/10.1080/14767058.2020.1732342
24. Mahapatra A, Dhal S, Jena PP, Mohapatra A, Dash D, Padhee A. Neonatal septicaemia due to a rare bacterium: Pantoea agglomerans (case series). Pediatr Infect Dis. 2014; 6(3): 102-4. DOI: https://doi.org/10.1016/j.pid.2014.02.001

ORIGINAL ARTICLE

Demographics and clinical manifestations of patients with *Raoultella terrigena* infections: A Retrospective Single Center Study from Karachi, Pakistan

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ABSTRACT

Background: *Raoultella terrigena* (formerly *Klebsiella terrigena*) is an environmental gram-negative rod. It can cause infections in humans, especially in immunosuppressed patients and tends to be multi-drug resistant, limiting treatment options. There is lack of data on clinical presentation and outcomes of infections due to this organism. In this study; we describe the clinical features (presenting complaints, co-morbid diseases, complications, etc.), available treatment options, and outcomes (hospital/ICU stay, mortality) of patients with *R. terrigena* infections, seen retrospectively over six years.

Material and Methods: A cross-sectional study was conducted on all adult hospitalized patients with clinical specimens positive for *Raoultella terrigena* at a 700-bedded tertiary care hospital in Karachi, Pakistan, from January 2013 to December 2018.

Results: We identified 58 patients with *R. terrigena* isolated from different cultures specimens, of which n=12 (22.6%) were colonizers. The median age was 61.5 years (IQR=43-71), and most were male (n=28). The most common site of infection was the respiratory tract in 28.3%, then urinary tract in 26%, and central line in 26.1%. Amongst infected cases, 37% had septic shock, 45.7% had respiratory failure.

Conclusion: *R. terrigena* is a multi-drug-resistant organism with a high mortality rate and can cause hospital-acquired respiratory tract infections in patients.

Keywords: *Raoultella terrigena, Klebsiella terrigena, Raoultella* species, *Klebsiella* species.

BACKGROUND

Raoultella terrigena, previously known as "*Klebsiella terrigena*," was discovered in 1981 and is a rare gram-negative organism, primarily found in soil and water.[1] It was distinguished from *Klebsiella* species in 2001 based on molecular analysis.[2] These organisms are oxidase negative, capsulated, immotile, facultatively anaerobic, and aerobic bacilli[2]. However, many microbiology laboratories continue to identify this organism as a "*Klebsiella species*," making it difficult to estimate its true incidence.[3] The first reported case as a human pathogen was in 2007 in a middle-aged post-liver transplant patient with endocarditis[4]. Another case report was published on sepsis secondary to *R. terrigena* in 2011[5]. A literature search reveals that most of the reported cases caused by genus *Raoultella* consist of *R. orinithinolytica* and *R.planticola*,[6,7] and are associated with diseases of the biliary tract and post-surgical interventions[6,8] as well as necrotizing fasciitis[9], soft tissue infection[10] and cystitis.[11] Our study describes the clinical presentations and outcomes of infections caused by *Raoultella terrigena*.

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This article can be cited as: Ahmed I, Nasir N, Farooqui F, Mahmood F. Demographics and clinical manifestations of patients with *Raoultella terrigena* infections: A Retrospective Single Center Study from Karachi, Pakistan. Infect Dis J Pak. 2024; 33(3): 118-124.
DOI: https://doi.org/10.61529/idjp.v33i3.284

Receiving date: **22 Jan 2024** Acceptance Date: **13 Aug 2024**
Revision date: **24 Jul 2024** Publication Date: **30 Sep 2024**

MATERIAL AND METHODS

We conducted a cross-sectional study on all adults greater than or equal to 18 years of age, admitted to Aga Khan University Hospital (AKUH) between January 2013 and December 2018, who had *Raoultella terrigena* isolated from culture specimens. Since this was a case series, formal sample size calculation was not performed. We excluded patients with recurrent *Raoultella* infections and colonization. *Raoultella* infection was defined as the presence of *R. terrigena* in a clinically relevant culture specimen along with the presence of signs and symptoms fulfilling criteria for a specific infection site as defined by CDC.[12] Colonization was defined as isolation of *R. terrigena*

from culture specimen but not causing any symptoms or disease. The site of infection was defined using CDC definitions.[12] Polymicrobial infections were defined as infection with two or more bacteria considered pathogens; isolated from a clinically relevant culture specimen. Multi-drug-resistant organisms (MDR) are defined as bacteria resistant to one or more key classes of antibiotics for that organism. Source control of infection comprised of all physical measures to remove foci of infection where applicable such as removal of infected lines or drains, drainage of the liquid component of infection, surgical debridement, in order to restore optimal function of the involved area. Relapse was defined as clinical deterioration after a temporary improvement in patients with the same organism within 1 month of initial infection. Patients who fulfilled the eligibility criteria were consecutively included in the study. Demographic and other categorical variables such as age, sex, co-morbid conditions, prior history of hospitalizations, invasive procedures, presence of lines and surgical drains, use of antibiotics, hospital stay, intensive care unit (ICU) stay, and in-hospital mortality were collected from the electronic health records (EHR) on a pre-tested structured proforma. Microbiological data on cultures which included, blood, sputum, tracheal aspirates, pus, urine, pleural fluid, peritoneal fluid, cerebrospinal fluid, and tissue specimen positive for *R. terrigena* was also extracted from EHR.

The study received exemption from approval by the Aga Khan University Ethics review committee. (ERC Reference No:2019-1232-3287). Patient confidentiality was maintained and no personal identifiers were obtained. As this was a retrospective study, the committee waived the requirement of informed consent.

Specimen processing for culture was performed in the microbiology laboratory at Aga Khan University Hospital Karachi according to guidelines provided by the American Society for Microbiology. This involved sample inoculation on MacConkey, chocolate, and 5% sheep blood agar at 37 degrees Celsius for up to 48 hours. Blood for sheep blood agar was acquired by phlebotomizing sheep in the animal house at AKUH. Upon growth of lactose fermenter mucoid colonies after incubation on MacConkey agar, further biochemical tests were performed which included the utilization of citrate, production of hydrogen sulfide, detection of urease, production of indole from tryptophan, motility, and Triple Sugar Iron Test. If the organism tested negative to these tests, and gave an acidic slant over an acidic butt on the triple sugar iron test, it was further subjected to further identification. This was carried out by API 20 E, which consists of 20 biochemical tests. Antibiotic susceptibility testing was initially carried out on Mueller-Hinton agar with Kirby-Bauer disk diffusion test or Vitek-2 MS automated system. Colistin minimum inhibitory concentrations were confirmed by colistin broth microdilution, which is currently the recommended method as per Clinical and Laboratory Standards Institute (CLSI). Results were interpreted as per CLSI guidelines.

Descriptive analysis was performed for all patient-related variables with frequencies and proportions reported for categorical variables like sex, comorbid, clinical features and median with interquartile range reported for continuous variables like age, hospital stay. Chi-square test or Fisher exact test were used as appropriate to determine the association between two categorical variables, e.g., chronic kidney disease and death. IBM® Statistical Package for Social Sciences (SPSS®, version 25.0) was used for data analysis. A *p*-value of less than 0.05 was considered significant.

RESULTS

A total of 58 patients with *R. terrigena* isolated from different culture specimens were identified. Out of those, 12 isolates were identified as colonizers and excluded, remaining (46) were included in the study. The median age was 61.5 years (IQR= 43-71), with more males than females (60.9 % vs. 39.1%). The most frequent co-morbid conditions were diabetes mellitus in n=18 (39.1%) patients. Most patients n= 34 (73.9%) had a previous history of hospitalization (within the past six months) for various medical and surgical conditions, and majority, n=24 (52.17%) had at least one hospital admission. 78% patients (n=36) reported antibiotic use in last 6 months.

Out of 46 patients included in the study, 31 (67.4%) had had a prior culture of blood or other body fluid specimens growing a multidrug-resistant organism in the last six months (Table-I), while n=35 (76.1%) had a urinary catheter in place, and central lines were present in n=22 (47.8%). There were 29 patients (63%) with this infection who had a recent history (past six months) of invasive procedures.

The most common site of infection was the respiratory tract in n=13 (28.3%) patients which included sputum and tracheal aspirates, followed by urinary tract infections n=12 (26%), and bloodstream infections n=12 (26.1%) (Table-II). Respiratory failure was seen in n=21 (45.7%) patients, of which n=11 (52.4%) required mechanical ventilation and n=10 (47.6%) needed non-invasive ventilation. Of the n=17 patients, (37%) who were in septic shock due to *R. terrigena*, n=13, (76.5%) required vasopressors and n=4 (23.5%) were treated with fluid resuscitation.

The most common sources of cultures positive for *R. terrigena* were blood in 32.6%, sputum in 28.3%, and urine in 21.7% of the patients. *Monomicrobial growth of R. terrigena* was identified from n=26 (56.5%) of culture specimens, and polymicrobial growth was identified from n=20 (43.5%) culture specimens. Polymicrobial growth was most frequently seen in sputum n=7 (35%), followed by blood in n=6 (30%). The organism was highly resistant to most of the commonly used antibiotics. Carbapenem resistance was present in 91.3%, colistimethate resistance 65.2% (Table-III). In most cases, sensitivities were checked for tigecycline and fosfomycin after they were resistant to colistimethate

Out of 46 cases, eight patients were lost to follow-up, and treatment information was not available. The remaining 38 patients, n=31 patients (81.6%) received combination therapy, and n=6 (15.8%) received monotherapy. One patient died before starting treatment. Antibiotics used as empiric therapy were carbapenems n=24 (77.4%), beta-lactam/lactamase inhibitors n=14 (45.2%), vancomycin n=15 (48.4%), colistimethate n=13 (41.9%) cases. The most frequent treatment combination was carbapenem and colistemethate in n=11 (28.9%), followed by a combination of carbapenem with colistemethate and tigecycline in n=8 (21.1%) Mortality association with monotherapy was (p= 0.672), and with combination therapy (p= 0.70). Out of n=14 (36.84%) cases in whom repeat cultures for clearance needed, bacteriological clearance was achieved in n=9 (64.2%) cases. There were 23 cases that needed source control of underlying infection. It was achieved in n=12 (52.1%). Two cases (5.3%) relapsed.

The average hospital stay was a median of 11.50 days (IQR=6-22), with a median of 3 days before positive culture. Approximately n=23 (60.52%) of patients were seriously ill, requiring intensive care unit care with a median ICU stay of 6 days (IQR=4-11). In-hospital mortality recorded in n=17 (44.7%) patients.

In the subgroup analysis of factors associated with death in *R. terrigena* infections (Table-IV), it was found that chronic kidney disease (CKD) (*p* value = 0.029) and septic shock (*p* value= 0.001) were significantly associated with mortality. Also, persons with a high (greater or equal to three) Charlson- comorbidity index had increased mortality (*p* value = 0.002).

Table-I: Demographics of patients infected with *R. terrigena* (n=46).

Age in years	**Median: (61.50) IQR 43-71**
Characteristic	**n (%)**
Gender:	Male: 28 (60.9)
	Female: 18 (39.1)
Prior antibiotics (6, months)	36 (78.3)
Carbapenems	26 (72.2)
Beta lactam/ lactamase inhibitors	23 (63.9)
Glycopeptides	20 (55.6)
Colistemethate	11 (30.6)
Prior hospitalization: (6, months)	34 (73.9)
Prior MDROs (6, months)	31 (67.4)
CRE *K. pneumoniae*	13 (28.2)
MDR *Acinetobacter*	11 (23.9)
MDR *P. aeruginosa*	11 (23)
Central lines	22 (47.8)
VP shunt	1 (2.2)
Surgical drains	14 (30.4)
Urinary catheter	35 (76.1)
Recent procedures	29 (63)
Skin, soft tissues	9 (31)
Abdomen	7 (24.1)

CNS	7 (24.1)
Genitourinary	8 (27.6)
Stent placement	4 (13.8)
Chest:	4 (13.8)
Others:	6 (20.7)
Co-morbid	
Diabetes mellitus	18 (39.1)
Chronic kidney disease	16 (34.7)
Malignancy	11 (23.9)
Cerebrovascular accident	7 (15.2)
Chronic liver disease	8 (17)
Steroid therapy	2 (4.3)
Connective tissue disease	1 (2.1)

S.E., standard Error; IQR, Inter Quartile Range; M, male; F, female; CLABSI, Central Line Associated Blood Stream Infection; BSI, Blood Stream infection; VP, ventriculo-Peritoneal; MDRO, Multi Drug Resistant Organism; CRE, Carbapenem Resistant Enterobacterales; *K. pneumoniae, Klebsiella pneumoniae; P. aeruginosa, Pseudomonas aeruginosa;* MDR, Multi Drug Resistance; CNS, Central Nervous System

Table-II: Clinical features (n=46) and outcomes of *R.terrigena* (n=38).

Clinical feature	N (%)
Site of infection:	
Pneumonia	9 (19.6)
Tracheitis	4 (8.7)
Cystitis	6 (13)
Pyelonephritis	6 (13)
CLABSI	7 (15.2)
Unspecified BSI	5 (10.9)
Necrotizing fasciitis	2 (4.3)
Bed sore infection	2 (4.3)
Cellulitis	1 (2.2)
Septic arthritis	1 (2.2)
Ventriculitis	1 (2.2)
Peritonitis	1 (2.2)
Cholangitis	1 (2.2)
Clinical presentation:	
Hypotension (Systolic < 90)	17 (36.9)
Respiratory failure	21 (29.2)
Altered mental status:	25 (54.3)
Alive	21 (55.3)
Dead	17 (44.7)
Hospital stay (days)	Median (11.50) IQR=6-22
ICU stay (days)	Median (6) IQR=4-11

ICU, Intensive Care Unit.

Table-III: Drugs susceptibility of *R-terrigena*

Antibiotics	Sensitivity	Resistance	Intermediate	Unchecked
Amoxicillin clavulanate	2.2%	93.5%	2.2%	2.2%
Amikacin	19.6%	76.1%	2.2%	2.2%
Imipenem	4.3%	89.1%	2.2%	4.3%
Piperacillin tazobactam	4.3%	91.3%	2.2%	2.2%
Gentamicin	8.7%	89.1%	2.2%	
Ceftriaxone	2.2%	97.8%		
Trimethoprim sulfamethoxazole	8.7%	89.1%		2.2%
Ciprofloxacin	6.5%	91.3%		2.2%
Meropenem	8.7%	91.3%		
Colistemethate	23.9%	65.2%		10.9%
Tigecycline	30.4%	10.9%	26.1%	32.6%
Fosfomycin	15.2%	28.3%	19.6%	37%

Table-IV: Association of mortality with clinical features.

Clinical feature	Alive	Expired n	P-value n(%)
Total 38	**21**	**17**	
Non CKD	17	8	0.029
Chronic kidney disease	4	9	
Non diabetic	14	9	0.38
Diabetes mellitus	7	8	
Non CVA	19	15	0.82
Cereberovascular accident	2	2	
No lung disease	17	14	0.91
Chronic lung disease	4	3	
No CTD	20	17	0.749
Connecive tissue disease	1	0	
No CLD	15	13	0.72
Chronic liver disease	6	4	
No malignancy	12	13	0.21
Malignancy	9	4	
No steroids	19	17	0.477
Steroids	2	0	
No resp. failure	5	4	0.98
Respiratory failure	16	13	
No septic shock	19	8	<0.01
Septic shock	2	9	
Charleson's comorbidity index <3	13	2	<0.01
Charleson's comorbidity index ≥3	8	15	

DISCUSSION

In our study, most patients had hospital-acquired pneumonia due to *Raoultella terrigena*, followed by urinary tract infection. Complications identified in the majority included respiratory failure and septic shock. Our results showed a multi drug resistant susceptibility profile. The organism was found to be resistant to beta-lactams, carbapenems, and colistimethate.

Infections caused by the genus *Raoultella* have been commonly reported in older-aged immunocompromised patients; those who were suffering from malignancy or had undergone surgical interventions. Many of them developed infections of the biliary tract and had variable mortality rates.[13] In contrast, in our study most of the patients were middle aged males and the most frequent co-morbid conditions included diabetes followed by CKD and malignancy. Around two-thirds of them had recent surgical interventions. Compared to previously published case reports,[5,13,14] we had only one patient with biliary tract involvement, and we found a greater number of patients having nosocomial pneumonia and urinary tract infections.

A summary of previous reports published on *R. terrigena* is given in Table 5. It shows that while cases from most parts of the world were carbapenem sensitive, those from the Pakistani case series were carbapenem resistant. A study done on carbapenem resistant enterobacterales at the Aga Khan University showed that of 215 carbapenem resistant enterobacterales tested, 15.9% were also resistant to colistin[17] and it is of interest to note that 15% of the tested isolates were *Raoultella* species. Our study shows a similar result to both the studies mentioned above from the same locale, wherein most cases were resistant to carbapenem, and additionally, 65% were also resistant to colistin, which is often used as a last resort antibiotic in carbapenem resistant cases. This highlights the importance of using correct microbiological methods for identification and susceptibility of organisms in accordance with CLSI guidelines,[18] to ensure that correct susceptibility profiles are provided to clinicians for adequate treatment of such cases.

Like other cases reported before,[8-11, 13-17, 19-20] ours too showed that patients who acquired resistant strains of *R. terrigena* had complicated medical histories such as diabetes, previous hospitalization, and/or antibiotic use, invasive procedures or cultures positive for (other) resistant organisms in the last six months. In addition, our study also showed that several patients had indwelling devices in place, pointing towards the propensity of these infections being hospital acquired. Further studies, of course, are required to confirm this

hypothesis. In contrast to the many case reports we found that showed intra-abdominal infections with *R. terrigena*, our study showed pneumonia as the predominant clinical presentation.

Interestingly, we found no difference of outcome in treating patients with monotherapy or combination therapy. Literature suggests treating carbapenem and colistin resistant enterobacterales with a combination of more than two antibiotics[21] but our study showed no difference when the mortality of patients treated with monotherapy and combination therapy was compared.

STRENGTH AND LIMITATIONS

Although our study has a limited sample size and is a single-center experience, it is the largest cohort of patients reported of this rare gram-negative infection to date. Because of the scarcity of available resources, we could not identify *R. terrigena* by molecular analysis (standard method).

CONCLUSION

Infections caused by *R. terrigena* are highly drug-resistant, mostly causing hospital-acquired respiratory tract infections that are difficult to treat, leading to prolonged hospital and ICU stays with a high mortality rate. Patients with underlying renal dysfunction, those on vasopressor support, and a high CCI score are at greater risk of death due to this infection. Patients with multiple comorbid conditions and immunosuppression are at risk of acquiring infections with opportunistic organisms, including *R. terrigena*, leading to increased mortality if not identified and treated correctly.

CONFLICT OF INTEREST

None

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHOR CONTRIBUTION

Ishfaque Ahmed: Drafting of article, acquisition of data: laboratory or clinical, analysis of data, critical revision, final approval of manuscript, accountable for all aspects of work

Nosheen Nasir: Conception and design of study, Drafting of article, final approval of manuscript, accountable for all aspects of work

Fizza Farooqui: Analysis of data, critical revision, drafting of article, final approval of manuscript, accountable for all aspects of work

Syed Faisal Mahmood: Conception and design of study, Drafting of article, final approval of manuscript, accountable for all aspects of work

REFERENCES

1. Izard D, Ferragut C, Gavini F, Kersters K, De Ley J, Leclerc H, *et al*. *Klebsiella terrigena*, a new species from soil and water. Int J Syst Bacteriol 1981; 31(2): 116-27. DOI: https://doi.org/10.1099/00207713-31-2-116
2. Drancourt M, Bollet C, Carta A, Rousselier P. Phylogenetic analyses of *Klebsiella* species delineate *Klebsiella* and *Raoultella* gen. nov., with description of *Raoultella ornithinolytica* comb. nov., *Raoultella terrigena* comb. nov. and *Raoultella planticola* comb. nov. Int J Syst Evol Microbiol. 2001; 51(3): 925-32. DOI: https://doi.org/10.1099/00207713-51-3-925
3. Walckenaer E, Poirel L, Leflon-Guibout V, Nordmann P, Nicolas-Chanoine M 2004. Genetic and Biochemical Characterization of the Chromosomal Class A β-Lactamases of *Raoultella* (formerly *Klebsiella) planticola* and *Raoultella ornithinolytica*. Antimicrob Agents Chemother 48:. https://doi.org/10.1128/aac.48.1.305-312.2004
4. Goegele H, Ruttmann E, Aranda-Michel J, Kafka R, Stelzmueller I, Hausdorfer H, *et al*. Fatal endocarditis due to extended spectrum betalactamase producing *Klebsiella terrigena* in a liver transplant recipient. Wien Klin Wochenschr. 2007; 119(11-12): 385-6. DOI: https://doi.org/10.1007/s00508-007-0812-5
5. Shaikh MM, Morgan M. Sepsis caused by *Raoultella terrigena*. J R Soc Med Sh Rep. 2011; 2: 49. DOI: https://doi.org/10.1258/shorts.2011.010127
6. Hajjar R, Ambaraghassi G, Sebajang H, Schwenter F, Su SH. *Raoultella ornithinolytica*: emergence and resistance. Infect Drug Resist. 2020: 1091-104. DOI: https://doi.org/10.2147%2FIDR.S191387
7. Appel TM, Quijano-Martínez N, De La Cadena E, Mojica MF, Villegas MV. Microbiological and clinical aspects of *Raoultella* spp. Front Public Health 2021; 9: 686789. DOI: https://doi.org/10.3389/fpubh.2021.686789
8. Chen X, Guo S, Liu D, Zhong M. Neonatal septicemia caused by a rare pathogen: *Raoultella planticola*-a report of four cases. BMC Infect Dis. 2020; 20: 1-6. DOI: https://doi.org/10.1186/s12879-020-05409-5
9. Sêkowska A, Bogiel T, Woźniak M, Gospodarek-Komkowska E. *Raoultella* spp.–reliable identification, susceptibility to antimicrobials and antibiotic resistance mechanisms. J Med Microbiol. 2020; 69(2): 233-8. DOI: https://doi.org/10.1099/jmm.0.001150
10. Goodman DT, Murphy D, Dorairaj J. Case study: Soft tissue infection with *Raoultella ornithinolytica*. J Plast Reconstr Aesthet Surg. 2022; 33: 17-20. DOI: https://doi.org/10.1016/j.jpra.2022.04.004
11. Olson DS, Jr., Asare K, Lyons M, Hofinger DM. A novel case of *Raoultella planticola* urinary tract infection. Infection. 2013;41(1): 259-61. DOI: https://doi.org/10.1007/s15010-012-0294-x
12. CDC/NHSN surveillance definitions for specific types of infections 2021 [updated January 2021. Available from:

https://www.cdc.gov/nhsn/pdfs/pscmanual/17pscnosinfdef_current.pdf.

13. Teo I, Wild J, Ray S, Chadwick D. A rare case of cholecystitis caused *by Raoultella planticola*. Case Rep Med. 2012;2012. 601641.
DOI: https://doi.org/10.1155%2F2012%2F601641
14. Goggins A, Lykins J, Aston A, Shaw J. Acute cholecystitis secondary to *Raoultella ornithinolytica* infection, complicated by sepsis, gallbladder perforation, hepatic abscess and bacteraemia. BMJ Case Rep. 2022; 15(12): e250766. DOI: https://doi.org/10.1136/bcr-2022-250766
15. Hong KW, Cheon YH, Moon K, Hong SI, Ryu BH, Cho OH, *et al*. Comparison of the clinical characteristics and outcomes of bloodstream infections caused by *Raoultella species* and *Klebsiella pneumoniae*. Infect Dis. 2020; 52(7): 489-97.
DO: https://doi.org/10.1080/23744235.2020.1758764
16. Wang Y, Jiang X, Xu Z, Ying C, Yu W, Xiao Y. Identification of *Raoultella terrigena* as a rare causative agent of subungual abscess based on 16S rRNA and housekeeping gene sequencing. Can J Infect Dis Med Microbiol. 2016; 2016: 3879635.
DOI: https://doi.org/10.1155/2016/3879635
17. Mal PB, Sarfaraz S, Herekar F, Ambreen R. Clinical manifestation and outcomes of multi-drug resistant (MDR) *Raoultella terrigena* infection - A case series at Indus Health Network, Karachi, Pakistan. ID Cases. 2019; 18: e00628.
DOI: https://doi.org/10.1016%2Fj.idcr.2019.e00628
18. Qamar S, Shaheen N, Shakoor S, *et al*. Frequency of colistin and fosfomycin resistance in carbapenem-resistant Enterobacteriaceae from a tertiary care hospital in Karachi. Infect Drug Resist. 2017; 10: 231-6.
DOI: https://doi.org/10.2147%2FIDR.S136777
19. CLSI, Performance Standards for Antimicrobial Susceptibility Testing. 28th ed. Vol. M100. Wayne, PA: Clinical Laboratory and Standards Institute; 2018
20. Kitagawa S, Tsushima Y, Ishikawa S. Acute obstructive suppurative pancreatic Ductitis caused by *Raoultella planticola*: An emerging pathogen. Pancreas. 2022; 51(10): e118-9.
DOI: https://doi.org/10.1097/mpa.0000000000002158
21. Huang Q, Zhang J, Liao G. Abdominal abscess caused by *Raoultella ornithinolytica* secondary to postoperative gastric fistula: Case report and review of literature. BMC Infect Dis. 2024; 24(1): 363.
DOI: https://doi.org/10.1186/s12879-024-09234-y
22. Rafailidis PI, Falagas ME. Options for treating carbapenem-resistant Enterobacteriaceae. Curr Opin Infect Dis: 2014; 27 (6): 479-83.
DOI: https://doi.org/10.1097/qco.0000000000000109

ORIGINAL ARTICLE

Clinical and microbiological characteristics and outcome of patients with healthcare associated ventriculitis and meningitis at a Public Sector Hospital in Pakistan

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ABSTRACT

Background: Healthcare-associated meningitis and ventriculitis (HAVM) is a serious complication of placement of ventriculoperitoneal shunt (VPS) and external ventricular device (EVD), and can incur high morbidity and mortality.

Material And Methods: This retrospective descriptive, single center study was conducted in patients who had placement of VPS or EVD between January 2018 and December 2021 and developed HAVM. A list of these patients was retrieved from records maintained by the neurosurgery department.

Results: Thirty patients with HAVM were included. Median age was 8.5(IQR 22.25-0.75), two thirds were pediatric, 50% were males. Indication for device placement was congenital hydrocephalus in 18 (60%), tumor in 6 (20%) with VPS insertion in 25 (83.3%), EVD in 5 (16.7%). Fever was documented in 29 (96.7%). Cerebrospinal fluid analysis (CSF) demonstrated median pleocytosis with IQR of 93.5 (17-12959), protein of 131 (IQR 2-285), glucose of 24.5 (IQR 0-72). 17 (56.6%) had ICU stay, 11 were on mechanical ventilation. Mortality occurred in 4 (13.3%). CSF cultures were positive in 28 (93%) patients. Of 32 bacterial isolates, 16 were gram positive and 16 gram negative. In 15 (93.7%) patients, gram negatives were resistant to carbapenems and included 7 (46.6%) Enterobacterales (CRE), 3 (20%) *Pseudomonas aeruginosa* and 5 (33.3%) *Acinetobacter spp*. They were treated with IV colistin and meropenem. Predictors of mortality were male sex and sepsis. ($p \leq 0.05$).

Conclusion: Male sex and sepsis were found to be predictors of mortality in HAVM. Gram negatives in CSF cultures of 15 (93.7%) patients were resistant to carbapenems and challenging to treat.

Keywords: Cerebrospinal fluid infection, Healthcare-associated meningitis and ventriculitis, Hydrocephalus management, Risk-factors, Shunt infections

BACKGROUND

Healthcare-associated meningitis and ventriculitis (HAVM) is a known complication of invasive neurosurgical procedures and is associated with high morbidity and mortality.[1] One of the most commonly employed neurosurgical intervention is placement of ventriculoperitoneal shunt (VPS) or external ventricular drainage (EVD) catheter in acute hydrocephalus.[2] Depending on the location and the kind of neurological device employed, ventriculitis epidemiology varies, with some studies showing a 20% prevalence rate.[3] VPS related cerebrospinal infection rates ranging from 2.2% to 41% have been reported.[4] Reported rates of EVD-related infections range from 3%-19%.[5] HAVM prolongs duration of hospitalization and overall cost, increases morbidity and mortality and worsens prognosis.[6-8] Moreover, in a critical care setting, HAVM is increasingly caused by multi-drug resistant organisms (MDRO) which are challenging to treat.[9] There is limited data from Pakistan regarding HAVM. This study was conducted in order to identify the clinical presentation, microbial etiology, management and factors associated with poor outcome in patients who develop HAVM with the goal to contribute to guidelines for the most appropriate empirical antimicrobial therapy, and to improve management and outcomes.

MATERIAL AND METHODS

This is a retrospective descriptive study in patients diagnosed with HAVM. The Benazir Bhutto Institute of Trauma is a 500-bed facility that is located in the

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This article can be cited as: Arshad N, Samad S, Memon S, Ishaque1 S, Baqi S. Clinical and microbiological characteristics and outcome of patients with healthcare associated ventriculitis and meningitis at a Public Sector Hospital in Pakistan. Infect Dis J Pak. 2024; 33(3): 125-132.
DOI: https://doi.org/10.61529/idjp.v33i3.316

Receiving date: **28 Apr 2024** Acceptance Date: **23 Jul 2024**
Revision date: **04 Jul 2024** Publication Date: **30 Sep 2024**

southern port city of Karachi in Sindh, Pakistan and is a major referral center for the provinces of Sindh and neighboring Baluchistan. It is a public sector government funded hospital, and one of few trauma centers in the country.

A list of all patients who had placement of either VPS or EVD between January 2018 and December 2021 was retrieved from records maintained by the neurosurgery department. Those patients that developed HAVM were further identified. from the list and their medical records reviewed to determine if they met our study criteria.

Our inclusion criteria incorporated patients that were admitted to the Institute of Trauma and had placement of the VPS or EVD at the facility, and subsequently developed HAVM. All ages, including infants, pediatric (1-17 years) and adults were included. Patients with HAVM were excluded if they had placement of VPS or EVD at an outside facility but had been transferred to our institute. In addition, patients with community-acquired meningitis, subdural empyema, brain abscess, or tuberculous meningitis were excluded even if they had placement of the ventricular catheter at our facility as part of their management. Those with missing pertinent data were also excluded.

We diagnosed healthcare-associated meningitis and ventriculitis if CSF culture was positive, or if there were at least 2 signs and symptoms such as fever >38.0 °C, headache or meningeal signs along with abnormal CSF analysis with pleocytosis, elevated protein, and decreased glucose. Diagnosis of HAVM by an Infectious Diseases specialist was also included in the definition, if above conditions were met.

Recovery from HAVM was documented when there was resolution of symptoms and signs of CNS infection and CSF cultures remained negative at completion of antimicrobial therapy with no relapse of infection during the admission.

The admission diagnosis, demographics, co-morbidities, type of ventricular catheter whether VP or EVD, indication for use, whether inserted in the emergency or elective setting, were recorded. Health care-associated infections such as pneumonia, central line bloodstream infection, and catheter-associated urinary tract infection were also entered into the data base. The number of intracranial surgeries, removal or revision of the device, empirical antimicrobial therapy, and duration of ICU and hospital stay were noted. Laboratory data including CSF parameters of cell count, biochemistry, and microbiology were abstracted from the patients' electronic medical records.

The primary outcome of this study was in-hospital mortality, whereas secondary outcomes included ICU admission, mechanical ventilation, sepsis, length of ICU stay, duration of hospitalization and readmission for the same diagnosis. Ethical approval was obtained from the institutional ERC prior to conducting the study. All data entered in the computer were password secured (Author/PI had access only). No personal identifiers of patients were recorded.

Data were stored and analyzed using IBM-Statistical Package for the Social Sciences version 23.0; counts with percentages were given on demographics, co-morbidities, indication for neurosurgical procedure, type of ventricular catheter, placement, pre-operative antibiotics, sign and symptoms at presentation of HAVM and other qualitative parameters of study, Median with Interquartile range was reported for age, length of ICU stay, white cell counts, protein and glucose. Fisher's Exact test was used to check the association of mortality with age, gender, diagnosis, comorbidities, type of device, CSF protein, Type of bacteria in CSF, Cather management, ICU admission, sepsis and mechanical ventilation. p-values less than 0.05 were considered statistically significant.

RESULTS

A total of 30 patients with HAVM were included in the study. The median age and IQR of patients was 7 years (22). Of 30, two-thirds (66.7%) were of the pediatric population, 50% were males.

The most common co-morbidity noted was diabetes mellitus in 6 (20%). None were diagnosed to be immunocompromised. The most common indications for placement of the device were congenital hydrocephalus in 18 (60%) and tumor in 6 (20%).

Initial ventricular catheter placement included a VP shunt in 25 (83.3%) and EVD in 5 (16.7%). Catheter placement procedures were performed in the emergency setting in 8 (26.7%). All patients received peri-operative ceftriaxone. Fever was the most common symptom of HAVM in 29 (96.7%); headache in 14 (46.7%). The most common sign of HAVM was neck stiffness in 6 (20%). Cerebrospinal fluid analysis was significant for a median pleocytosis with IQR of 93.5 cells/mm^3 (17-

12959), protein of 131 mg/dl (2-285) and glucose of 24.5 mg/dl (0-72).

Of 30, 17 (56.6%) had ICU stay and of these 11 were placed on mechanical ventilation. Mortality occurred in 4 (13.3%) patients whereas 26 (86.7%) were considered to have made good recovery and were discharged. None of those discharged were readmitted. Duration of hospital stay was a median of 26 (10-332) (Table-I).

Of 30 patients with HAVM, CSF cultures were positive in 28 (93%) patients. Of 32 bacterial isolates recovered, 16 (50%) were gram positive and 16 (50%) gram negative. The most commonly reported bacteria overall was *Coagulase negative staphylococcus* in 9 (56.25%). Gram negative multi-drug-resistant organisms that were resistant to the carbapenems were isolated in 15 (93.7%) patients and included 7 (46.6%) Enterobacterales (CRE), 3 (20%) "difficult-to-treat" *Pseudomonas aeruginosa* and 5 (33.3%) *Acinetobacter spp*.

All patients were started empirically on intravenous meropenem and vancomycin and therapy was adjusted in accordance with culture and sensitivity results, if indicated. Of 30, 15 (50%) patients received combination of intravenous colistin and meropenem for treatment of gram-negative multi-drug-resistant organisms isolated in CSF culture. None of the patients received intrathecal or intraventricular colistin. Repeat CSF studies were performed, on average, every 72 hours, to document clearance in those with positive CSF cultures, as well as to monitor CSF parameters while on antimicrobial therapy.

Initial catheter management of HAVM included immediate removal of VP shunt or EVD in 4 (13.3%) patients, exteriorization of distal end in 22 (73.3%) of whom 10 (33.3%) subsequently required shunt removal, whereas in 4 (13.3 %) the VP shunt and EVD were left in-situ and treated conservatively with antibiotics alone. Of 30 patients, sepsis was diagnosed in 5 (16.6%). The most common healthcare associated infection documented was pneumonia. Infection at the device insertion site occurred in 2 (6.7%) patients (Table-II).

Four (13.3%) patients died and good recovery was documented in 26 (86.7%) patients. Predictors of mortality were found to be male sex and sepsis. ($p \leq 0.05$). Though not found to be statistically significant, 3 of 4 patients that died had gram negative multidrug resistant organisms recovered in CSF culture. Age, comorbidities, immune status, concomitant infection and mode of procedure, whether elective or emergent, were not found to be statistically significant (Table-III).

Table-I: Demographics, clinical and laboratory characteristics of patients with healthcare associated ventriculitis and meningitis (n=30).

Characteristics	Frequency (%)
Demographics:	
Median Age (IQR):	07 (22)
< 1 year	09 (30%)
1-17 Years	11 (36.7%)
18-65 Years	10 (33.3%)
Gender:	
Male	15 (50%)
Female	15 (50%)
Co-morbidities	
Diabetes Mellitus	06 (20%)
Hypertension	01 (3.3%)
Malignancy	03 (10%)
Indication for Neurosurgical Procedure:	
Tumor	06 (20%)
Post Traumatic hydrocephalus	04 (13.3%)
Intraventricular bleed	01 (3.3%)
Congenital hydrocephalus	18 (60%)
Normal pressure hydrocephalus	01 (3.3%)
Type of Ventricular Catheter:	
VP	25 (83.3%)
EVD	05 (16.7%)
Placement:	
Emergency	08 (26.7%)

Elective	22 (73.3%)
Peri-operative Antibiotics	30 (100%)
Signs and Symptoms at Presentation of HAVM:	
Fever	29 (96.7%)
Altered mental status	09 (30%)
Nausea/Vomiting	03 (10%)
Neck Stiffness	06 (20%)
Headache	14 (46.7%)
Seizures	06 (20%)
Laboratory Parameters	
Positive CSF Culture: (n %)	28 (93%)
Cerebrospinal Fluid: (median ,IQR)	93.5 (17-12959)
Leukocytes (per mm^3)	72
Neutrophils percentage	24.5 (0-72)
Glucose (mg/dl)	131 (2-285)
Protein (mg/dl)	04 (13.3%)
Blood: (n %)	
Positive blood cultures	
Number of neurosurgeries during admission (median, IQR)	2(0-3)
ICU stay	17 (56.6%)
Duration of ICU stay (median, IQR)	7 (1-112)
Mechanical Ventilation	11 (36.7%)
Duration of hospitalization (median, IQR)	26 (10-332)
Outcome	
• Died	04 (13.3%)
• Recovered	26 (86.6%)

Table-II: Microbiological Data and Management of Healthcare Associated Ventriculitis and Meningitis (n=30)

Pathogen	No (%)
Cerebrospinal (CSF) Culture	
No growth	02 (6.7%)
Positive Culture:	28 (93.3%)
• Monomicrobial Growth	24 (85.7%)
• Polymicrobial Growth	04 (14.3%)
Gram Positives:	16 (50%)
Staphylococcus aureus (all MRSA)	05 (31.25%)
Coagulase negative staphylococcus	09 (56.25%)
Corynebacterium spp	02 (12.5%)
Gram Negatives:	16 (50%)
Acinetobacter spp.	05 (31.25%)
Enterobacter spp.	01 (6.25%)
Escherichia Coli	03 (18.75%)
Klebsiella spp	03 (18.75%)
Pseudomonas aeruginosa	03 (18.75%)
Serratia Marcescens	01 (6.25%)
MDRO Gram Negatives	15 (93.7%)
CRE[1]	07 (46.6%)
CRA[2]	05 (33.3%)
DTR-PA[3]	03 (20%)
Blood Culture	
No growth	26 (86.6%)
Coagulase Negative Staphylococcus	02 (6.7%)
Pseudomonas aeruginosa	02 (6.7%)
Concomitant Healthcare Associated Infection:	
None	14 (46.6%)
CLABSI	02 (6.7%)
HAP/VAP	10 (33.3%)
CAUTI	02 (6.7%)

SSSI at VP or EVD Insertion Site	02 (6.7%)
Sepsis	05 (16.6%)
IV Antibiotics given:	
IV Colistin	15 (50%)
Meropenem	18 (60%)
Vancomycin	17 (56.6%)
Catheter Management:	
• Immediate removal of VP shunt or EVD	4 (13.3%)
• Exteriorization of distal end alone.	12 (40%)
• Exteriorization of distal end followed by VP shunt removal.	10 (33.3%)
• VP and EVD not removed with conservative management alone.	4 (13.3%)

Table-III: Predictors of mortality in patients with HAVM (n=30).

Variable	Total Number (n=30)	Mortality n (%) (n=4)	*p* -value
Age			
< 1 year	9 (30%)	0	
1-17 years	11 (36.7%)	1 (25%)	0.13
18-65 years	10 (33.3%)	3 (75%)	
Gender			
Male	15 (50%)	4 (100%)	0.032*
Female	15 (50%)	0	
Diagnosis			
Tumor	6 (20%)	0	0.14
Post Trauma	4 (13.3%)	1 (25%)	
Intraventricular Bleed	1 (3.3%)	1 (25%)	
Congenital Hydrocephalus	18 (60%)	2 (50%)	
Normal Pressure Hydrocephalus	1(3.3%)	0	
Co-morbidities			
Diabetic Mellitus	5 (16.7%)	1 (25%)	0.63
Type of Device			
Extra-Ventricular Drain	5 (16.7%)	1 (25%)	0.63
Ventriculoperitoneal Shunt	25 (83.3%)	3(75%)	
CSF Protein>100mg/dl	18 (60%)	3 (75%)	0.51
Type of bacteria in CSF:			0.80
No Growth	02(6.7%)	0	
Gram Positive	12 (40%)	01 (25%)	
Gram Negative	12 (40%)	02 (50%)	
	04 (13.3%)	01 (25%)	
Multidrug resistant gram negative (n=15):			
CRE	07 (46.7%)		0.34
CRA	05 (33.3%)	02 (66.7%)	
DTR-PA	03 (20%)	01 (33.3%)	
Positive Blood Culture:			
Negative	26 (86.6%)	4 (100%)	
Pseudomonas Aeruginosa	02 (6.7%)	0	0.70
Coagulase negative Staphylococcus	02 (6.7%)	0	
Symptoms:			0.69
Fever	29 (96.7%)	4 (100%)	
Catheter Management:			
• Immediate removal of VP shunt or EVD with revision.	4 (13.3%)	3 (75%)	0.001*
	12 (40%)	0	
• Exteriorization of distal end alone.	10 (33.3%)	1 (25%)	

• Exteriorization of distal end followed by VP shunt removal.	4 (13.3%)	0	
VP and EVD not removed with conservative management alone.			
ICU admission	17 (56.7%)	4 (100%)	0.06
Sepsis	5 (16.7%)	2 (50%)	0.055*
Mechanical Ventilation	11 (36.7%)	3 (75%)	0.87

1. CRE carbapenem resistant Enterobacterales
2. CRA carbapenem resistant *Acinetobacter*
3. "difficult-to-treat" *Pseudomonas aeruginosa* defined as isolates testing intermediate or resistant to all reported carbapenems, beta-lactams, fluoroquinolones and monobactam

DISCUSSION

Healthcare associated ventriculitis and meningitis is associated with significant mortality and morbidity. We reported a mortality rate of 13%. C Srihawan *et al* reported an overall mortality rate of 9.3% and adverse outcomes in 78% of patients.[10]

We found that male sex and sepsis were predictors of mortality in our patients with HAVM. Prior studies have reported factors associated with mortality. In a study by Rodriguez Guardado *et al*, of 51 patients with EVD-related *Acinetobacter* meningitis, 17 died from the infection. They found that lack of removal of intraventricular catheters, high CSF pleocytosis (4988.35 vs 1341 cells/mm3), and older age (50 vs 40 years) were significantly associated with mortality.[11] However, Kim *et al* did not report similar factors associated with mortality in their study of 27 patients with *Acinetobacter* meningitis.[12] Srihiwan C *et al* identified age ≥45 years, abnormal neurological exam, and mechanical ventilation as poor prognostic factors. In their study, CSF parameters and removal of intraventricular catheters were not associated with adverse outcomes, as was also demonstrated in our study.[10]

We had equal proportion of gram positive and gram-negative bacteria isolated in CSF cultures of our patients with HAVM. *Staphylococcus aureus* was the most frequently encountered pathogen, found in 53.3% of the cases in a pediatric study of shunt infections.[13] Yakut N *et al* reported that in 148 out of 290 VPS infections, coagulase negative staphylococcus was isolated in 42.5%, *Pseudomonas aeruginosa* in 14.9%, *Klebsiella pneumoniae* in 10.1% and *Staphylococcus aureus* in 10.1% of cases.[14]

Of great concern is that half of our patients had highly resistant gram negatives isolated from the CSF, which included Enterobacterales, *Acinetobacter spp*. and *Pseudomonas aeruginosa*, all of which were resistant to the carbapenems. This has implications for empirical therapy for HAVM, which should be guided by the antibiogram of the institution. In our facility, based on the findings of this study, empirical therapy for HAVM with meropenem alone, without colistin, for gram negative coverage is no longer a viable option. Colistin has limited penetration into the cerebrospinal fluid and therefore it is recommended to additionally administer intrathecal and intra-ventricular colistin for the treatment of CNS infections.[15]

In a retrospective analysis by Chen *et al*, 28 patients who had MDRO gram negative CNS infection were treated with intraventricular polymyxin B supplemented by continuous external ventricular drainage. The duration of treatment was 14.96 ± 4.28 days and negative CSF culture were achieved by 8.23 ± 4.02 days. They found a bacterial clearance rate from cerebrospinal fluid of 92.9% (26/28) with a clinical cure rate of 82.1% (23/28), thus concluding that intraventricular polymyxin B supplemented by continuous external ventricular drainage is a safe and effective treatment strategy for MDRO gram negative CNS infection. [16]

Several β-lactam/β-lactamase inhibitors (BLBLIs) have been developed for the treatment of pneumonia, urinary tract, intra-abdominal and bloodstream infections due to carbapenem-resistant Enterobacterales, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. These include ceftolozane-tazobactam, ceftazidime-avibactam and cefiderocol as well as meropenem-vaborbactam, and imipenem-relebactam. However, there is limited data for the use of these agents in CNS infections.

Guidelines for management of HAVM recommend immediate removal of the catheter . [(17)]However, Brown *et al* treated 43 of 122 patients with CSF shunt infections conservatively with intraventricular and systemic antibiotics. The study reported that 84% of these patients were cured. A 92% success rate was reported in infections caused by bacteria other than *Staphylococcus aureus*, and included 30 patients with coagulase-negative staphylococcal infections .[(18)] This treatment approach cannot be recommended for more virulent pathogens such as gram negative and *S. aureus*. In our study, over half of the patients did not have removal of the device. None of these patients received intrathecal

or intraventricular colistin. We were not able to follow patients after discharge, and therefore cannot comment on the long-term success of conservative management. Limitations of our study is that it is a single-center retrospective study and the number of cases is relatively small. Moreover, the study design was unable to incorporate follow-up of patients after discharge, which is logistically challenging at the Trauma Institute since patients are discharged back to referring healthcare facilities that are often located in distant towns or provinces.

CONCLUSION

This retrospective, single center study examined 30 patients with healthcare associated ventriculitis and meningitis. Male sex and sepsis were found to be predictors for mortality. Cerebrospinal cultures demonstrated equal representation of gram positive and gram-negative bacteria. Almost all gram negatives isolated were resistant to the carbapenems, which is challenging to treat due to limited antimicrobial options. A prospective study with larger sample size is recommended for further analysis of clinical characteristics, microbiology, outcome and risk factors for mortality and morbidity in patients with HAVM.

CONFLICT OF INTEREST

None

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHOR CONTRIBUTION

Nazish Arshad: Conception, frame work, literature search, data interpretation and drafting, accountable for all aspects of the work

Saima Samad: Data collection, data Analysis, accountable for all aspects of the work

Sughand Memon: Literature search, accountable for all aspects of the work

Sadia Ishaque: Review of manuscript, accountable for all aspects of the work

Shehla Baqi: Study design, critical review, final approval of the article, accountable for all aspects of the work

REFERENCES

1. Hader WJ, Steinbok P. The value of routine cultures of the cerebrospinal fluid in patients with external ventricular drains. Neurosurgery. 2000; 46(5): 1149-55. DOI:https://doi.org/10.1097/00006123-200005000-00025
2. Lilley D, Munthali P. Analysis of the management of ventriculitis cases at a UK neurosurgery centre. Infect Prev Pract. 2022;4(4):100240. DOI: https://doi.org/10.1016%2Fj.infpip.2022.100240
3. Hasbun R. Central nervous system device infections. Curr Infect Dis Rep. 2016; 18 (11): 34. DOI: https://doi.org/10.1007/s11908-016-0541-x
4. Humphreys H, Jenks P. Surveillance and management of ventriculitis following neurosurgery. J Hosp Infect. 2015; 89(4): 281-6. DOI: https://doi.org/10.1016/j.jhin.2014.12.019
5. Mikhaylov Y, Wilson TJ, Rajajee V, Thompson BG, Maher CO, Sullivan SE, *et al*. Efficacy of antibiotic-impregnated external ventricular drains in reducing ventriculostomy-associated infections. J Clin Neurosci. 2014; 21(5): 765-8. DOI: https://doi.org/10.1016/j.jocn.2013.09.002
6. Fried HI, Nathan BR, Rowe AS, Zabramski JM, Andaluz N, Bhimraj A, *et al*. The insertion and management of external ventricular drains: an evidence-based consensus statement: a statement for healthcare professionals from the Neurocritical Care Society. Neurocrit Care. 2016;24:61-81. DOI: https://doi.org/10.1007/s12028-015-0224-8
7. Arabi Y, Memish ZA, Balkhy HH, Francis C, Ferayan A, Al Shimemeri A, *et al*. Ventriculostomy-associated infections: incidence and risk factors. Am J Infect Control. 2005; 33(3): 137-43. DOI: https://doi.org/10.1016/j.ajic.2004.11.008
8. Hagel S, Bruns T, Pletz M, Engel C, Kalff R, Ewald C. External ventricular drain infections: risk factors and outcome. Interdiscip Perspect Infect Dis. 2014; 2014. 708531. DOI: https://doi.org/10.1155/2014/708531
9. Pandey S, Li L, Deng XY, Cui DM, Gao L. Outcome following the treatment of ventriculitis caused by multi/extensive drug resistance gram negative bacilli; Acinetobacter baumannii and Klebsiella pneumonia. Front Neurol. 2019; 9: 1174. DOI: https://doi.org/10.3389/fneur.2018.01174
10. Srihawan C, Castelblanco RL, Salazar L, Wootton SH, Aguilera E, Ostrosky-Zeichner L, *et al*., editors. Clinical characteristics and predictors of adverse outcome in adult and pediatric patients with healthcare-associated ventriculitis and meningitis. Open Forum Infectious Diseases; 2016: Oxford University Press.
11. Rodriguez Guardado A, Blanco A, Asensi V, Pérez F, Rial J, Pintado V, *et al*. Multidrug-resistant Acinetobacter meningitis in neurosurgical patients with intraventricular catheters: assessment of different treatments. J Antimicrob Chemother. 2008; 61(4): 908-13. DOI: https://doi.org/10.1093/jac/dkn018
12. Kim H-I, Kim S-W, Park G-Y, Kwon E-G, Kim H-H, Jeong J-Y, *et al*. The causes and treatment outcomes of 91 patients with adult nosocomial meningitis. Korean J Intern Med. 2012; 27(2): 171. DOI: https://doi.org/10.3904%2Fkjim.2012.27.2.171
13. Bastian RA, Pramusinto H, Basuki E, Marianne M. Ventriculoperitoneal shunt infection: a study about age as a risk factor in hydrocephalus pediatrics. Open Access Macedonian J Med Sci. 2022;10(B):314-9. DOI: http://dx.doi.org/10.3889/oamjms.2022.8251
14. Yakut N, Soysal A, Kadayifci KE, Dalgic N, Yılmaz Ciftdogan D, Karaaslan A, *et al*. Ventriculoperitoneal shunt infections and re-infections in children: A

multicentre retrospective study. Br J Neurosurg. 2018; 32(2): 196-200.
DOI: https://doi.org/10.1080/02688697.2018.1467373

15. Markantonis S, Markou N, Fousteri M, Sakellaridis N, Karatzas S, Alamanos I, *et al*. Penetration of colistin into cerebrospinal fluid. Antimicrob Agents Chemother. 2009; 53(11): 4907-10.
DOI: https://doi.org/10.1128%2FAAC.00345-09
16. Chen H, Guo X, Xie D, Dong X, Niu J, Chen G. A clinical study on the use of intraventricular Polymyxin B supplemented by continuous external ventricular drainage in the treatment of drug-resistant gram-negative bacilli intracranial infection. Infect Drug Resist. 2020: 2963-70.
DOI: https://doi.org/10.2147/idr.s261510
17. Tunkel AR, Hasbun R, Bhimraj A, Byers K, Kaplan SL, Scheld WM, *et al*. 2017 Infectious Diseases Society of America's clinical practice guidelines for healthcare-associated ventriculitis and meningitis. Clin Infect Dis. 2017; 64(6): e34-e65.
DOI: https://doi.org/10.1093/cid/ciw861
18. Brown EM, Edwards RJ, Pople IK. Conservative management of patients with cerebrospinal fluid shunt infections. Neurosurgery. 2008; 62: SHC-661-SHC-9.
DOI:https://doi.org/10.1227/01.neu.0000204126.54417.46

ORIGINAL ARTICLE

In vitro efficacy of colistin against multidrug-resistant *Pseudomonas aeruginosa* in burn patient by minimum inhibitory concentration with broth dilution

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ABSTRACT

Background: *Pseudomonas aeruginosa* strains cause 86% of sepsis mortality in burn victims. Therefore, to combat the multi-drug resistance *in Pseudomonas aeruginosa*, colistin is the new drug, and it has recently been introduced. To analyze the in vitro efficacy of colistin against multidrug-resistant *Pseudomonas aeruginosa* in burn patients by determining the minimum inhibitory concentration with broth dilution.

Material And Methods: The cross-sectional study was performed from March 2021 to February 2022 in the Burn Centre at Nishter Hospital in Multan, Pakistan. 300 burn patients (≥20% burn) were selected, and their pus samples were collected and processed in the microbiology laboratory. The Kirby-Bauer disc diffusion method was applied to check the antibiotic susceptibility against isolated strains. The colistin sensitivity against multi-drug resistant (MDR) strains was estimated by employing the broth dilution method at two-fold serial dilutions from 0.5 µg/mL to 0.003 µg/mL.

Results: 124 (55.3%) strains were identified as *Pseudomonas aeruginosa*, while the remaining strains were identified as *Escherichia coli* (13.0%), *Streptococci* (13.0%), *Klebsiella pneumoniae* (8.9%), *Staphylococci* (6.6%), and *Candida albicans* (2.2%). All isolated strains of *Pseudomonas aeruginosa* showed resistance against antibiotics: aztreonam (60.4%), cefepime (72.5%), ceftazidime (71.7%), ciprofloxacin (62.0%), imipenem (33.0%), levofloxacin (58.0%), meropenem (19.3%), piperacillin/tazobactam (66.1%), and tobramycin (63.7%). The significantly calculated MIC value of colistin against MDR strains was 0.35-0.5 mg/L (CLSI and EUCAST recommended value = ≤2 mg/L).

Conclusion: Colistin can be a good option to treat nosocomial infections of MDR *Pseudomonas aeruginosa* in burn patients.

Keywords: Burn patients, Colistin, Multi-drug resistance, Minimum inhibitory concentration

BACKGROUND

Skin is the largest organ of the human body and regulates homeostasis and acts as a protective barrier against infections.[1] Damage to the skin causes loss of the skin barrier and subsequent pathogenic inflammatory activation.[2] The high mortality rate from skin burns is unacceptable and underreported.[3] Direct or indirect contact with chemicals, fire, and electric current can cause mild or severe skin burn injuries.[4] According to the World Health Organization (WHO), burn injuries cause 180,000 deaths globally each year. Pakistan's 6.5% burn death rate is a result of inadequate burn patient care and inadequate infrastructure.[5] Patients with burns are also more susceptible to bacterial and fungal infections during their course of treatment due to an infirm immune system.[6, 7]

The opportunistic pathogenicity of *Pseudomonas aeruginosa* (*P. aeruginosa*) is widely recognized in patients with impaired immune systems.[8] Nosocomial infections (NIs) are frequently caused by *P. aeruginosa* in burn patients.[9] Bloodstream infections (BSIs), surgical site infections (SSIs), and urinary tract infections (UTIs) are examples of these NIs.[10,12] Approximately 10-11% of NIs are caused by *P. aeruginosa*.[13] In 2017, the WHO identified *P. aeruginosa* as a pathogen that needed to be studied more closely to develop new antibiotics.[14]

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This article can be cited as: Neelam B, Malik S, Naqvi SMA, Khan MH, Javeed M. In vitro efficacy of colistin against multidrug-resistant Pseudomonas aeruginosa in burn patient by minimum inhibitory concentration with broth dilution. Infect Dis J Pak. 2024; 33(3): 133-139
DOI: https://doi.org/10.61529/idjp.v33i3.338

Receiving date: **15 Jul 2024** Acceptance Date: **26 Sep 2024**
Revision date: **11 Sep 2024** Publication Date: **30 Sep 2024**

Antibiotic resistance is one of the major reasons for the high mortality rate in burn patients from *P. aeruginosa* NIs.[15] 86% of sepsis deaths in burn patients are caused by multi-drug resistant (MDR) strains of *P. aeruginosa*.[16] It was discovered that carbapenems were the most successful antibiotics against *P. aeruginosa* NIs.[17] However, *P. aeruginosa* strains that are resistant to β-lactams, carbapenems, cephalosporins, aminoglycosides, and quinolones have been reported in previous years.[18]

Colistin and polymyxin B (previously believed to be toxic for clinical use) are currently being brought back to be used as "last option" antibiotics against Gram-negative bacteria.[19] In 1947, Koyama discovered colistin as a byproduct of the Gram-positive soil bacteria *Paenibacillus polymyxa* subsp. *Colistinus* in Japan.[20] Colistin began to be used in both humans and animals in 1952. But between the 1970s and 1980s, its utilization in medicine nearly disappeared. However, recently, to treat infections caused by the MDR *P. aeruginosa*, colistin has started to be used in humans.[20] The WHO recommended colistin as critically important human medicine in 2018.[21] Therefore, the present study was conducted to determine the frequency of MDR *P. aeruginosa* in burn patients and the minimum inhibitory concentration (MIC) value of colistin using the broth dilution method against MDR strains

MATERIAL AND METHODS

The cross-sectional investigation was carried out at the Microbiology Laboratory, Department of Pathology, Nishter Medical University, in association with the Pak Italian Burn Unit in Multan. The convenient non-probability sampling technique was used. A sample size of 124 MDR *P. aeruginosa* isolates was calculated by taking a 95% confidence level, 5% absolute precision, and an expected prevalence of 22.7% of *P. aeruginosa* in burn patients.[22] The burn patients (male and female), representing any type of burn and ranging from 15 to 60 years of age, were included in this study. Patients with less than 20% burn, MLC cases as per hospital records, and patients taking antibiotics were excluded.

For this study, a total of 300 patients with severe burns were chosen. Every patient was admitted to the Nishter Hospital Burn Centre. The basic information, medical history, and history of infections were gathered using a specified questionnaire. Furthermore, each patient provided written approval for the use of their samples.

The aspirators or drainage tubes were used to gather pus samples in sterile containers from each patient. The sterile cotton swabs were used to help obtain pus samples from the burn wounds of some patients who did not have any drainage. Afterward, every pus sample was appropriately labeled and sent to the microbiology lab for further investigation (Figure-I).

The selected antibiotics for this study are mentioned in Table-I. The sterile and freshly prepared Muller-Hinton agar was dispensed into the sterile petri plates, and the poured plates were kept at room temperature until solidification. The in vitro antibiotic susceptibility of each strain was ascertained using the Kirby-Bauer disc diffusion method. Using aseptic methods, bacterial suspensions (0.5 MacFarland) were swabbed onto each agar plate. The sterile syringes were used to impregnate antibiotic discs on agar plates at equal distances. Following incubation, zones of inhibition (ZOIs) were recorded and interpreted in accordance with Clinical and Laboratory Standard Institute (CLSI) recommendations of 2022. For MIC determination, a stock solution of colistin (Sigma-Aldrich) was prepared in a clean test tube. For this, 10 mg was added to 100 mL of sterile deionized water and thoroughly mixed. From this tube, 1 mL was transferred to another test tube containing 100 mL of sterile deionized water. This test tube was labelled as A. 11 clean test tubes were taken, and labelled from 1 to 11. 0.5 mL of freshly prepared nutrient broth was added to test tubes 2 to 11. 0.5 mL of antibiotic solution (test tube A) was added to test tubes 1 and 2. Two-fold serial dilutions of the antibiotic solution were prepared. From test tube 2, 0.5 mL was transferred to test tube 3 and mixed well. The same procedure was continued up to test tube 9. From tube 9, 0.5 mL was discarded to equal the volume (0.5 mL) in each tube. 0.5 mL of *P. aeruginosa* overnight-grown culture suspension (turbidity equal to 0.5 MacFarland standard) was added in each test tube except test tube 11. Finally, each test tube received a total volume of 1 mL, while consecutive test tubes received one and half of the original concentration of antibiotic. The test tubes 01, 10, and 11 were considered controls. Test tube 01 was an antibiotic control tube (it received 0.5 mL of bacterial suspension and 0.5 mL of antibiotic). The test tube was labelled C_1. Test tube 10 was a growth control

tube (it received 0.5 mL of bacterial suspension and 0.5 mL of nutrient broth). The test tube was labelled C_2. Test tube 11 was a sterility control tube (it received only 0.5 mL of nutrient broth). The test tube was labelled C_3. All test tubes were kept in an incubator at 37 °C for 22-24 hours, and the next day, visible turbidity in the test tubes was observed (Figure-II). To calculate the MIC value, the following formula was used:

MIC value=(lowest dilution inhibits growth + consecutive highest dilution allows growth) / 2

Excel spreadsheet version 22 and SPSS software version 25 were used to enter and analyze data, respectively. We noted the percentages or frequencies of all variables (age, gender, burn types, and isolated strains) accordingly.

RESULTS

The age distribution results showed that 20 (13.0%), 43 (28.7%), 56 (37.3%), 24 (16.0%), and 7 (4.6%) burn patients belonged to the age range of <20 years, 20-30 years, 31-40 years, 41-50 years, and >50 years old, respectively. The occurrence of burning was observed more in males (56%) than females (43%). In the present study, 23 (15.3%) patients of acid burn, 18 (12.0%) patients of accident burn, 16 (10.6%) patients of electrical burn, 40 patients (26.6%) of flame burn, 21 patients (14.0%) of liquid burn, 23 patients (15.3%) of scald burn, and 8 (5.3%) patients of suicide burn cases were observed. After biochemical testing, 124 (55.3%) bacterial strains were characterized as *P. aeruginosa*. Similarly, 100 strains out of the remaining 150 were identified as *Escherichia coli* (13.3%), *Streptococci* spp. (13.3%), *Klebsiella pneumoniae* (8.9%), *Staphylococcus* spp. (6.6%), and *Candida albicans* (2.2%).

The susceptibility of 124 strains of *P. aeruginosa* was further tested against aztreonam, cefepime, ceftazidime, ciprofloxacin, levofloxacin, piperacillin/tazobactam, and tobramycin. The measured ZOIs were compared with the CLSI guidelines of 2021, and it was observed that all 124 strains of *P. aeruginosa* were MDR. Out of a total of 124, 75 (60.4%), 90 (72.5%), 89 (71.7%), 77 (62.0%), 41 (33.0%), 72 (58.0%), 24 (19.3%), 82 (66.1%), and 79 (63.7%) *P. aeruginosa* strains were resistant to aztreonam, cefepime, ceftazidime, ciprofloxacin, imipenem, levofloxacin, meropenem, piperacillin/tazobactam, and tobramycin, respectively.

The broth dilution method was employed to calculate the MIC value of colistin against MDR strains of *P. aeruginosa*. Two-fold serial dilutions from 0.5 µg/mL to 0.07 µg/mL of colistin were prepared in test tubes. After the subsequent incubation period, all broth test tubes were clearly observed for the presence or absence of turbidity. The optical density (OD_{600}) values of each test tube were measured using a spectrophotometer (Table-II). According to the visual observation and OD_{600} values, test tube no. 2 showed no turbidity with an OD_{600} value of 0.12 as compared to other dilutions of colistin. After repetitive experiments, an average MIC value of 0.375-0.5 µg/mL (0.35-0.5 mg/L) was estimated for MDR strains of *P. aeruginosa* (Figure-III). This MIC value falls within the colistin MIC breakpoints (Table-III) of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical Laboratory and Standards Institute (CLSI) against MDR strains.

Table-I: Antibiotic discs used for antibacterial susceptibility testing.

Sr. No.	Name of Antibiotic	Disc content
1.	Aztreonam (ATM)	30 µg
2.	Cefepime (FEP)	10 µg
3.	Ceftazidime (CAZ)	30 µg
4.	Ciprofloxacin (CIP)	5 µg
5.	Levofloxacin (LEV)	5 µg
6.	Piperacillin/Tazobactam (TZP)	(100/10 µg)
7.	Tobramycin (TOB)	10 µg
8.	Meropenem (MEM)	5 µg

Table-II: OD_{600} values of different colistin concentrations and controls.

Test tube number	Colistin concentration (µg/ml)	Optical density (OD) value
C_1	0.5	0.1
C_2	0	1.4
C_3	0	0.03
2	0.5	0.12
3	0.25	0.23
4	0.125	0.34
5	0.06	0.48
6	0.03	0.57
7	0.015	0.69
8	0.007	0.85
9	0.003	0.97

Table-III: Colistin MIC breakpoints.

Bacteria	EUCAST Breakpoints (mg/L)		CLSI Breakpoints (mg/L)	
	S≤	R>	I≤	R>
Enterobacteriaceae	2	2	2	4
Pseudomonas	2	2	2	4
Acinetobacter	2	2	2	4

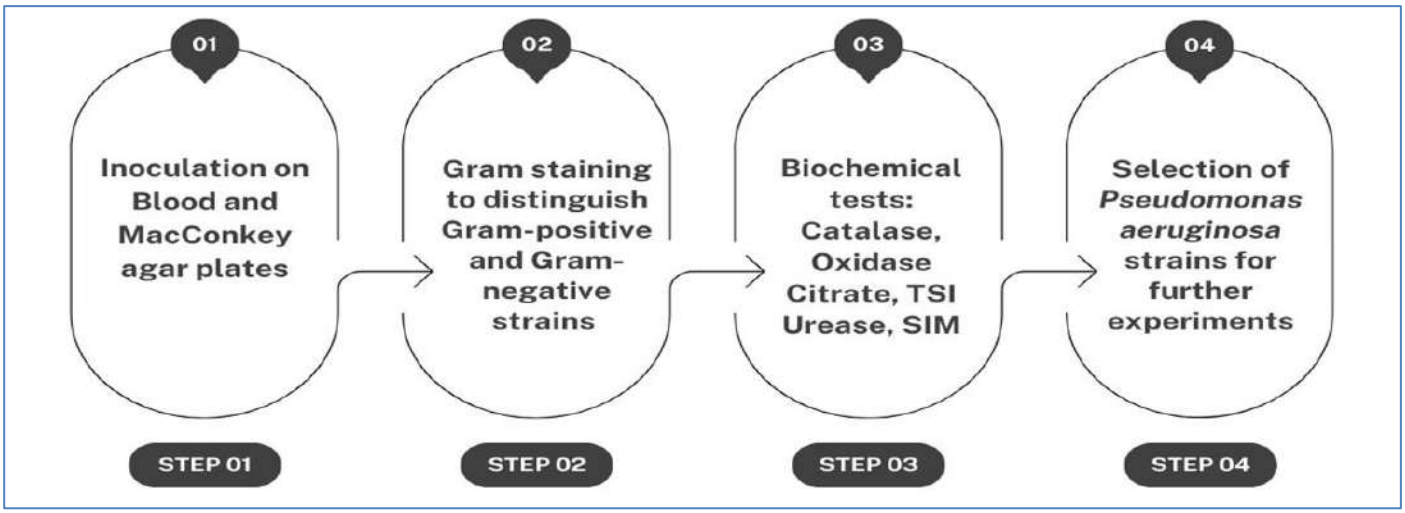

Figure-I: Flowchart shows the processing of the collected pus samples.

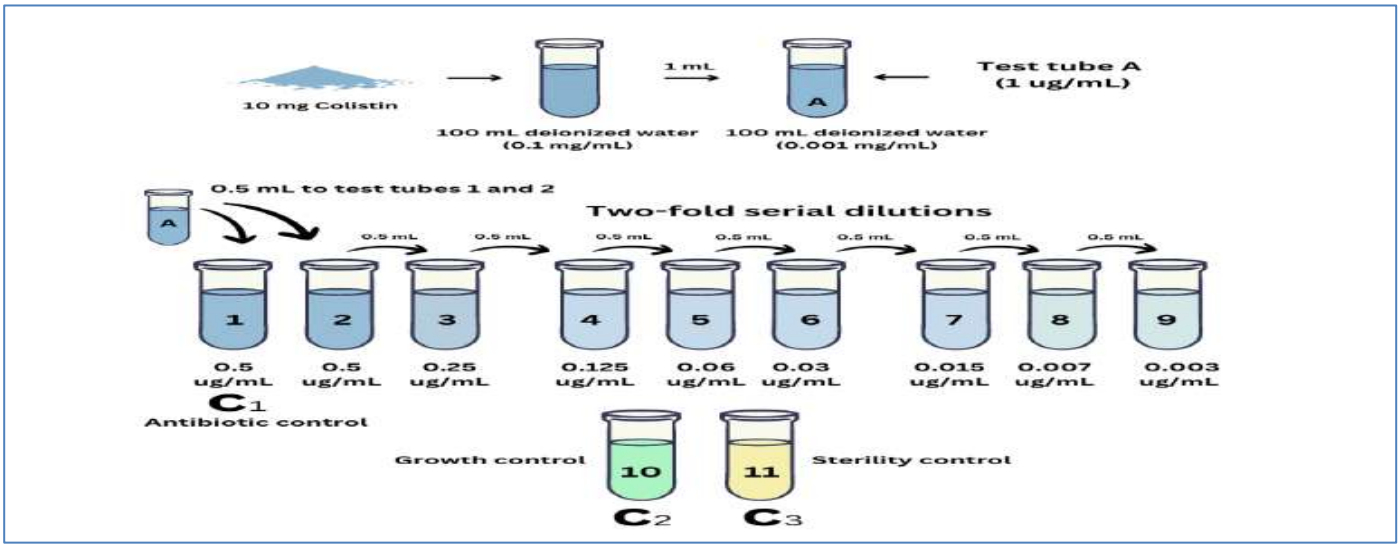

Figure-II: Graphical representation of the MIC determination by broth dilution method.

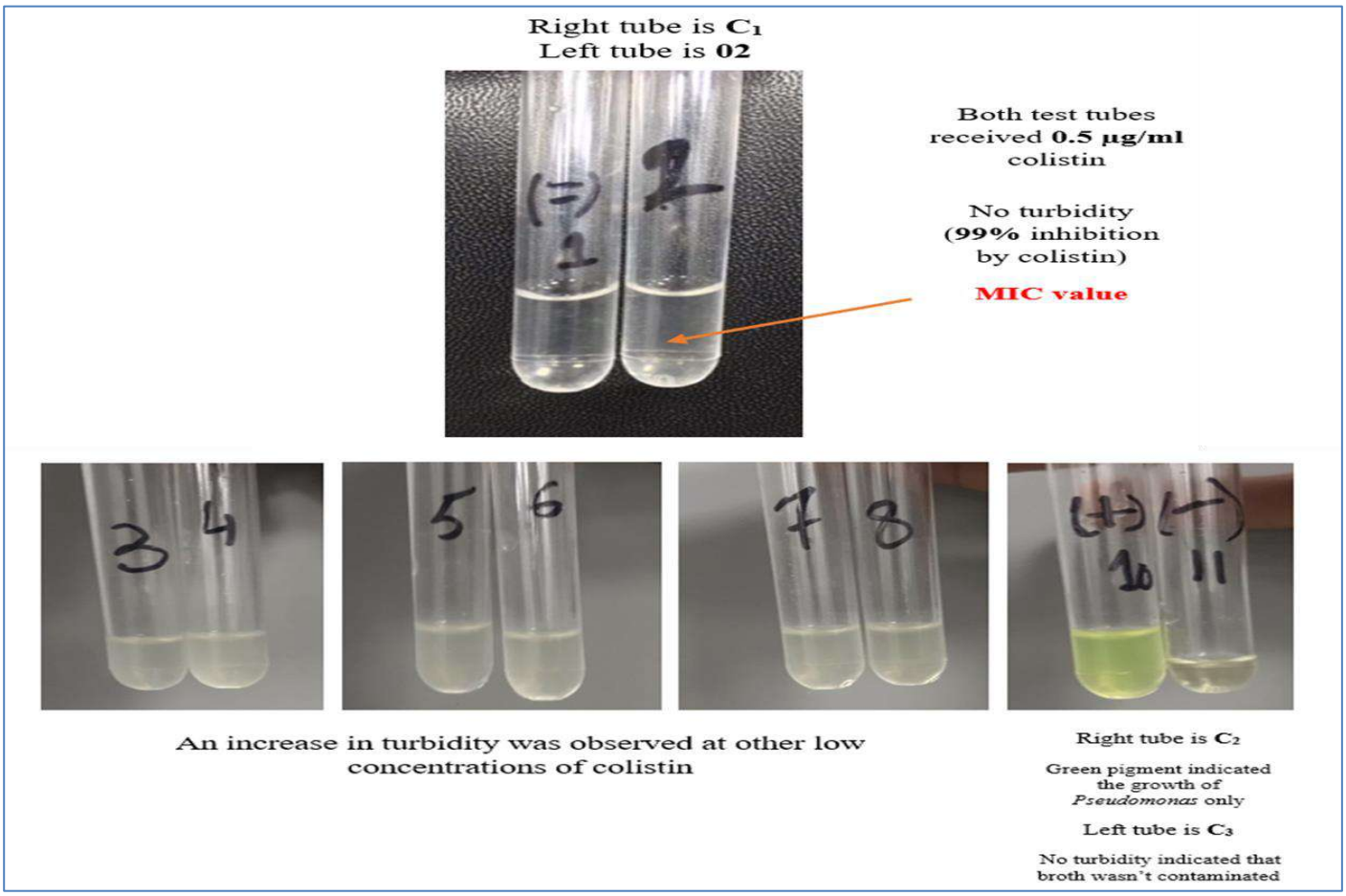

Figure-III: Results of the broth dilution method to determine the colistin MIC value against MDR *P. aeruginosa*.

DISCUSSION

A burn percentage of >20 was observed in all age groups and both genders. It was interpreted from these results that the burn percentage among study subjects didn't depend on gender or age, while it may depend on burn types and total body surface area (TBSA). Although findings on the age factor revealed significant differences in *P. aeruginosa* infections across the groups, patients between the ages of 16 and 30, 31, and 45 were more likely to become infected with *P. aeruginosa* infections.[23] In the present study, flame, scald, acid, and liquid burn types were more prevalent among study subjects, which is comparably similar to the results of a previous study.[24] Another similar study reported that the majority of burn patients belonged to the age group of 15-60 years, accounting for 55.2%. The primary factor, which accounted for 39% of the cases, was scald burn, followed by 33.6% (flame burn), 26.6% (electrical burn), and 0.8% (chemical burn).[25]

In the present study, 124 (55.3%) pus samples presented the growth of *P. aeruginosa* on the agar plates. A previous study reported the isolation of 118 *P. aeruginosa* strains from burn patients. Similar to this, another study reported the isolation of 45 *P. aeruginosa* strains from 101 burn victims.[9,26] A recent study observed a 53.3% prevalence of *P. aeruginosa* in 2nd and 3rd degree burn patients.[27] According to the results of another recent study, the most frequently isolated MDR strain from burn patients was *P. aeruginosa*, accounting for 38%.[28] The efflux pumps, modifying enzymes, horizontal gene transfer among bacterial species, and very stable biofilm formation are responsible for the multi-drug resistance in *P. aeruginosa* clinical strains.[29] In the present study, 0.35-0.5 mg/L was estimated as the MIC value against MDR *P. aeruginosa*. According to a previous study, MIC values ≤ 2 mg/L for colistin were considered significant, while MIC values ≥ 2 mg/L were considered non-significant.[30] Another previous study t reported colistin MIC values from 0.25-2 mg/L against 12 strains of *P. aeruginosa*.[31] Another relevant study reported 99% colistin sensitivity in comparison with the antibiotics tazobactam (62%), amikacin (65%),

cefepime (65%), imipenem (62.50%), and meropenem (54%), against all clinical strains of *P. aeruginosa*.[32] Lipopolysaccharides are the main target of the colistin antibiotic in Gram-negative bacteria. The positive-charge molecule colistin has great affinity for negative-charge lipids in bacteria. The lipopolysaccharide molecules in the outer membrane cause cations to be dislodged by electrostatic interactions, rupturing the membrane, releasing lipopolysaccharide molecules into the environment, and ultimately causing cell death.[33, 34]

CONCLUSION

The present study checked the colistin sensitivity patterns among MDR strains of *P. aeruginosa*. 300 burn patients were selected for this study who were admitted to the Burn Centre at Nishter Hospital Multan, Pakistan. The pus samples were collected and aseptically transferred to the laboratory. The bacterial strains isolated from samples were identified as MDR, and a very significant MIC value of colistin was calculated by using the broth dilution method against MDR strains. In conclusion, colistin can be utilized as an effective drug against *P. aeruginosa*.

CONFLICT OF INTEREST

None

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHOR CONTRIBUTION

Blossom Neelam: Conception, manuscript writing, data collection, accountable for all aspects of the work

Sumera Malik: Data collection, study design, interpretation of the work, accountable for all aspects of the work

Syed Muhammad Abbas Naqvi: Study design, proofreads, accountable for all aspects of the work

Mahnoor Haidar Khan: Critical review, revisions, accountable for all aspects of the work

Mehvish Javeed: Revisions, proofreads, accountable for all aspects of the work

REFERENCES

1. Abdel-Sayed P, Hirt-Burri N, de Buys Roessingh A, Raffoul W, Applegate LA. Evolution of biological bandages as first cover for burn patients. Adv Wound Care. 2019;8(11):555-64. DOI: https://doi.org/10.1089%2Fwound.2019.1037
2. Dolivo D, Rodrigues A, Sun L, Galiano R, Mustoe T, Hong SJ. Reduced hydration regulates pro-inflammatory cytokines via CD14 in barrier function-impaired skin. Biochim Biophys Acta Mol Basis Dis. 2022; 1868(10): 166482. DOI: https://doi.org/10.1016/j.bbadis.2022.166482
3. Zhang P, Zou B, Liou YC, Huang C. The pathogenesis and diagnosis of sepsis post burn injury. Burns Trau. 2021; 9: tkaa047. DOI: https://doi.org/10.1093/burnst/tkaa047
4. Torbey A, Shibani M, Alzabibi MA, Eddin AS, Ammar A. Epidemiology of in-hospital burn patients in a tertiary hospital in Damascus, Syria. A retrospective cohort study DOI: https://doi.org/10.1016/j.injury.2022.11.067
5. Ali MB, Ali MB. Psychological and physiological complications of post-burn patients in Pakistan: A narrative review. Sultan Qaboos Uni Med J. 2022;22(1):8-13. DOI: https://doi.org/10.18295/squmj.8.2021.118
6. Dang J, Goel P, Choi KJ, Massenzio E, Landau MJ, Pham CH, *et al*. Mucormycosis following burn injuries: A systematic review. Burns. 2023; 49(1): 15-25. DOI: https://doi.org/10.1016/j.burns.2022.05.012
7. Tiruneh CM, Belachew A, Mulatu S, Emiru TD, Tibebu NS, Abate MW, *et al*. Magnitude of mortality and its associated factors among burn victim children admitted to South Gondar zone government hospitals, Ethiopia, from 2015 to 2019. Ital J Pediatr. 2022;48(1):12. DOI: https://doi.org/10.1186/s13052-022-01204-x
8. Hoque MN, Jahan MI, Hossain MA, Sultana M. Genomic diversity and molecular epidemiology of a multidrug-resistant *Pseudomonas aeruginosa* DMC30b isolated from a hospitalized burn patient in Bangladesh. J Glob Antimicrob Resist. 2022; 31: 110-8. DOI: https://doi.org/10.1016/j.jgar.2022.08.023
9. Fang Y, Baloch Z, Zhang W, Hu Y, Zheng R, Song Y, *et al*. Emergence of carbapenem-resistant ST244, ST292, and ST2446 *Pseudomonas aeruginosa* clones in burn patients in Yunnan province. Infect Drug Resist. 2022: 1103-14. DOI: https://doi.org/10.2147%2FIDR.S353130
10. Botelho J, Grosso F, Peixe L. Antibiotic resistance in *Pseudomonas aeruginosa*–Mechanisms, epidemiology and evolution. Drug Resist Updat. 2019; 44: 100640. DOI: https://doi.org/10.1016/j.drup.2019.07.002
11. Haque M, Sartelli M, McKimm J, Bakar MA. Health care-associated infections–an overview. Infect Drug Resist. 2018: 2321-33. DOI: https://doi.org/10.2147%2FIDR.S177247
12. Xin XF, Kvitko B, He SY. Pseudomonas syringae: what it takes to be a pathogen. Nat Rev Microbiol. 2018; 16(5): 316-28. DOI: https://doi.org/10.1038%2Fnrmicro.2018.17
13. Labovská S. Pseudomonas aeruginosa as a Cause of Nosocomial Infections [Internet]. *Pseudomonas aeruginosa*-Biofilm Formation, Infections and Treatments. IntechOpen; 2021.
14. World Health Organization. Prioritization of pathogens to guide discovery, research and development of new antibiotics for drug-resistant bacterial infections, including tuberculosis. WHO. 2017.
15. Pang Z, Raudonis R, Glick BR, Lin TJ, Cheng Z. Antibiotic resistance in *Pseudomonas aeruginosa*: mechanisms and alternative therapeutic strategies. Biotechnol Adv. 2019; 37(1): 177-92. DOI: https://doi.org/10.1016/j.biotechadv.2018.11.013

16. Reynolds D, Kollef M. The epidemiology and pathogenesis and treatment of *Pseudomonas aeruginosa* infections: An update. Drugs. 2021; 81(18): 2117-31. DOI: https://doi.org/10.1007/s40265-021-01635-6
17. Heidari R, Sheikh FA, Hashemzadeh M, Farshadzadeh Z, Salmanzadeh S, Saki M. Antibiotic resistance, biofilm production ability and genetic diversity of carbapenem-resistant *Pseudomonas aeruginosa* strains isolated from nosocomial infections in southwestern Iran. Mol Biol Rep. 2022; 49(5): 3811-22. DOI: https://doi.org/10.1007/s11033-022-07225-3
18. Azam MW, Khan AU. Updates on the pathogenicity status of *Pseudomonas aeruginosa*. Drug Discov Today. 2019; 24(1): 350-9. DOI: https://doi.org/10.1016/j.drudis.2018.07.003
19. El-Sayed Ahmed MA, Zhong LL, Shen C, Yang Y, Doi Y, Tian GB. Colistin and its role in the Era of antibiotic resistance: an extended review (2000–2019). Emerg Microb Infect. 2020;9(1):868-85. DOI: https://doi.org/10.1080/22221751.2020.1754133
20. Andrade FF, Silva D, Rodrigues A, Pina-Vaz C. Colistin update on its mechanism of action and resistance, present and future challenges. Microorg. 2020;8(11):1716. DOI: https://doi.org/10.3390/microorganisms8111716
21. Aidara-Kane A, Angulo FJ, Conly JM, Minato Y, Silbergeld EK, McEwen SA, Collignon PJ. WHO Guideline Development Group Hanan Balkhy Peter Collignon John Conly Cindy Friedman Aidan Hollis Samuel Kariuki Hyo-Sun Kwak Scott McEwen Gérard Moulin Antoinette Ngandjio Bernard Rollin Flávia Rossi David Wallinga. World Health Organization (WHO) guidelines on use of medically important antimicrobials in food-producing animals. Antimicrob Resist Infect Control. 2018; 7: 1-8. DOI: https://doi.org/10.1186/s13756-017-0294-9
22. Mater ME, Yamani AE, Aljuffri AA, Binladen SA. Epidemiology of burn-related infections in the largest burn unit in Saudi Arabia. Saudi Med J. 202041(7): 726. DOI: https://doi.org/10.15537/smj.2020.7.25141
23. Al-Kabi NH, Al-Essa RA, Hussain KA. Detection of *Pseudomonas aeruginosa* with biofilm formation in burn patients: A study in Al-Hussein Teaching Hospital of Iraq. IJCBS. 2022; 21: 207-11.
24. Ziabari SM, Mobayen MR, Rimaz S, Nejat DR, Rimaz S. Evaluation of patterns, cause and risk factors of burns in patients with seizure. Int J Burns Trauma. 2022; 12(1): 23-7.
25. Mulatu D, Zewdie A, Zemede B, Terefe B, Liyew B. Outcome of burn injury and associated factor among patient visited at Addis Ababa burn, emergency and trauma hospital: a two years hospital-based cross-sectional study. BMC Emerg Med. 2022; 22(1): 199. DOI: https://doi.org/10.1186/s12873-022-00758-7
26. Elnagar RM, Elshaer M, Osama Shouman O, Sabry El-Kazzaz S. Type III secretion system (exoenzymes) as a virulence determinant in *Pseudomonas aeruginosa* isolated from burn patients in Mansoura University hospitals, Egypt. Iranian J Med Microbiol. 2022;16(6):520-7. DOI: http://dx.doi.org/10.30699/ijmm.16.6.520
27. Al-Emara AS, Jalil MB. The prevalence of *Pseudomonas aeruginosa* as a risk factor among burn patients in Basrah–Iraq. Al-Kunooze Sci J. 2022; 4(1): 34-45.
28. Ruegsegger L, Xiao J, Naziripour A, Kanumuambidi T, Brown D, Williams F, *et al*. Multidrug-resistant gram-negative bacteria in burn patients. AAC. 2022; 66(9): e00688-22. DOI: https://doi.org/10.1128/aac.00688-22
29. Contreras-Gómez MJ, Martinez JR, Rivas L, Riquelme-Neira R, Ugalde JA, Wozniak A, *et al*. Role of the multi-drug efflux systems on the baseline susceptibility to ceftazidime/avibactam and ceftolozane/tazobactam in clinical isolates of non-carbapenemase-producing carbapenem-resistant *Pseudomonas aeruginosa*. Front Pharmacol. 2022; 13: 1007162. DOI: https://doi.org/10.3389/fphar.2022.1007162
30. Javed M, Ueltzhoeffer V, Heinrich M, Siegrist HJ, Wildermuth R, Lorenz FR. Colistin susceptibility test evaluation of multiple-resistance-level *Pseudomonas aeruginosa* isolates generated in a morbidostat device. J Antimicrob Chemother. 2018; 73(12): 3368-74. DOI: https://doi.org/10.1093/jac/dky337
31. Matuschek E, Åhman J, Webster C, Kahlmeter G. Antimicrobial susceptibility testing of colistin–evaluation of seven commercial MIC products against standard broth microdilution for *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter* spp. Clin Microbiol Infect. 2018; 24(8): 865-70. DOI: https://doi.org/10.1016/j.cmi.2017.11.020
32. Katoch P, Roach V. Pseudomonas aeruginosa positivity and sensitivity in invasive bloodstream infections using automated bactec in tertiary care teaching hospital of North India. Asian J Res Infect. 2021; 7(4): 15-20. DOI: https://doi.org/10.9734/bpi/etdhr/v2/15163D
33. Yassin MT, Mostafa AA, Al-Askar AA, Al-Otibi FO. Synergistic antibacterial activity of green synthesized silver nanomaterials with colistin antibiotic against multidrug-resistant bacterial pathogens. Crystals. 2022; 12(8): 1057. DOI: https://doi.org/10.3390/cryst12081057
34. Yi K, Liu S, Liu P, Luo X, Zhao J, Yan F, *et al*. Synergistic antibacterial activity of tetrandrine combined with colistin against MCR-mediated colistin-resistant *Salmonella*. Biomed Pharmacother. 2022; 149: 112873. DOI: https://doi.org/10.1016/j.biopha.2022.112873

ORIGINAL ARTICLE

Association between demographic factors and side effects associated with COVID 19 vaccination

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ABSTRACT

Background: COVID vaccines were crucial in tackling the COVID 19 pandemic. However, they were also associated with side effects in the vaccinated population. These side effects included mild symptoms of COVID, fever, pain and swelling at the injection site. Hence, we evaluated the association between demographic factors and side effects in individuals following COVID vaccination.

Material and methods: A cross- sectional study was conducted in 93 participants for a period of 1 year, from January 2022 to January 2023. This was a retrospective study in which information regarding the side effects of two COVID vaccines was collected from the participants post-vaccination. Demographic data of vaccinated individuals and side effects associated with COVID vaccination were documented on a standardized proforma. Statistical analysis was performed to ascertain any association between the demographic variables and vaccination-associated side effects.

Results: Total 93 participants were evaluated during the study period. Our findings revealed higher frequency of vaccination-associated side effects in females and individuals above 20 years of age. The most common side effects included mild symptoms of COVID, fatigue and tiredness, fever, pain and swelling at the injection site and allergic reaction. None of these associations were found to be statistically significant.

Conclusion: Our findings suggested relatively higher frequency of vaccination-associated side effects in females and in individuals above 20 years of age which according to our inference, might be attributable to certain immune mechanisms in the affected population. In order to develop effective and safer vaccines, we recommend multi-center studies to explore the immunogenesis of such side effects.

Keywords: COVID, Demographics, Immunization, Vaccination-associated side effects

BACKGROUND

In 2020, when severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was rapidly proliferating throughout the world and was at its peak level of transmission, vaccines were most crucial in combating the COVID pandemic.[1-3] Various vaccines were developed and entered clinical trials by early 2020.[1,4,5] Owing to the situation many of these vaccines received emergency approval by FDA and governments worldwide launched vaccination campaigns to combat the spread of SARS-CoV-2.[1] There are four main categories of COVID vaccines viz. nucleic acid-based vaccines (DNA or mRNA), viral vector vaccines (replicating or non-replicating), live inactivated (or attenuated) virus vaccines, and protein-based vaccines.[6] Global vaccine safety studies have reported that recipients of the mRNA COVID vaccine typically experience local reactions more frequently than systemic ones.[1] While serious adverse effects have been rarely reported[1,7] in randomized clinical trials, most common vaccination-associated adverse effects include pain, redness and swelling at the injection site.[8,9] Systemic effects include fatigue, headache, muscle and joint pain.[8] While these adverse effects are mild and transient, studies have reported that approximately 50-90% of the participants experienced some form of adverse effect following vaccination.[8] In the Pfizer-BioNTech mRNA vaccine trial it was found that most

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This article can be cited as: Abdullah SM, Shumyle DE, Sheikh HR, Huda NU, Shakoor A, Butt H. Association between demographic factors and side effects associated with COVID 19. Infect Dis J Pak. 2024; 33(3): 140-145.

DOI: https://doi.org/10.61529/idjp.v33i3.173

Receiving date: **25 Dec 2023** Acceptance Date: **08 Aug 2024**
Revision date: **02 May 2024** Publication Date: **30 Sep 2024**

frequently observed side effects included mild to moderate fatigue and headache.[1] AstraZeneca and Sputnik vaccine trails gave the same conclusion of mild to moderate side effects.[1] Some of the more severe effects of the vaccines, such as myocarditis and pericarditis, were later observed to occur almost exclusively after immunization with mRNA vaccines.[10] These rare vaccine related conditions could only be identified after the vaccines were authorized, as their low frequency made them undetectable even in large trials[10].

Among the COVID vaccines Sinopharm and Sinovac vaccines are both developed by growing SARS-CoV-2 virus in the lab and then chemically inactivating it.[11,12] Although inactivated, the virus has the active spike protein in its surface which serves as the primary target of the immune system.[11] Introduction of the inactivated virus leads to a cascade of events that triggers humoral and cellular immunity providing long term protection from the disease.[12] Both vaccines have same mechanisms of immunogenicity but variations in their efficacy have been reported.[13] Sinovac has reported efficacy of 50-84% compared to Sinopharm (79%).[13] The commonly reported side effects of both vaccines include pain at injection site, fatigue[14] ,headache, muscle and joint pain, fever, chills, redness at injection site and nausea.[14] Sinopharm trials have shown that the most commonly observed side effect is pain at the injection site followed by fever.[1] All the effects reported are mild, self-limiting and not requiring medical attention.[1]

Some preliminary studies have suggested that vaccination-associated side effects may vary based on gender and age.[15] Available evidence indicates that the innate, humoral, and cell-mediated responses to viral vaccines can vary between females and males.[16] A study reported that pain at the injection site was experienced more in males as compared to females.[17] In addition multiple studies reported an association of age with side effects of COVID vaccines.[18] Younger participants were were found to have symptoms of flu following vaccination[18] whereas other studies reported no such association.[19]

As a result, there has been growing interest in understanding the mechanisms behind these differences.[16] Hence we evaluated the association of gender and age with the development of side effects associated with COVID vaccines viz. Sinopharm and Sinovac vaccines.

MATERIAL AND METHODS

After obtaining clearance from ethical committee of Sharif Medical Research Centre (Ref # SMDC/SMRC/270-22) a cross-sectional study was conducted from January 2022 to January 2023. Data collection was done after taking informed consent from the study participants. Sample size was calculated using an online[20] sample size calculator Scalex Sp 1.0.01.[20] Keeping the precision 5%, prevalence of COVID vaccination side effects to be 7%[21] and confidence interval 95%, the sample size was calculated to be 100. After data collection it was analyzed that individuals who had received PFIZER Biotech (2), Cansino (3) and Moderna (2) were very few in comparison to Sinopharm (47) and Sinovac (46). Therefore, only participants(n=93) administered with Sinopharm and Sinovac vaccines were included in the study as the major recipients of COVID vaccines during the study period. This study was a retrospective study in which information about the side effects of the vaccine was collected from the participants six months after they received the vaccination on a standardized proforma.

Participants who had received COVID vaccination irrespective of their age and gender were included in the study and those who refused to give consent to be included in the study, and those who had any illness and were on medication, were excluded. Data was collected using a pre-validated questionnaire with a Cronbach alpha value of 0.7.[22] Fisher exact test was used to assess any association between gender and age of the study participants with side effects due to Sinopharm and Sinovac vaccines. Chi-square test was used to ascertain any association between gender and age with pain and swelling at the injection site after sinovac injection. Statistical Package for Social Sciences 23 was used for data collection. A p value of ≤ 0.05 was taken as significant.

RESULTS

Regarding study participant, most were females (77.4%) compared to males (22.6%). More participants were above 20 years of age (78.5%) than those less than 20 years (21.5%). Sinopharm vaccine was received by 50.5% individuals while 49.5% received Sinovac.

The side effects experienced by individuals after administration of Sinopharm vaccine were not significantly associated with gender. A higher percentage of females developed symptoms of COVID,

fatigue, tiredness, fever, pain and swelling at the injection site, and allergic reaction as compared males (Table 1).

The association between age and side effects after administration of Sinopharm vaccine was not significant. A higher percentage of individuals above the age of 20 years developed symptoms of COVID, fatigue and tiredness, fever, pain and swelling at the injection site and allergic reaction as compared to those below 20 years of age (Table-II).

The association between age and side effects after administration of Sinovac was not significant. A higher percentage of females developed symptoms of COVID, fatigue and tiredness, fever, pain and swelling at the injection site and allergic reaction as compared to the males (Table-III).

The association between age and side effects after administration of Sinovac was not significant. A higher percentage of individuals above the age of 20 years developed fatigue and tiredness, fever, pain and swelling at the injection site and allergic reaction as compared to those below the age of 20 years. Symptoms of COVID infection were observed more in individuals less than 20 years of age (Table-IV).

Table-I: Association of gender with vaccination-associated side effects (Sinopharm).

Side effects after Sinopharm vaccination		Gender		Total	p value
		Male (n=10)	Female (n=37)		
COVID infection post vaccination	**Yes**	0 (0%)	3 (100%)	3 (100%)	1.00
	No	10 (23%)	34 (78%)	44 (100%)	
Tiredness and fatigue	**Yes**	3 (12%)	23 (89%)	26 (100%)	0.08
	No	7 (33%)	14 (67%)	21 (100%)	
Fever	**Yes**	0 (0%)	5 (100%)	5 (100%)	0.56
	No	10 (1%)	32 (76%)	42 (100%)	
Pain and swelling at injection site	**Yes**	5 (18%)	23 (82%)	28 (100%)	0.49
	No	5 (26%)	14 (74%)	19 (100%)	
Allergic reaction at the injection site	**Yes**	1 (33%)	2 (67%)	3 (100%)	0.52
	No	9 (21%)	35 (80%)	44 (100%)	

Table-II: Association of age with post COVID vaccine side effects (Sinopharm).

Side effects after Sinopharm vaccination		Age		Total	p value
		Less than 20 Years (n=7)	Above 20 years (n=40)		
COVID infection post vaccination	**Yes**	1 (33%)	2 (67%)	3 (100%)	0.39
	No	6 (14%)	38 (86%)	44 (100%)	
Tiredness and fatigue	**Yes**	5 (19%)	21 (81%)	26 (100%)	0.43
	No	2 (10%)	19 (91%)	21 (100%)	
Fever	**Yes**	0 (0%)	5 (100%)	5 (100%)	1.00
	No	7 (17%)	35 (83%)	42 (100%)	
Pain and swelling at injection site	**Yes**	4 (14%)	24 (86%)	28 (100%)	1.00
	No	3 (16%)	16 (84%)	19 (100%)	
Allergic reaction at the injection site	**Yes**	0 (0%)	3 (100%)	3 (100%)	1.00
	No	7 (16%)	37 (84%)	44 (100%)	

Table-III: Association of gender with post COVID vaccine side effects (Sinovac).

Side effects after Sinovac vaccination		Gender		Total	p value
		Male (n=11)	Female (n=35)		
COVID infection post vaccination	**Yes**	0 (0%)	5 (100%)	5 (100%)	0.31
	No	11 (27%)	30 (73%)	41 (100%)	
Tiredness and fatigue	**Yes**	6 (19%)	25 (81%)	31 (100%)	0.46
	No	5 (33%)	10 (67%)	15 (100%)	
Fever	**Yes**	3 (21%)	11 (79%)	14 (100%)	1.00
	No	8 (25%)	24 (75%)	32 (100%)	
Pain and swelling at injection site	**Yes**	5(23%)	17 (77%)	22 (100%)	0.85
	No	6 (25%)	18 (75%)	24 (100%)	
Allergic reaction at the injection site	**Yes**	0 (0%)	1 (100%)	1 (100%)	1.00
	No	11 (24%)	34 (76%)	45 (100%)	

Table-IV: Association of age with post COVID vaccine side effects (Sinovac).

Side effects after Sinovac vaccination		Age		Total	p value
		Less than 20 years (n=13)	Above 20 Years (n=33)		
COVID infection post vaccination	**Yes**	3 (60%)	2 (40%)	5 (100%)	0.12
	No	10 (24%)	31 (76%)	41 (100%)	
Tiredness and fatigue	**Yes**	10 (32%)	21 (68%)	31 (100%)	0.49
	No	3 (20%)	12 (80%)	15 (100%)	
Fever	**Yes**	5 (36%)	9 (64%)	14 (100%)	0.49
	No	8 (25%)	24 (75%)	32 (100%)	
Pain and swelling at injection site	**Yes**	4 (18%)	18 (82%)	22 (100%)	0.14
	No	9 (38%)	15 (63%)	24 (100%)	
Allergic reaction at the injection site	**Yes**	0 (0%)	1 (100%)	1 (100%)	1.00
	No	13 (29%)	32 (71%)	45 (100%)	

DISCUSSION

The findings of this study reveal several important insights into the side effects experienced by individuals following the administration of COVID vaccines (Sinopharm and Sinovac) in relation to their demographic data.

According to our study, relatively higher percentage of females developed symptoms of COVID, fatigue and tiredness, fever, pain and swelling at the injection site and allergic reaction as compared to males. Similar results were seen in individuals who received Sinovac. However, no significant association between gender and side effects after administration of Sinopharm and Sinovac vaccines was found in our study. These findings are in accordance with existing literature, where female participants often reported higher rates of vaccination-associated side effects compared to males[23] A potential explanation for this could be hormonal differences between the two genders.[18] The influence of estrogen in females enhances their immune response making them more prone to vaccination-associated side effects.[24] However, it is important to note that the association between gender and side effects was not statistically significant, which suggests that while females may experience side effects more frequently, the difference may not be robust across populations or settings.[24] Another study reported similar results where higher percentage of females (19%) reported reaction at the injection site as compared to the males (7.9%). In contrast to our study, this association was significant (p=.018).[25] Similarly, higher incidence of immune reaction was seen in females (20%) as compared to males (10.9%) following vaccination though the association wasn`t statistically significant (p=0.066).[25]

A relatively higher percentage of individuals in our study having administered both vaccines and above 20 years of age developed symptoms of COVID, fatigue and tiredness, fever, pain and swelling at the injection site, and allergic reaction as compared to those below 20 years of age. With regards to Sinovac vaccine, symptoms of COVID were seen more frequently in individuals less than 20 years of age.

Ganesan et al suggested that older individuals may experience more pronounced immune response which could lead to more frequent or severe side effects.[8] Although in contrast to other studies, these findings corroborate our study results.[26] Another study reported that individuals above 24 years of age experienced more reactions at the injection site (20%) as compared to those younger than 24 years (13.3%),[25] similar to our study. However, in contrast to our study, individuals below 24 years of age (19.4%) experienced more immune reactions after vaccination as compared to those above 24 years (12%).[25]

Even though extensive literature exists on the side effects of COVID vaccines, specific vaccines have not been analyzed. In this regard we have made an effort in exploring the association of side effects related to specific COVID vaccines in terms of their participant demographics.

LIMITATION

Our study had few limitations. Majority of the participants received Sinopharm and Sinovac vaccines and hence, the study was based on the side effects of these two vaccines only. Furthermore, there might be a recall bias in our study as the study participants were investigated six months post-vaccination and therefore, may not have accurately recalled the development of immediate side effects following vaccination. With regards to these

limitations, we recommend a multi-center study with larger sample size and more defined timeframes post-vaccination for accurately investigating the safety of COVID vaccines on a wider population.

CONCLUSION

The prevalence of side effects of Sinopharm and Sinovac vaccines was higher in females as compared to males and in individuals above 20 years of age which according to our inference, might be attributable to certain immune mechanisms in the affected population. In order to develop effective and safer vaccines, we recommend multicenter center studies to explore the immunogenesis of such side effects.

CONFLICT OF INTEREST

None

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHOR CONTRIBUTION

Syed Muhammad Abdullah: Literature review and data collection, final approval, accountable for all aspects of the work

Dur E Shumyle: Manuscript write-up, final approval, accountable for all aspects of the work

Hina Rafiq Sheikh: Literature review and supervision

Noor Ul Huda: Data collection, final approval, accountable for all aspects of the work

Asma Shakoor: Data Collection, final approval, accountable for all aspects of the work

Hira Butt: Data Collection, concept and design, Manuscript write-up, statistical analysis critical revision, Supervision, final approval, accountable for all aspects of the work

REFERENCES

1. Ganesan S, Al Ketbi LMB, Al Kaabi N, Al Mansoori M, Al Maskari NN, Al Shamsi MS, *et al*. Vaccine side effects following COVID-19 vaccination among the residents of the UAE-An observational study. Front Public Health. 2022; 10: 876336. DOI: https://doi.org/10.3389/fpubh.2022.876336
2. Greenhalgh T, Sivan M, Perlowski A, Nikolich JŽ. Long COVID: A clinical update. The Lancet, 2024; 404 (10453): 707-24. DOI: https://doi.org/10.1016/s0140-6736(24)01136-x
3. Padilla-Flores T, Sampieri A, Vaca L. Incidence and management of the main serious adverse events reported after COVID-19 vaccination. Pharmacol Res Perspect. 2024; 12(3): e1224. DOI: https://doi.org/10.1002/prp2.1224
4. Panagiotakopoulos L, Moulia DL, Godfrey M, Link-Gelles R, Roper L, Havers FP, et al. Use of COVID-19 Vaccines for Persons Aged ≥6 Months: Recommendations of the Advisory Committee on Immunization Practices - United States, 2024-2025. Morb Mortal Wkly Rep. 2024; 73(37): 819-24. DOI: https://doi.org/10.15585/mmwr.mm7337e2
5. Li Y, Lundin SK, Li J, Tao W, Dang Y, Chen Y, Tao C, Unpacking adverse events and associations post COVID-19 vaccination: a deep dive into vaccine adverse event reporting system data. Expert Rev Vaccines. 2024; 23(1): 53-9. DOI: https://doi.org/10.1080/14760584.2023.2292203
6. Hosseini R, Askari N. A review of neurological side effects of COVID-19 vaccination. Eur J Med Res. 2023; 28(1): 102. DOI: https://doi.org/10.1186/s40001-023-00992-0
7. Ciotti M, Ciccozzi M, Terrinoni A, Jiang WC, Wang CB, Bernardini S. The COVID-19 pandemic. Crit Rev Clin Lab Sci 2020 Aug 17;57(6):365-88. DOI: https://doi.org/10.1080/10408363.2020.1783198
8. Beatty AL, Peyser ND, Butcher XE, Cocohoba JM, Lin F, Olgin JE, *et al*. Analysis of COVID-19 vaccine type and adverse effects following vaccination. JAMA Netw Open. 2021; 4(12): e2140364. DOI: https://doi.org/10.1001/jamanetworkopen.2021.40364
9. Li Y, Li J, Dang Y, Chen Y, Tao C. Adverse events of COVID-19 vaccines in the United States: Temporal and spatial analysis. JMIR Public Health Surveill, 2024. 10(1): 51007. DOI: https://doi.org/10.2196/51007
10. Buoninfante A, Andeweg A, Genov G, Cavaleri M *et al*. Myocarditis associated with COVID-19 vaccination. NPJ Vaccines, 2024. 9(1): 122. DOI: https://doi.org/10.1038/s41541-024-00893-1
11. Mascellino MT, Di Timoteo F, De Angelis M, Oliva A. Overview of the main anti-SARS-CoV-2 vaccines: mechanism of action, efficacy and safety. Infect Drug Resist. 2021; 143: 4501-4502. DOI: https://doi.org/10.2147/idr.s315727
12. Chary M, Barbuto AF, Izadmehr S, Tarsillo M, Fleischer E, Burns MM. COVID-19 therapeutics: use, mechanism of action, and toxicity (vaccines, monoclonal antibodies, and immunotherapeutics). J Med Toxicol. 2023. 19(2): p. 205-18. DOI: https://doi.org/10.1007/s13181-023-00931-9
13. Meo SA, ElToukhy RA, Meo AS, Klonoff DC. Comparison of biological, pharmacological characteristics, indications, contraindications, efficacy, and adverse effects of inactivated whole-virus COVID-19 vaccines Sinopharm, CoronaVac, and Covaxin: an observational study. Vaccines. 2023. 11(4): 826. DOI: https://doi.org/10.3390/vaccines11040826
14. Ghasemiyeh P, Mohammadi-Samani S, Firouzabadi N, Dehshahri A, Vazin A. A focused review on technologies, mechanisms, safety, and efficacy of available COVID-19 vaccines. Int Immunopharmacol. 2021. 100: p. 108162. DOI: https://doi.org/10.1016/j.intimp.2021.108162
15. Alemayehu A, Demissie A, Yusuf M, Abdullahi Y, Abdulwehab R, Oljira L, et al. COVID-19 vaccine side effect: age and gender disparity in adverse effects following the first dose of AstraZeneca COVID-19 vaccine among the vaccinated population in Eastern Ethiopia: a community-based study. SAGE Open Med. 2022; 10: 20503121221108616. DOI: https://doi.org/10.1177/20503121221108616
16. Riad A, Sağıroğlu D, Üstün B, Pokorná A, Klugarová J, Attia S, et al., Prevalence and risk factors of CoronaVac side effects: an independent cross-sectional study among

healthcare workers in Turkey. J Clin Med. 2021. 10(12): 2629. DOI: https://doi.org/10.3390/jcm10122629
17. Mohsin S, Zahoor M, Mashhadi SF, Ali U, Yousaf U, Malaika N- e-, et al. Safety of COVID-19 Vaccines; Frequency of Side Effects and Association with Sociodemographic Characteristics among Medical Students. Pak Armed Forces Med J. 2024; 72(SUPPL-4): S688-92. DOI: https://doi.org/10.51253/pafmj.v72iSUPPL-4.9639
18. Saeed BQ, Al-Shahrabi R, Alhaj SS, Alkokhardi ZM, Adrees AO. Side effects and perceptions following Sinopharm COVID-19 vaccination. Int J Infect Dis. 2021. 111: 219-26. DOI: https://doi.org/10.1016/j.ijid.2021.08.013
19. Abbas S, Abbas B, Amir S, Wajahat M. Evaluation of adverse effects with COVID-19 vaccination in Pakistan. Pak J Med Sci. 2021; 37(7); 1959. DOI: https://doi.org/10.12669/pjms.37.7.4522
20. Daniel WW and CL. Cross, Biostatistics: A foundation for analysis in the health sciences. 2018: Wiley.
21. Qureshi A, Syed Sulaiman SA, Rehman W, Mehmood A, Idrees S, Kumar N. Prevalence of post-vaccine side effects among COVID-19 immunized community of Southern Pakistan. PlosOne, 2023. 18(5): e0285736. DOI: https://doi.org/10.1371/journal.pone.0285736
22. Hatmal MM, Al-Hatamleh MAI, 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
23. Jayadevan R, Shenoy R, Anithadevi TS. Survey of symptoms following COVID-19 vaccination in India. Medrxiv, 2021. DOI: https://doi.org/10.1101/2021.02.08.21251366
24. Klein SL, Marriott I, Fish EN. Sex-based differences in immune function and responses to vaccination. Trans R Soc Trop Med Hyg. 2015. 109(1): 9-15. DOI: https://doi.org/10.1093/trstmh/tru167
25. Wibowo J, Heriyanto RS, Wijovi F, Halim DA, Claudia C, Marcella E, *et al.* Factors associated with side effects of COVID-19 vaccine in Indonesia. Clin Exp Vaccine Res. 2022; 11(1): 89-95. DOI: https://doi.org/10.7774/cevr.2022.11.1.89
26. Riad A, Pokorná A, Klugarová J, Antalová N, Kantorová L, Koščík M, Klugar M. Side effects of mRNA-based COVID-19 vaccines among young adults (18–30 years old): An independent post-marketing study. Pharmaceuticals, 2021. 14(10): p. 1049. DOI: https://doi.org/10.3390/ph14101049

CASE REPORT

Sudden splenic rupture in a *Plasmodium vivax* infected patient: A case report

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ABSTRACT

Malaria is a significant public health concern in Pakistan, with *Plasmodium vivax* being a prevalent strain. While the disease typically follows a benign course, it can lead to severe complications including anemia, hepatic dysfunction, jaundice, acute lung injury, acute respiratory distress syndrome, pulmonary edema, shock, acute renal failure, thrombocytopenia and splenic rupture. This case report documents the presentation, diagnosis and management of a 32-year-old male patient. He was admitted with acute febrile illness and diagnosed with *P. vivax* infection. On the fifth day of illness he developed severe abdominal pain and hypotension, necessitating intensive care unit (ICU) admission. Labs revealed precipitous drop in hemoglobin and platelet count. Imaging studies revealed hemoperitoneum with splenic rupture. An emergency laparotomy was performed followed by splenectomy as a therapeutic intervention to manage the acute clinical crisis. Blood transfusions were administered during surgery and post-splenectomy vaccination was given. The patient's condition improved gradually and he was discharged from the hospital. This case report highlights the critical importance of early diagnosis, vigilant monitoring and timely intervention in managing severe complications associated with *P. vivax* infection. Prompt recognition and appropriate management are essentials to prevent morbidity and mortality in patients with severe malaria.

Keywords: *Plasmodium vivax*, Hemoperitoneum, Complication, Splenic rupture, Splenectomy

BACKGROUND

Malaria is endemic in Pakistan and after devastating floods the number of cases increased about four folds i.e. from 40,000 cases in 2021 to more than 1.6 million reported cases in 2022 with actual toll exceeding this number.[1] From January to August 2022, of 170,000 lab confirmed cases in Pakistan, 77% were due to *Plasmodium vivax* (*P. vivax*).[2]

P. vivax malaria poses the greatest global threat among human malaria species, with an estimated 2.5 billion individuals at risk of infection.[3] It exists in dormant stage in liver in form of hypnozoites and can reactivate to blood-stage infection after weeks, months or even years. Primaquine prophylaxis can eliminate this hypnozoite stage.[4]

P.vivax malaria typically follows a benign course but may cause multiple organ dysfunctions and severe life threatening issues such as severe anemia, hepatic dysfunction, jaundice, acute lung injury, acute respiratory distress syndrome, pulmonary edema, shock, acute renal failure, thrombocytopenia and splenic rupture.[5]

We present here case of a patient with *P. Vivax* malaria who developed spontaneous splenic rupture on 5th day of fever onset and was managed with prompt splenectomy and blood products replacement.

CASE REPORT

We present case of a 32-year-old sanitary worker, resident of Karachi Pakistan. He was taking over the counter medicines for fever for last five days when he got admitted to the male ward of PAF Hospital Karachi with complaint of high-grade fever associated with myalgias, headache, anorexia and nausea. Laboratory results revealed a hemoglobin level of 13.6 g/dL, total leukocyte count (TLC) of 3.4 × 10^3/µL, and a platelet count of 47,000/µL, with *P. vivax* infection confirmed on Immunochromatographic test (ICT).

On admission his condition escalated prompting transfer to the intensive care unit (ICU) due to severe abdominal pain and hypotension. The patient was kept on intravenous (IV) fluids and inotropic support. Despite the intensity of symptoms, he remained

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This case report can be cited as: Afzal H, Hussain A, Fatima A, Iqbal MM, Hameed H. Sudden splenic rupture in a *plasmodium vivax* infected patient: A case report. Infect Dis J Pak. 2024; 33(3): 146-149.
DOI: https://doi.org/10.61529/idjp.v33i3.274

Receiving date: **16 Dec 2023** Acceptance Date: **31 Jul 2024**
Revision date: **03 Jul 2024** Publication Date: **30 Sep 2024**

conscious and oriented, with a Glassgow Coma Scale (GCS) of 15/15.

The subsequent day witnessed a precipitous drop in platelets to 28,000/µL and hemoglobin to 7.7 g/dL, which further dropped to 6.1g/dL on same day. His glucose-6-phosphate dehydrogenase (G6PD) levels were normal with normal liver function tests (LFTs), lactate dehydrogenase (LDH) and urine routine. His reticulocyte count was 0.9. Peripheral film revealed trophozoites of *P. Vivax*, normocytic anemia and absence of schistocytes. All these labs were indicating anemia secondary to loss of blood instead of hemolysis. Abdominal ultrasound revealed ascites and pleural effusion, leading to urgent abdominal contrast enhanced computed tomography (CECT) (Figure-I), ultimately diagnosing hemoperitoneum with splenic rupture.

Urgent intervention took the form of an emergency laparotomy, culminating in splenectomy due to the splenic rupture (Figure-II).

Transfusions, including 4 pints of red cell concentrates (RCC), 4 bags of fresh frozen plasma (FFPs) and 1 mega platelet were administered before and during the procedure. Postsplenectomy vaccination was administered. Patient's clinical trajectory gradually improved leading to his discharge.

This case highlights the intricacies of managing sudden splenic rupture in the context of *P. vivax* infection, emphasizing the significance of meticulous monitoring, timely surgical intervention and supportive care for optimal patient outcomes.

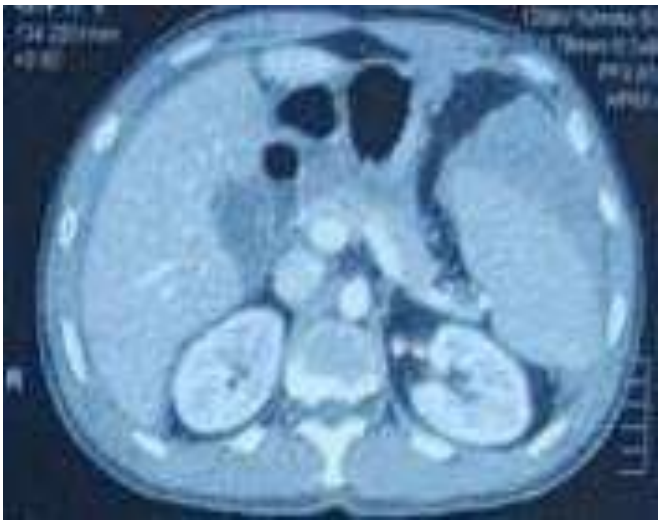

Figure-I: Large sub-capsular hematoma, superoposterior intraparenchymal hematoma (7x6 cm in size) with a parenchyml laceration indicating splenic rupture.

Figure-II: Ruptured spleen.

DISCUSSION

The presented case of a sudden splenic rupture in a *P. vivax* infected patient serves as a poignant reminder of the unpredictable and potentially life-threatening complications associated with malaria. Patient's journey from initial symptoms to a critical state necessitating emergency splenectomy highlights the need for heightened awareness and clinical vigilance in regions where *P. vivax* is endemic.

Splenomegaly is one of the commonest features of malaria, with more marked enlargement with *P. vivax* as compared to other plasmodium species.[5,6] Proportion of palpable spleens in a given population was used by World Health Organization (WHO) in 1950s to 60s, as a metric in deployment of control measures against malaria.[6]

Commonest cause of splenic rupture is trauma followed by hematological malignancies, infections, vascular, genetic and haematological disorders.[5] Malaria related splenic rupture mostly occurs in patients with low or no immunity towards disease and is more prevalent with *P. vivax* than other species.[5, 6] The rate of splenic rupture with malaria might increase with malaria elimination efforts as this complication mostly affects non immune individuals.[7]

It seems that rupture follows rapid enlargement of spleen with preceding infarction. Other reported mechanisms are compression of spleen by abdominal muscles while performing routine activities and stasis of blood in splenic sinuses by deformed erythrocytes and activated lymphatic tissue, though exact pathophysiological mechanism is still unknown.[5,6,7,8] A reported case of splenic rupture after fall from a tree with no direct abdominal trauma and demonstration of

P.vivax in thick smear after splenectomy supports the theory that minor trauma can lead to splenic rupture in malaria.[6]

A previous review of literature reveals 22 cases of spontaneous splenic rupture since 1960 while 15 of these cases were with *P.vivax.*[8] In a reported case patient was initially managed conservatively but his condition deteriorated requiring prompt decision of laparotomy.[8]

The significant drop in platelet count and haemoglobin levels necessitated prompt and aggressive management. Inotropic support and transfusions played a crucial role in stabilizing patient's condition during the perioperative period. The decision to perform an emergency laparotomy and subsequent splenectomy was driven by the urgent need to address the hemoperitoneum resulting from splenic rupture as splenic rupture is a rare but life-threatening complication with 38% mortality.[9]

Splenic rupture was confirmed with computed tomography (CT), which has at least 95% sensitivity and specificity for detecting splenic injury and splenectomy is the treatment of choice in patients with hemoperitoneum and persistent instability.[8] However, the aim of management in malarial splenic rupture should be spleen conservation as it heals in most stable cases and preserving the spleen can help avoid further severe malaria attacks.[8,10] A Brazilian case report demonstrated 2 more episodes of malaria at 2 months intervals after splenectomy in malarial splenic rupture.[5] A study analysing 55 cases of splenic rupture in malaria stated that 14 out of 55 cases were managed conservatively.[7]

The successful outcome in this case underscores the effectiveness of a multidisciplinary approach involving medical specialists, surgeons and anesthetist, as the collaboration between them plays a pivotal role in the managements of such patients.

Additionally, postsplenectomy vaccination should be administered promptly, considering long-term consequences and preventive measures for them.

This case emphasizes the need for healthcare providers to be vigilant about potential severe complications of *P. vivax* infections, even in the absence of typical markers of severity. Further research and case studies are warranted to elucidate the underlying mechanisms leading to splenic rupture in *P. vivax* infections and to refine management strategies for such rare but critical occurrences.

CONCLUSION

Although, *P. vivax* is traditionally perceived as causing milder forms of malaria, this case highlights the importance of considering atypical and severe manifestations. The scarcity of reported cases of splenic rupture associated with *P. vivax* underscores the need for further research to elucidate the underlying mechanisms and risk factors contributing to such complications.

This case provides valuable insights into the complexities of managing severe complications arising from *P. vivax* infection. It emphasizes the critical role of early recognition, vigilant monitoring, timely intervention and multidisciplinary team involvement in optimizing outcomes. This contribution to the evolving understanding of severe manifestations of *P. vivax* malaria encourages on-going research and underscores the importance of continued education for healthcare providers working in malaria-endemic regions.

CONFLICT OF INTEREST

None

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHOR CONTRIBUTION

Hania Afzal: Finding resources, data curation, writing and reviewing original draft, accountable for every aspect of the work

Muhammad Aamir Hussain: Treating physician, data curation, investigation and writing**,** accountable for every aspect of the work

Feroze Fatima: Draft proof reading, editing, accountable for every aspect of the work

Muhammad Mansoor Iqbal: Treating surgeon, data proof reading, editing, accountable for every aspect of the work

Huma Hameed: Diagnosis, data curation and investigation, accountable for every aspect of the work

REFERENCES

1. World Health Organization. "It was just the perfect storm for malaria" – Pakistan responds to surge in cases following the 2022 floods. 2023 Apr 18.
2. World Health Organization. Disease Outbreak News; Malaria - Pakistan. 2022 Oct 17.
3. Howes RE, Battle KE, Mendis KN, Smith DL, Cibulskis RE, Baird JK, et al. Global epidemiology of *plasmodium vivax*. Am J Trop Med Hyg. 2016; 95(6 Suppl): 15-34. DOI: https://doi.org/10.4269/ajtmh.16-0141

4. Mikolajczak SA, Vaughan AM, Kangwanrangsan N, Roobsoong W, Fishbaugher M, Yimamnuaychok N, et al. *Plasmodium vivax* liver stage development and hypnozoite persistence in human liver-chimeric mice. Cell Host Microbe. 2015; 17(4): 526-35. DOI: https://doi.org/10.1016/j.chom.2015.02.011
5. Elizalde-Torrent A, Val F, Azevedo ICC. et al. Sudden spleen rupture in a *Plasmodium vivax*-infected patient undergoing malaria treatment. Malar J. 2018; 17(1): 79. DOI: https://doi.org/10.1186/s12936-018-2228-2
6. Siqueira AM, Magalhães BML, Melo GC, Ferrer M, Castillo P, Martin-Jaular L, *et al*. Spleen rupture in a case of untreated *Plasmodium vivax* infection. PLoS Negl Trop Dis. 2012; 6(12): e1934. DOI: https://doi.org/10.1371/journal.pntd.0001934
7. Imbert P, Rapp C, Buffet PA. Pathological rupture of the spleen in malaria: Analysis of 55 cases (1958-2008). Travel Med Infect Dis. 2009; 7(3): 147-59. DOI: https://doi.org/10.1016/j.tmaid.2009.01.002
8. Hussein BMA, Ani AMA, Al-Mayoofi O, Mehraj M, Joher AA, Bonilla JA, et al. Spontaneous rupture of splenic hematoma in a malaria patient: Case report and review of literature. Int J Surg Case Rep. 2016; 29: 241-4. DOI: https://doi.org/10.1016/j.ijscr.2016.10.017
9. Alansari S, Abdulghani M, Zakaullah M, Vadlamudi R, *et al*. A rare twist: Spleen rupture in *plasmodium vivax* malaria. Cureus. 2023; 15(11): e48546. DOI: https://doi.org/10.7759%2Fcureus.48546
10. Hamel CT, Blum J, Harder F, Kocher T. Nonoperative treatment of splenic rupture in malaria tropica: Review of literature and case report. Acta Trop. 2002; 82(1): 1-5. DOI: https://doi.org/10.1016/s0001-706x(02)00025-6

The urgent need for enhanced surveillance and research on avian influenza possible pandemic

Dear Editor,

Zoonotic influenza usually results from sporadic transmission from poultry to humans, but such infections are usually self-limiting. However, some strains of avian influenza viruses (AIVs), such as avian H5 and H7 viruses, are known to cause hundreds of thousands of infections with very high morbidity (30% to 50%). Therefore, some of these highly pathogenic avian influenza (HPAI) viruses have the capacity to be reproduced in humans, mediating beneficial human-to-human transmission and possible transmission, and thus pose a significant public health risk.[1] A few years after an outbreak of human HPAI H5N1 virus was reported in Hong Kong in 1997, the virus re-emerged in Asia, causing numerous deaths in poultry as well as making it difficult for humans to breathe. It is noteworthy that the Asian H5N1 influenza virus also infects and kills non-human primates and birds. These animals include large cats (tugs), domestic cats, and other felines. Thailand has reported several fatal infections caused by Asian H5N1 influenza virus in large cats (lions and leopards) as well as domestic dogs and cats.[2]

Sporadic cases of HPAI, A(H5N1) virus infections in humans, exhibiting varying levels of clinical severity and a cumulative case fatality rate exceeding 50%, have been documented in 23 countries over a span of more than 20 years. The HPAI A(H5N1) clade 2.3.4.4b viruses have undergone widespread dissemination among wild bird populations globally since around 2020–2021, resulting in outbreaks among poultry and other animal species. Recently, instances of HPAI A(H5N1) clade 2.3.4.4b viruses have been detected in dairy cows and unpasteurized milk samples across multiple U.S. states.[3] In February 2024, veterinarians were alerted to an outbreak in lactating cows in the North Texas Panhandle. Affected animals develop non-specific diseases, including reduced food intake, reduced rumination and reduced milk production. Milk from affected cows often has a thick, creamy yellow appearance, like colostrum. Milk cultures are usually negative, blood tests show mild values for aspartate aminotransferase, gamma-glutamyl transferase, creatine kinase, and bilirubin, and a complete blood count. It shows anemia and leukopenia levels.[4]

AIV surveillance has improved significantly since HPAIV H5N1 infected humans. AIVs that circulate and thrive in poultry may have the best potential for direct transmission from the chicken-human interface to the same individuals. The H3N8 G25 virus has increased binding to human receptors but low herd immunity, raising concerns about the potential for a pandemic. Dual receptor binding features and mutations associated with increased virulence and disease in animals have also been identified in many H3 AIVs. Surveillance and research on H3 avian influenza, including the use of drugs and vaccines, should be strengthened to prepare for the pandemic.[5]

Challenges such as low antibody response to AIV and diversity of AIV antigens can be overcome by developing new vaccines with broad-spectrum antibodies against AIV subtypes. The development of new vaccines is one of the most important steps in preventing such events. Antibodies produced after vaccination can be evaluated by obtaining human serum from clinical studies and testing them against selected pathogens. Since ferrets are considered the "gold standard" animal model of influenza, there is an urgent need for the development of vaccines and new tools to assess ferret immunology. The development of antibodies that are broadly cross-reactive against all IAVs of a subtype or UIV that protect against all IAV subtypes is both the intermediate and ultimate goal of vaccine research and success. Because the transmission and spread of the highly contagious influenza virus in farm animals such as cattle is not understood, further research and research into H5N1 infection in farm animals such as cattle is needed to fully understand this. their disease. We must rethink the relationship between humans, domestic animals, and wildlife to prevent the emergence of dangerous diseases that impact biodiversity and human health. The government should be responsible for protecting biodiversity and human health from diseases resulting from human activities, particularly those initiated by intensive production, such as H5N1 avian influenza. If we hope to preserve biodiversity and protect human health, we must change the way we produce food and the way we

interact with and impact wildlife.

REFERENCES

1. Tang CY, Ramesh A, Wan XF. Avian and swine influenza viruses. In Molecular Medical Microbiology. Academic Press. 2024; 2375-2411.
2. Ly H. Highly pathogenic avian influenza H5N1 virus infection of companion animals. Virulence. 2024; 15(1): 2289780. DOI: https://doi.org/10.1080/21505594.2023.2289780
3. Uyeki TM, Milton S, Hamid AC, Webb RC, Presley S, Shetty V, et al. Highly Pathogenic Avian Influenza A(H5N1) Virus Infection in a Dairy Farm Worker. N Eng J Med. 2024; 390(21): 2028-9. DOI: https://doi.org/10.1056/NEJMc2405371
4. Burrough ER, Magstadt DR, Petersen B, Timmermans SJ, Gauger PC, Zhang J, et al Highly pathogenic avian influenza A(H5N1) clade 2.3.4.4b virus infection in domestic dairy cattle and cats, United States, 2024. Emerg Infect Dis. 2024; 30(7): 10.3201/eid3007.240508. DOI: https://doi.org/10.3201/eid3007.240508
5. Yang J, Zhang Y, Yang L, Li X, Bo H, Liu, J, et al. Evolution of avian influenza virus (H3) with spillover into humans, China. Emerg Infect Dise. 2023; 29(6): 1191–201. DOI: https://doi.org/10.3201/eid2906.221786

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Receiving date: **30 May 2024** Acceptance Date: **18 Aug 2024**
Revision date: **23 Jul 2024** Publication Date: **30 Sep 2024**