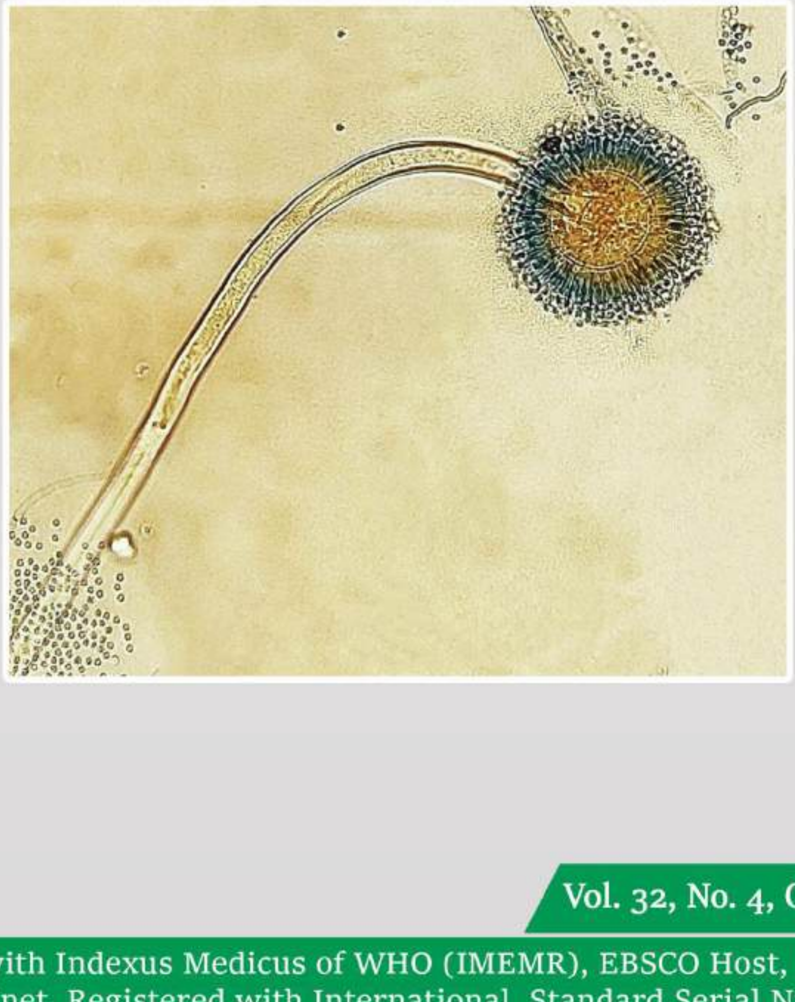
ISSN 1027-0299

INFECTIOUS DISEASES JOURNAL OF PAKISTAN | IDJP

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



Quarterly

Vol. 32, No. 4, Oct - Dec 2023

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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REVIEW ARTICLE

THE ALARMING RESURGENCE OF DIPHTHERIA IN PAKISTAN: A WAKE-UP CALL

Samreen Kulsoom Zaidi[1], Fizza Farooqui[2]

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Post COVID era, Pakistan has witnessed a concerning resurgence of diphtheria[1], a potentially deadly but vaccine preventable disease.[2] Global public health efforts has once succeeded in nearly eliminating this disease, however this re-emergence emphasizes upon the importance of continuous commitment to vaccination efforts and stringent public health policies. The resurgence of diphtheria in Pakistan demands urgent attention from policymakers, healthcare professionals, and the public at large.

Recent data indicates a distressing increase in diphtheria cases across the country.[1,2] Factors contributing to this resurgence may include lapses in vaccination coverage specially during the COVID pandemic, weakened healthcare infrastructure, and challenges in reaching remote or underserved populations. Additionally unvaccinated immigrant influx has contributed to sporadic outbreaks in the country.

As per unofficial reports, more than hundred clinical cases of diphtheria have been treated at hospitals during the last year.[3] There may be multiple contributing factors to this rise of diphtheria cases. As seen in other vaccine preventable disease resurgences, one of the primary factors attributed to the resurgence of diphtheria may be a decline in vaccination coverage. In the population currently affected, this decline may be due to a combination of factors, including vaccine hesitancy, limited access to healthcare facilities, misinformation and difficulty in vaccine availability during COVID-19 era. A very active awareness campaign to educate communities about the importance of vaccination and improve accessibility to healthcare services must be prioritized. To encourage vaccination, public must be made cognizant of the grave consequences associated with diphtheria complications.[4]

A persistent factor for all communicable diseases in resources limited countries such as Pakistan is weakened healthcare infrastructure, particularly in rural and underserved areas. Due to an inadequate health budget, the country faces challenges like insufficient resources, shortage of healthcare workers, and inadequate facilities hinder the delivery of essential health services, including immunization. Strengthening healthcare infrastructure, particularly in remote regions, is critical to effectively combat diphtheria.[5]

A significant barrier to effective vaccination efforts is the hindrance in reaching remote areas either due to transportation or due to aggressive resistance from locals.[6] Secure mobile clinics, use of innovative approaches like telemarketing and social media can play a vital role in achieving our goal.[7]

Encouragement can be provided to the general public with a concerted effort to combat misinformation and build their trust in vaccination. Using religious and other famous public figures to aid our cause by educating communities about the safety and efficacy of vaccines is essential in dispelling myths and misconceptions. Public awareness campaigns, led by healthcare professionals and supported by community leaders, can serve as powerful tools in this endeavor.[8]

The resurgence of diphtheria in Pakistan is an alarming situation and needs to be addressed actively. This requires a committed multi-faceted approach, including improving vaccination coverage, strengthening healthcare infrastructure, and enhancing public awareness. Swift and sustained action from policymakers, healthcare professionals, and communities is essential to combat this concerning trend and protect the health and well-being of the nation.

This resurgence is a reminder that constant vigilance even after elimination of outbreaks is the key to keep vaccine preventable diseases at bay.

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ORIGINAL ARTICLE

AZITHROMYCIN SUSCEPTIBILITY TESTING FOR *SALMONELLA ENTERICA* ISOLATES: COMPARING DISK DIFFUSION RESULTS WITH MIC GRADIENT STRIPS

Aqib Sultan, Nasrullah Malik, Summiya Nizamuddin, Nida Safdar

Shaukat Khanum Memorial Cancer Hospital and Research Centre, Lahore Pakistan

ABSTRACT

Background: Enteric fever remains an imperative public health problem in developing countries. After the emergence of cephalosporin resistance in *Salmonella enterica* subsp. enterica serovar Typhi, azithromycin is increasingly being used for oral treatment of enteric fever. Reports of sporadic azithromycin resistance have been reported across the country, additionally, misuse of azithromycin during the COVID-19 pandemic has concerns regarding emerging azithromycin resistance. This study evaluated the reliability of the disc diffusion method as a screening test for detecting azithromycin resistance by comparing it with the minimum inhibitory concentrations (MIC) gradient strip results, in 231 typhoidal salmonellae.

Material and Methods: This prospective study was conducted in the Section of microbiology of the Shaukat Khanum Memorial Cancer Hospital and Research Centre, Lahore from March 2022 to March 2023. Isolates recovered from blood cultures of patients, suffering from enteric fever were selected. Azithromycin susceptibility testing was performed both by disk diffusion and as well as gradient strips and their results were compared.

Results: Among typhoidal salmonellae, a significant portion consisted of extensively drug resistant *Salmonella* Typhi (61.9%). Only one XDR *S*. Typhi was found to be resistant to azithromycin both by disk diffusion method and MIC gradient strip method, with a MIC value of 64µg/ml. The study found no discrepancy between the disk diffusion and gradient strip methods.

Conclusion: The current study found no discordance between disk diffusion and gradient strip test methods for evaluating azithromycin susceptibility among typhoidal salmonellae.

Keywords: Azithromycin, disk diffusion testing, enteric fever, Minimum inhibitory concentration, *Salmonella* Typhi

BACKGROUND

Typhoid fever is the leading cause of morbidity and mortality worldwide. It is a potentially lethal bacterial infection caused by *Salmonella enterica* serotype Typhi, if not promptly treated and managed.[1]

According to recent WHO figures, typhoid fever causes upto up to 21 million illnesses and 161,000 fatalities annually.[2] Since typhoid fever is usually acquired by consuming food or water contaminated with *Salmonella* Typhi, nations with inadequate infrastructure and sanitation facilities are more likely to experience a high incidence of the illness. Typhoid fever is characterized by persistently high temperature, headache, nausea, abdominal pain, and diarrhea or constipation.[3] *Salmonella* Paratyphi A, B, or C is the cause of comparable frequently less severe paratyphoid fever.[4]

Due to the development of antimicrobial resistance among typhoidal salmonellae, typhoid fever has become difficult to treat over the years. Resistance to first and second line antibiotics i.e. chloramphenicol, ampicillin, cotrimoxazole and fluoroquinolones had led to the rise in multidrug resistant (MDR) strains leaving behind ceftriaxone as the treatment option.[5] However, with the advent of extremely drug-resistant (XDR) strains, this treatment option has also been rendered ineffective and azithromycin and meropenem are left as antibiotics of last resort. Azithromycin has gained significant prominence in recent years as the sole oral antibiotic available for the treatment XDR *S.* typhi.[6]

Unfortunately, we now face the emergence of azithromycin resistance from various parts of the world including Pakistan, India, Nepal and Bangladesh.[7]

Additionally, the blatant misuse and overuse of azithromycin during the COVID-19 pandemic have only made matters worse and led to heightened concerns about the increasing resistance to the said antibiotic.[8]

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This article can be cited as: Sultan A, Malik N, Nizamuddin S, Safdar N. Azithromycin susceptibility testing for Salmonella enterica isolates: comparing disk diffusion results with mic gradient strips. Infect Dis J Pak. 2023; 32(4): 121-125. DOI: 10.61529/idjp.v32i4.242

Receiving date: **04 Sep 2023** Acceptance Date: **05 Dec 2023**
Revision date: **27 Nov 2023** Publication Date: **29 Dec 2023**

Azithromycin susceptibility testing guidelines for *S*. Typhi were published in 2015 by the Clinical and Laboratory Standards Institute (CLSI). Though, there are still no guidelines available for the same for ***S***. Paratyphi.[9]

CLSI M100 33rd edition (2023) provides both zone diameters as well as MIC breakpoints for azithromycin susceptibility testing against *Salmonella* Typhi. Either of the methodologies can be opted for by laboratories, depending on their feasibility. However, several studies in the past found discordance between the two methodologies when tested against typhoidal salmonellae. These studies concluded that the disk diffusion susceptibility testing method can misinterpret azithromycin sensitive strains as resistant and therefore, is not an accurate method for checking the susceptibility of azithromycin against *Salmonella* isolates.[10]

Hence, our objective was to assess the reliability of disc diffusion test for the detection of azithromycin resistance by comparing it with the MIC gradient strips, in randomly selected typhoidal salmonellae recovered from blood cultures received in our laboratory.

MATERIAL AND METHODS

From March 2022 to March 2023, a prospective study was carried out in the microbiology section of Shaukat Khanum Memorial Cancer Hospital and Research Centre (SKMCH&RC), Lahore after the approval of Institutional Review Board Committee. A waiver for informed consent was also sought and approved.

Using the laboratory network of SKMCH&RC, all blood culture samples from patients suspected of having typhoid fever were collected and incubated in an automated blood culture system BACT/ALERT VIRTUO (BioMérieux, France). Positively flagged bottles were subcultured onto chocolate, blood, and MacConkey agar plates. Duplicate isolates were excluded.

Using API 20E (*bioMérieux*), colonies suggestive of Salmonellae were identified using biochemical tests, serotyping and colony morphology. *Salmonella* Typhi and *Salmonella* Paratyphi A isolates were selected from these blood cultures by random consecutive sampling. Interpretive breakpoints for *Salmonella* Typhi from CLSI M100 33rd edition (2023) were extrapolated and applied to Salmonella Paratyphi A.

The Kirby–Bauer disc diffusion method on Muller–Hinton agar was used to test 231 *Salmonella* isolates for antibiotic susceptibility in compliance with Clinical Laboratory Standards Institute (CLSI) M100, 33rd edition (2023).[11] A 90 mm Mueller Hinton agar plate containing a 15 µg disk was inoculated with 0.5 McFarland suspension of Salmonellae isolates, and the plates were then left to incubate overnight at 37°C. Zones of inhibitions were measured after 18-24 hours of incubation. A zone of inhibitions of ≥ 13 mm was considered as sensitive and ≤ 12 mm was considered as resistant.[11]

Using E-test (BioMérieux), the minimum inhibitory concentrations of azithromycin were found. Mueller Hinton agar plates were inoculated with 0.5 McFarland bacterial suspensions were incubated overnight at 37 °C after placing azithromycin E-strips on their surfaces. MICs were measured after 18-24 hours of incubation. According to interpretive breakpoints provided by the CLSI, isolates having MICs ≤ 16 µg/ml were considered susceptible while isolates having MICs ≥32 µg/ml were considered resistant (11). All results were reassessed by a second reader to remove any bias.

The Statistical Package for Social Sciences (SPSS) version 24.0 (IBM, Armonk, NY) was used to compile and analyze all the clinical ad microbiological data. The frequencies and percentages used to represent the descriptive data were displayed. Frequencies of azithromycin MICs and distribution of XDR, MDR and multi-drug susceptible strains among *Salmonella* isolates were represented by using percentages and graphs and p-value of less than 0.05 was deemed statistically significant.

RESULTS

A total of 231 typhoidal salmonellae recovered from blood cultures were tested. Out of these, 184 isolates (80%) were *Salmonella* Typhi and 47 isolates (20%) were *Salmonella* Paratyphi A. Among the 184 Salmonella Typhi isolates; 143 (78 %), 9 (5 %) and 32 (17%) were XDR, MDR and multi-drug susceptible strains, respectively (Figure-1). Whereas, all *Salmonella* Paratyphi A isolates were multi-drug susceptible. The significant portion of *Salmonella* isolates were found to be XDR *Salmonella* Typhi (62%).

Analysis of demographic data showed that of the isolates, 94 (40.7%) came from female patients and 137 (59.3%) from male patients with mean age of 16.53±11.8 years. The strains originated from various parts of the country, with 48.5% (112) coming from

Khyber Pakhtunkhwa, followed by Punjab with 38% (88), Sindh 2.5% (6), FATA 5.7% (13), Balochistan 4.9% (11) and the Federal Capital 0.4% (1).

Currently, CLSI M100, 33rd edition (2023) provides the interpretive criteria for azithromycin susceptibility testing by disk diffusion method as well as MIC testing for *Salmonella* Typhi only, the same criteria were extrapolated and applied for *Salmonella* Paratyphi A isolates. Among all *Salmonella* isolates tested, only one XDR *Salmonella* Typhi strain (0.43%) was found to be resistant to azithromycin both by disk diffusion method (no zone of inhibition) as well as the gradient strip method (MIC= 64µg/ml). The strain was recovered from the blood culture of a patient belonging to Peshawar, Khyber Pakhtunkhwa. The azithromycin resistant strain was sent for further analysis to the UK Health Security Agency reference laboratory and was found to be positive for the *mph*A gene.

The lowest and the highest MICs of azithromycin were found to be 0.75 µg/ml (***S***. Paratyphi A) and 64 µg/ml (XDR *S*. Typhi), respectively (Figure-2). The mean MIC of *Salmonella* isolates by gradient strip was found to be 2.84 ± 4.46 µg/ml whereas, the mean zone of inhibition by disk diffusion testing was 22.17 ± 2.64 mm. An inverse correlation between zone of inhibitions and MICs was found among *Salmonella* isolates with a statistically significant p-value of 0.01.

MIC50 and MIC90 were also calculated and found to be 2 µg/ml and 4 µg/ml, respectively.

The study found no discrepancy between the disk diffusion and gradient strip methods. There was no discordance in azithromycin susceptibility results obtained by both methods.

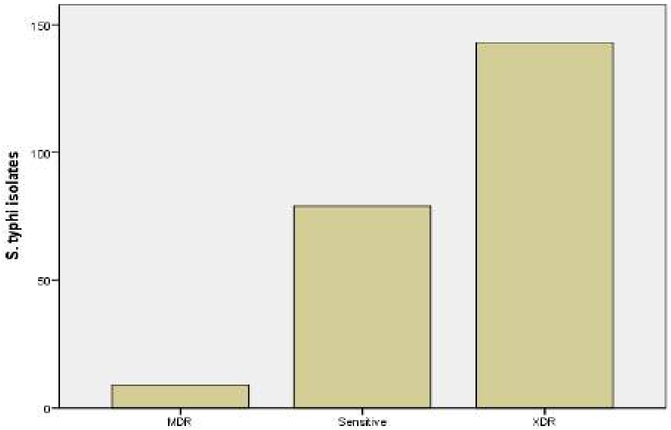

Figure-1: Distribution of XDR, MDR and multi-drug susceptible strains among *S*. Typhi isolates.

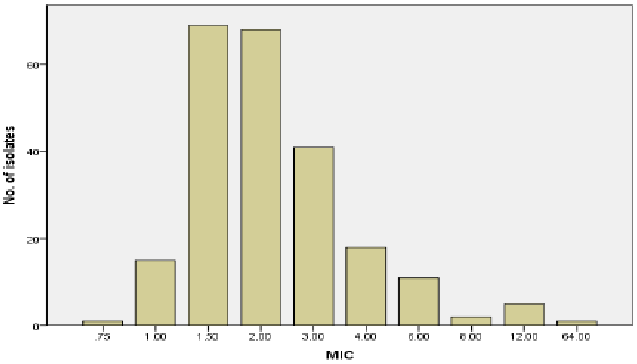

Figure-2: Distribution of minimum inhibitory concentrations (µg/ml) among *Salmonella* isolates.

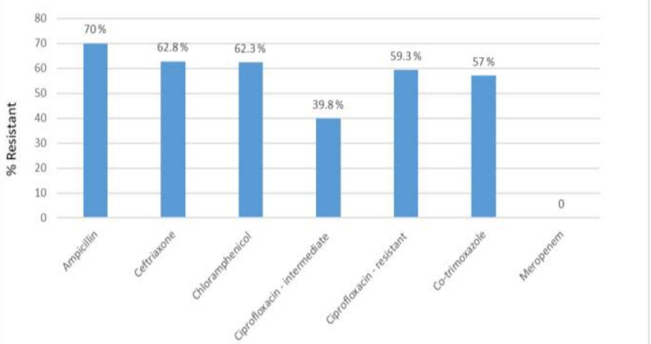

Figure-3: Antibiogram of typhoidal salmonellae.

DISCUSSION

The emergence of XDR ***S***. Typhi has posed a significant burden on the healthcare settings of low-middle income countries including Pakistan and has rendered typhoid fever difficult to treat. Consequently, carbapenems and azithromycin are left as antibiotics of last resort to treat XDR *S*. Typhi. Azithromycin being the only oral option available has gained significant importance during the last few years in the management of enteric fever caused by extensively drug resistant *S*. Typhi.

CLSI recommends either disk diffusion method or gradient strip method to evaluate azithromycin susceptibility in *Salmonella* isolates, however, data on azithromycin MICs among typhoidal salmonellae is limited from Pakistan.

According to the current study, a significant portion of Salmonella isolates were found to be XDR *Salmonella* Typhi (62%). Previously published data from our center reported a prevalence of 50% of XDR *S*. Typhi from all positive blood cultures.[12] Hence, reaffirming the predominance of XDR *S*. Typhi strains in our sample group.

The current study found only one azithromycin resistant *S*. Typhi isolate (0.43%) which indicate that the rate of azithromycin resistant Salmonellae is still low in the region. This is the first azithromycin resistant strain encountered at our centre.

The isolate was also found to have the highest azithromycin MIC value (64 µg/ml) among all *Salmonella* isolates. A recent retrospective cross-sectional multi-centric study conducted on 150 XDR *Salmonella* Typhi isolates at University of Health Sciences, Lahore from January, 2012 to June, 2021 found 3 (2%) azithromycin resistant isolates with MICs of 32 µg/ml by E-test method. A comparative study conducted on 60 *Salmonella* isolates in the Microbiology department of Pakistan Navy Ship Shifa Hospital Karachi (PNS Shifa) from June, 2020 to December 2020, found one azithromycin resistant isolate with a high MIC of 96 µg/ml checked by E-strip method and a zone of inhibition of 9 mm on disk diffusion method.[10] Another cross-sectional observational study conducted in the medicine department of Combined Military Hospital (CMH) Lahore, from April 2019 to October 2019 on 52 *Salmonella* isolates, also found 1 (1.9%) azithromycin resistant *S*. Typhi isolate with a high MIC of 64 µg/ml.[13]

A study conducted at a tertiary care hospital in Southern India from January, 2013 to December, 2017 on 100 *Salmonella enterica* strains found 6 (6%) azithromycin resistant strains with MICs ≥ 32 µg/ml.[14] Another cross-sectional study performed on 66 *S*. Typhi isolates in Northern India found 7 (10.6%) azithromycin resistant isolates by E-test method.[7] Similarly, azithromycin resistant *S*. Typhi isolates have also been reported in other parts of the world including Nepal and Bangladesh.[15]

The current study found a low resistance rate (0.432%) of azithromycin against typhoidal salmonellae. However, ongoing vigilance and surveillance is required for evaluating its resistance to minimize the public health risk. Increasing azithromycin resistance among typhoidal salmonellae in the recent past is attributable to COVID-19 pandemic. During 2020-2022, Pakistan faced dual epidemic of COVID-19 and typhoid. Azithromycin was misused during COVID-19 pandemic due to its broad-spectrum respiratory coverage. Unjudicial empirical use of azithromycin and non-adherence to antimicrobial stewardship practices may also have led to the emergence of azithromycin resistance among typhoidal salmonellae.[16]

The azithromycin resistant *Salmonella* Typhi strain in the current study was found positive for the *mph*A gene. It is usually located on plasmids and is the main gene involved in macrolide among *Salmonella spp.* Macrolide-2'-phosphotransferase encoded by *mphA* gene confers azithromycin resistance in *Salmonella* isolates.[17] This gene is associated with high level azithromycin resistance among *Salmonella spp*. A previous study found a single high-level azithromycin resistant *Salmonella* strain (MIC: 64µg/ml) harbouring *mph*A gene.[14] According to another study conducted in United Kingdom 12 out of 15 azithromycin resistant non-typhoidal *Salmonella* strains encoded *mph*A gene.[18] However, literature on genetic analysis of azithromycin resistant salmonellae is scarce from Pakistan. Emergence of *mph*A mediated azithromycin resistance among *Salmonella spp.* and its ability to transmit horizontally is worrisome and renders the use of azithromycin for treating typhoid fever at risk.[17]

The current study found no discordance in azithromycin susceptibility results obtained by disk diffusion and E-test methods. However, few studies in the past showed discrepancies between disk diffusion and E-test methods. In 2020, a study conducted in Karachi found discordance in 10 (16.6%) out of 60 *Salmonella* isolates.[10] Another study conducted in India found discordance in 12 (12%) out of 100 isolates.[14] Similarly, in 2020 a study conducted in Karachi found discordance in 5 (0.23%) out of 2104 ***S***. Typhi isolates.[19] In 2021, a study conducted in Lahore also found discrepancy in 7 out of 150 (4.6%) isolates between disk diffusion and E-test methods.[20] These studies concluded that disk diffusion method provide false resistant results and does not provide accurate results for azithromycin susceptibility testing particularly in XDR ***S***. Typhi.

According to a study conducted in London between May, 2011 and April, 2019 concluded that E-strips for azithromycin MICs are difficult to interpret due to possible reader bias errors. So, prior training and a second reader system should be ensured at the institute level to mitigate this problem.[21]

As the current study showed no discrepancy between disk diffusion and E-test methods, hence, we can consider disk diffusion a reliable method for evaluating azithromycin susceptibility among *Salmonella* isolates as it is cost-effective and easy to interpret as compared to E-test method. Nonetheless, the present investigation has various constraints. The study is a single-centered. Additionally, as part of this investigation, we did not tack the clinical response of patients using azithromycin.

CONCLUSION

The current study concluded no discordance between disk diffusion and E-test methods for azithromycin susceptibility among *Salmonella* isolates. Hence, disk diffusion test can be considered a reliable method and can be used to evaluate azithromycin susceptibility among *Salmonella* isolates.

CONFLICT OF INTEREST

None

AUTHOR CONTRIBUTION

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

Nasrullah Malik, Summiya Nizamuddin: Final approval of the version to be published

Nida Safdar: Acquisition, analysis of data, second reader

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ORIGINAL ARTICLE

FREQUENCY OF CRYPTOCOCCAL ANTIGENEMIA IN HIV TREATMENT NAIVE PATIENTS WITH CD4 COUNT BELOW 200/ML

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ABSTRACT

Background: Cryptococcosis is a dangerous opportunistic fungal infection that poses a significant threat to individuals with advanced HIV/AIDS and compromised cellular immunity. The objective was to determine the frequency of asymptomatic cryptococcal antigenemia in treatment naïve HIV patients.

Material and Methods: This cross-sectional study was conducted at The Indus Hospital and Health Network, Karachi from July to December 2022. We included all consecutive HIV patients of age > 18 years, either gender, who were treatment naïve, with CD4 counts ≤200/μL, and did not have any symptoms of cryptococcal infection. Cryptococcal antigen (CrAg) was tested in blood based on the Direct Agglutination test.

Results: A total of 100 HIV patients were enrolled. The mean age was 35.12 ± 10.93 years. The majority (78%) of the subjects were males, and 2/3rd were sexually active; exposure to birds was identified in 31% of patients. Most of the patients enrolled belonged to WHO HIV stage 1 (61%), followed by stage 4 (30%). The median CD4 count was 103.5 (IQR: 56.5 – 126.5) /μL. 48 patients had CD4 count between 101 to 200/μL, while 51 patients had CD4 count ≤100/μL. Only 2 patients were found to have CrAg positive making an overall prevalence of 2%. Both patients had a CD4 count ≤ 100/μL (n = 2/51, 3.9%). 30% of patients had other opportunistic co-infections (n = 30).

Conclusion: The prevalence of asymptomatic cryptococcosis in the treatment naïve HIV is low in the Pakistani population. A routine CrAg screening can be individualized and avoided in selected patients with CD4 count >100/μL in a resource-limited country like Pakistan.

Keywords: AIDS, HIV stages, Asymptomatic, Opportunistic infection.

BACKGROUND

Cryptococcosis is a severe systemic disease that poses a life-threatening risk to infected individuals. The disease is caused by a group of encapsulated yeast known as Cryptococcus species.[1,2] Cryptococcus is frequently found in the environment, particularly in association with pigeon excreta, soil, and other avian waste products. The pathogen is present worldwide,[3] and is frequently inhaled by individuals. In immunocompetent individuals, cryptococcus generally does not give rise to severe illness. However, in immunocompromised individuals, this fungus can cause a serious opportunistic infection.[4,5] The inhalation of spores from yeast primarily leads to pulmonary infection presenting as symptoms of pneumonia. In individuals who are immunosuppressed, the disease can spread to various other regions of the body, notably the meninges, potentially leading to the development of life-threatening cryptococcal meningitis (CM).[6]

A significant proportion of the population infected with Human Immunodeficiency Virus (HIV) continues to seek healthcare services at a late stage, resulting in acquired immune deficiency syndrome (AIDS).[7,8] This fungal infection affects a considerable proportion of AIDS patients, estimated to be between 60% and 70%.[9,10] Individuals infected with HIV/AIDS who exhibit CD4+ T cell counts below 100 cells/μL are particularly susceptible to Cryptococcosis.[11] Cryptococcal disease is a leading cause of mortality amongst individuals afflicted with HIV in developing regions.[12-15] The incidence of morbidity and mortality resulting from CM exhibits regional variability that is primarily dictated by the prevalence of AIDS.[16] Recent estimates suggest that CM contributes to approximately 15-20% of AIDS-related fatalities worldwide, with the majority of these cases concentrated in the sub-Saharan African region.[8, 13, 15, 17, 18].

Early screening and treatment of cryptococcosis in individuals afflicted with HIV/AIDS is of paramount

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This article can be cited as: Shaikh N, Salahuddin N. Frequency of cryptococcal antigenemia in HIV treatment naive patients with CD4 count below 200/μL. Infect Dis J Pak. 2023; 32(4): 126-131.
DOI: 10.61529/idjp.v32i4.243

Receiving date: **20 Aug 2023** Acceptance Date: **29 Nov 2023**
Revision date: **16 Oct 2023** Publication Date: **29 Dec 2023**

importance.[11] The definitive approach for the identification of cryptococcal disease is predicated on culture obtained from bodily fluids. However, the identification of cryptococcal antigens (CrAg) within the bloodstream is the most frequently utilized technique, with sensitivity and specificity reaching 100%. The CrAg has been observed to exhibit a positive presence in blood for an average duration of 22 days prior to the onset of CM. Additionally, it has been observed that approximately 11% of patients display a positive CrAg test result over 100 days prior to experiencing the onset of CM.[10] There exist multiple procedures for detecting CrAg in cerebrospinal fluid (CSF) or plasma/serum, including latex agglutination (LA), enzyme immunoassay (EIA), and lateral flow assay (LFA) techniques.[7] Screening individuals for subclinical cryptococcal infection upon admission into antiretroviral therapy (ART) programs via point-of-care methods such as CrAg immunoassays exhibits great efficacy in the detection of patients predisposed to the development of cryptococcal meningitis (CM). Early identification facilitates targeted preemptive antifungal therapy to be administered to these individuals, ultimately minimizing the onset of severe illness and mortality.[19] Considering this, we decided to enroll all treatment naïve patients in this study.

The present study sought to determine the frequency of cryptococcal antigenemia in HIV patients with no clinical features of cryptococcal infection on history or examination, with CD4 count below 200/µL. To the best of our knowledge, there has been no research conducted in Pakistan on the cryptococcal antigen rate in HIV patients. Therefore, our research aims to not only assist in gathering relevant regional data but also provide a valuable resource for future references when dealing with individuals affected by cryptococcus among the HIV-infected population within our society.

MATERIAL AND METHODS

This cross-sectional study was conducted at The Indus Hospital and Health Network (IHHN), Korangi campus, Karachi from July to December 2022. Established in 2007, the Indus Hospital, Korangi campus, is a 300-bed state-of-the-art tertiary care facility providing 100% free and quality healthcare to approximately 400,500 patients annually. The IHHN Korangi campus runs an HIV clinic supported through the UNDP Fund, where investigations and anti-retroviral treatment (ART) are provided free of cost to the patients. The sample size was calculated by Open Epi 3.01 using a 95% confidence level, 7.5% margin of error, and 12.7% prevalence of Cryptococcal antigen.[20] Considering the above, the minimum sample size required for this study was 76 patients.

We included all consecutive HIV patients of age > 18 years, either gender, who were treatment naïve, with CD4 counts 200/µL or below. Patients on immunosuppressive medications for any other reason, active malignancy, known history or treatment of cryptococcal infection in the past, any symptoms of cryptococcal meningitis (headache, neck stiffness), and those who received solid organ transplant were not considered eligible.

After approval from the institutional review board, all eligible patients were enrolled in the study. Informed written consent was obtained from patients. Their clinical and demographic data **(**age, gender, absolute CD4 count, ethnicity, socioeconomic status, bird exposure, and comorbidities such as diabetes, and hypertension**)** were entered in a preformed questionnaire followed by Cryptococcal antigen testing in blood. Cryptococcal antigenemia was defined as a positive cryptococcal antigen (CrAg) test in blood, based on Direct Agglutination test using Thermo Scientific™ Remel™ Cryptococcus Antigen Test Kit. Cryptococcus Antigen Test Kit incorporates the use of latex particles sensitized with murine (mouse) IgM monoclonal antibodies. Cryptococcal polysaccharide antigen in patient serum interacts with sensitized latex particles producing visible agglutination. The use of IgM monoclonal antibodies and treatment of serum with protease reduce the potential for false-positive reactions, eliminating the need to perform a companion control latex test to verify the specificity of results.[21,22] Patients were grouped according to their CD4 counts and WHO HIV stages ranging from stage 1 (asymptomatic) to stage 4 (AIDS).[23]

Data were analyzed by using SPSS v26.0. Mean ±SD / Median (IQR) as appropriate were calculated for quantitative variables such as age, years of education, CD4 counts, etc. Shapiro-Wilk test for normality was used to see the data normality. Frequency (n) and percentage (%) were calculated for categorical variables such as gender, ethnicity, comorbidities, etc. Independent t-test was used to compare the means. Chi-square and Fischer Exact tests were used to compare the baseline characteristics among males vs females. Chi-square test was applied to assess the association of cryptococcal antigen with CD4 count and WHO HIV staging.

RESULTS

A total of 100 HIV patients were enrolled who presented to IHHN during the study period. The mean age was 35.12 ± 10.93 years. The majority (78%) were males. 61% were married; nearly half belonged to the Urdu-speaking ethnicity. 30% were unemployed while 15% were housewives. Exposure to birds was identified in 32% of patients. Most (n = 60, 61%) of the patients belonged to WHO HIV stage 1, followed by stage 4 (n = 30, 30%). Table 1 describes the basic demographic details. The median CD4 count was 103.5 (IQR: 56.5 – 126.5) /μL. 48 patients had CD4 count between 101 to 200/μL, while 51 patients had CD4 count ≤100/μL. Only 2 patients were found to have CrAg positive making an overall prevalence of 2%. Both patients had a CD4 count ≤ 100/μL (n = 2/49, 4.1%). Thirty percent of overall patients had other opportunistic co-infections; of them, tuberculosis was the most common opportunistic infection (21%) followed by syphilis (3%).

Table-1 also provide the comparison of baseline characteristics with gender. Significant difference was observed between employment and marital status among male’s vs female’s. No difference was observed in education status, comorbidities, bird exposure, opportunistic infections, cryptococcal antigenemia, WHO HIV stage CD4 count among the two genders.

Table-2 describes the association of CrAg HIV stages. None of the patients with HIV stage 1, 2 or 3 was positive for cryptococcal antigen, while cryptococcal antigen positive rate in WHO HIV stage 4 was 6.3%. Table-3 describes the association of CrAg with CD4 counts. The CrAg positive rate was 3.7% and 4.5% in patients with CD4 count 51-100/μL and ≤50/μL respectively. However, the p value was not significant due to low number of cases.

Table-1: Comparison of baseline characteristics with gender.

		Total n = 100	Male n = 78	Female n = 22	p-value
Age (Years)	Mean ± SD	35.12 ± 10.93	35.22 ±11.5	34.77±8.87	0.867
Ethnicity	Sindhi	8 (8%)	5 (6.41%)	3 (13.7%)	0.528
	Punjabi	38 (38%)	32 (41%)	6 (27.3%)	
	Urdu speaking	51 (51%)	38 (48.7%)	13 (59%)	
	Baloch	1 (1%)	1 (1.28%)	--	
	Pakhtoon	2 (2%)	2 (2.56%)	--	
Education	Illiterate	--	--	--	0.328
	Primary	9 (9%)	8 (10.2%)	1 (4.5%)	
	Secondary	24 (24%)	20 (25.6%)	4 (18.2%)	
	Matric	15 (15%)	12 (15.4%)	3 (13.7%)	
	Intermediate	14 (14%)	8 (10.2%)	6 (27.2%)	
	Graduate	38 (38%)	30 (38.5%)	8 (36.4%)	
Marital Status	Single	37 (37%)	33 (42.3%)	4 (18.2%)	0.005
	Married	61 (61%)	45 (57.7%)	16 (72.7%)	
	Widowed	2 (2%)	--	2 (9.1%)	
Employment Status	Employed	53 (53%)	50 (64.1%)	3 (13.7%)	<0.001
	Unemployed	30(30%)	27 (34.6%)	3 (13.7%)	
	Housewife	15(15%)	--	15 (68.1%)	
	Student	2 (2%)	1 (1.3%)	1 (4.5%)	
Comorbid	Hypertension	1 (1%)	1 (1.3%)	--	0.829
	Diabetes mellitus	2 (2%)	2 (2.6%)	--	
	COPD	1 (1%)	1 (1.3%)	--	
	End stage renal disease	1 (1%)	1 (1.3%)	--	
Bird Exposure	Present	32 (32%)	23 (29.5%)	9 (40.9%)	0.310
Opportunistic Infection	Candidiasis	2 (2%)	1 (1.3%)	1 (4.5%)	0.710
	Tuberculosis	20 (20%)	14 (17.9%)	6 (27.2%)	
	CMV	2 (2%)	1 (1.3%)	1 (4.5%)	
	Pneumocystis	1 (1%)	1 (1.3%)	--	
	Syphilis	3 (3%)	3 (3.9%)	--	

	Toxoplasmosis	2 (2%)	2 (2.6%)	--	
Cryptococcal Antigen	Positive	2(2%)	1 (1.3%)	1 (4.5%)	0.393
WHO HIV Stage	Stage 1	60 (60%)	49 (62.8%)	11 (50%)	0.441
	Stage 2	7 (7%)	4 (5.1%)	3 (13.7%)	
	Stage 3	1 (1%)	1 (1.3%)	--	
	Stage 4	32 (32%)	24 (30.8)	8 (36.3%)	
CD4 Count	≤50	22 (22%)	15 (19.2%)	7 (31.8%)	0.183
	51-100	27 (27%)	23 (29.5%)	4 (18.2%)	
	101-150	34 (34%)	29 (37.2%)	5 (22.7%)	
	151-200	17 (17%)	11 (14.1%)	6 (27.3%)	

Table-2: Relationship of cryptococcal antigenemia with WHO HIV stage.

WHO HIV Stage	Cryptococcal Antigen Positive	Cryptococcal Antigen Negative	p-Value
Stage 1	--	60 (100)	0.227
Stage 2	--	7 (100)	
Stage 3	--	1 (100)	
Stage 4	2 (6.3)	30 (93.8)	

Table-3. Relationship of cryptococcal antigenemia with CD4 count.

	Cryptococcal Antigen Positive	Cryptococcal Antigen Negative	p-Value
151-200	--	17 (100)	0.538
101-150	--	34 (100)	
51-100	1 (3.7)	26 (96.3)	
≤50	1 (4.5)	21 (95.5)	

DISCUSSION

Cryptococcal antigenemia correlates with reduced CD4 counts.[24] Further, its prevalence demonstrates geographical variations.[16] The true incidence and prevalence of CrAg in HIV patients in Pakistan is not known.

In the current study, we showed that in treatment naïve HIV patients, the overall asymptomatic cryptococcal antigenemia rate was only 2%, with CD4 count 200 or below. It is estimated that the annual incidence of cryptococcal infection in Pakistan lies in the range of 501-1000. [16] A retrospective analysis of all HIV/AIDS since 1986 to 1998 including 67 patients reported a rate of 2.5%.[25] Another study from 2012 showed 9% for cryptococcal meningitis.[26] However, these numbers reflect symptomatic patients.

The prevalence of cryptococcosis in HIV patients is high in African countries and varies from 1.7 to 15.8% in sub-Saharan Africa.[16] This high variation within African countries, and low level of cryptococcosis in Pakistan could be attributed to covert clinical and environmental factors affecting Cryptococcus spp growth. Despite the fact that environmental factors are not well elaborated, it appears that Cryptococcus Spp is linked with certain types of soil, flora, and fauna,[27] and exhibit a wide diversity with specific ecological niches.[28] Furthermore, the virulence of the organism also differs based on lineage and species.[29]

The rate of cryptococcal antigenemia appears to increase significantly with drop in CD4 count. The global prevalence of cryptococcal antigenemia is estimated to be around 2% in adults with CD4 counts between 101 – 200 /µL.[30] However, it increases to approximately 6% for patients with CD4 counts ≤100 /µL. (16) One study conducted in Indonesia found that among ART naïve, asymptomatic, newly diagnosed HIV-infected adult outpatients with CD4 T-cell counts below 100 cells/µL, the prevalence of cryptococcal antigenemia was 7.1%.[31] In a recent metanalysis, the pooled prevalence of cryptococcal antigenemia was 6.5% (95% CI, 5.7%–7.3%) in individuals with CD4 count ≤100 cells/µL and 2.0% (95% CI, 1.2%–2.7%) in those with CD4 count 101–200 cells/µL. In our study, the cryptococcal antigenemia rate was 4% in patients with CD4 count <100/µL. None of our patients with CD4 count >100/µL had Cryptococcal antigenemia.

HIV stage is also correlated with cryptococcal antigenemia. As the HIV infection progresses to advance stages, the likelihood of developing cryptococcal antigenemia becomes greater. In a study from Malwai, the prevalence of cryptococcal antigenemia among patients with WHO clinical HIV/AIDS stage 3 was 1/80 (1.3%) and with WHO clinical HIV/AIDS stage 4 was 1/20 (5.0%). In our study, both of our cryptococcal antigen positive patients belonged to WHO HIV stage 4, making the prevalence of 6.9% in this group. None of the patients with HIV stage 1, 2 or 3 was positive for cryptococcal antigen.

In our study, both patients with positive CrAg had CD4 count <100 cells/µL. Since the numbers identified were low, no other demographic factors like age or gender could be established, which is consistent with previous studies.[32, 33] Gender-specific differences in HIV have been reported.[34] The majority of our patients were male, in contrast to some previous reports of female preponderance of HIV in African countries.[35] Both of our patients were started on antifungal treatment after ruling out central nervous system involvement by cerebrospinal fluid examination, followed immediately by ART for HIV.

STRENGTHS OF THE STUDY

IHHN runs a dedicated HIV clinic with a large catchment area. Since IHHN provides free diagnostics and treatment, the ease of testing made it possible for us to undertake the study. As far as we could research, this was the first study of its kind from Pakistan.

LIMITATIONS OF THE STUDY

Our study was limited by single center design; we were unable to repeat tests or follow patients longitudinally to detect false negatives; given the low number of CrAg-positive patients, we were unable to perform risk analysis. Finally, we did not check HIV viral load, hence could not assess its relationship with cryptococcal antigenemia.

CONCLUSION

The prevalence of asymptomatic cryptococcosis in the treatment naïve HIV is low in the Pakistani population. A routine CrAg screening can be individualized and avoided in selected patients with CD4 count >100/µL in a resource-limited country like Pakistan.

AUTHOR CONTRIBUTION:

Noorulsaba Shaikh: Conception of the work, acquisition, analysis, and interpretation of data, and drafting the manuscript.

Naseem Salahuddin: Participated in design of the work and critical revision for important intellectual content

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ORIGINAL ARTICLE

PATTERN OF ORGANISMS CAUSING CENTRAL NERVOUS SYSTEM SHUNT RELATED INFECTIONS AND THEIR ANTIBIOTIC SUSCEPTIBILITY PROFILE IN A TERTIARY CARE SETUP

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ABSTRACT

Background: Central nervous system (CNS) shunt-associated infections pose a significant threat, necessitating an in-depth understanding of the frequency and antimicrobial sensitivity patterns of the implicated pathogens. This research aimed to ascertain the frequency and antimicrobial sensitivity patterns of pathogens isolated from central nervous system shunt associated infections.

Material and Methods: This cross-sectional study was conducted from April to September 2020 at the Neurosurgical department of Combined Military Hospital, Rawalpindi, and the Department of Microbiology, Armed Forces Institute of Pathology. 95 cerebrospinal fluid samples obtained through direct aspiration. The study employed standard microbiological techniques for pathogen isolation, identification, and antimicrobial susceptibility testing following CLSI guidelines 2019.

Results: Pathogens isolated from central nervous system shunt associated infections included *Staphylococcus* spp. (29.47%), *Escherichia coli* (16.84%), *Acinetobacter baumannii* (13.68%), *Klebsiella pneumoniae* (10.53%), *Pseudomonas* spp. (8.42%), *Enterococcus* spp. (7.37%), *Morganella morganii*, *Enterobacter cloacae*, and *Candida* spp. (2.11% each), *Stenotrophomonas maltophila* (1.05%), and mixed infections. Among Gram-negative pathogens, 95% were susceptible to Polymixin B, 93% to Meropenem, and 55% to Cefepime. All Gram-positive organisms were sensitive to Vancomycin and Linezolid (100%).

Conclusion: *Escherichia coli*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* were prevalent Gram-negative rods in infections related to CNS shunts, with *Staphylococcus* spp. following. Gram-negative bacteria exhibited sensitivity to Polymixin B, Meropenem, and Cefepime, while Gram-positive organisms were responsive to Vancomycin and Linezolid. The significant occurrence of Gram-negative rods emphasizes their consideration in empirical antimicrobial therapy recommendations for minimizing death and disability.

Keywords: Shunt-associated infections of central nervous system, Antibiotic sensitivity, Gram Negative Rods, Meropenem

BACKGROUND

CSF shunts are mechanical channels that drain CSF from the ventricles to a distal site for absorption. The aim of shunt is to decrease the raised intracranial pressure and is indicated to alleviate the symptoms of congenital hydrocephalus, brain tumors, intracranial hemorrhage, post traumatic hydrocephalus, myelomeningocele, dandy walker syndrome and Neurocysticercosis to name a few.[1,2] Shunt devices are mainly of two types: internalized shunts and externalized shunts. Internalized shunts are further of many types depending upon the location of drainage of their distal portion, i.e., Ventriculoperitoneal (VP), Ventriculopleural (VPL), Ventriculoatrial (VA), Ventriculosubgaleal (VSG) and Lumbar Peritoneal (LP) types. Externalized devices include temporary external ventricular drains (EVDS) and Ommaya reservoirs for administration of antibiotics and chemotherapeutic agents.[3,4] Ventriculoperitoneal (VP) shunts and External ventricular drains are most commonly used in our setup. The most common causes of improper functioning of shunts

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This article can be cited as: Irfan R, Mirza IA, Zaman G, Zulfiqar A, Qamar U, Sajjad Z. Pattern of organisms causing central nervous system shunt related infections and their antibiotic susceptibility profile in a tertiary care setup. Infect Dis J Pak. 2023; 32(4): 132-37.
DOI: 10.61529/idjp.v32i4.246

Receiving date: **05 Sep 2023** Acceptance Date: **24 Nov 2023**
Revision date: **23 Oct 2023** Publication Date: **29 Dec 2023**

are shunt blockade, hemorrhage into brain Parenchyma and infections. The clinical manifestations of shunt infections are subtle and can range from few or no symptoms to fever, progressive deterioration of conscious level, new headache, lethargy, nausea, vomiting and seizures. Signs of meningeal irritation are usually lacking. As with any implanted foreign hardware, infection is a major squeal leading to long term Cognitive Impairment, Neurological defects even death. Thus, it is essential to start appropriate therapy for treatment of CSF shunt infections for better patient outcome. The incidence of CNS shunt infections in various studies ranges from 5-15%.(5-7)The common pathogens isolated from CSF shunt infections (SI) include Coagulase negative *Staphylococcus*, *Staphylococcus aureus*, Gram negative Bacilli like *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Cutibacterium acnes*. Mixed infections i-e two isolates were also accounted for infection in few cases.(8)This study's purpose was to evaluate the range of organisms involved in CNS shunt infections and their antibiotic susceptibility profiles in our setup and thus will help in designing Empirical antimicrobial therapy for future.

MATERIAL AND METHODS

This descriptive, cross-sectional study was conducted for a period of six months, from 1st April 2020 to 31st September 2020 at Department of Microbiology / Armed Forces Institute of Pathology Rawalpindi and Neurosurgical department of Combined Military Hospital Rawalpindi. Non-probability, consecutive sampling was done. By using WHO sample size calculator, taking prevalence 40%(5), confidence interval 95%, margin of error (d) 10%, and response rate 100%, the estimated sample size is n=95. CSF Shunt infection will be defined by presence of bacteria in gram stain and culture of CSF obtained either from shunt, ventricles and CSF shunt hardware i-e shunt tip, shunt valve along with other symptoms and signs (fever, altered sensorium, seizures, nausea and vomiting) and no less than one of the following laboratory parameters. CSF cell count >5 cells/µl, CSF glucose < 2.25 mmol/l. CSF Proteins >45mg/dl, CRP >10mg/l. TLC <4.5 OR >11X10^9/l. The study included all CSF samples that were obtained through lumbar puncture with a CSF shunt in situ, ventricular tap, or direct aspiration from a CSF shunt. No discrimination was made on the basis of age and gender. Excluded from the analysis were repeat cerebrospinal fluid (CSF) samples from the same patient, as well as contaminated specimens exhibiting growth of more than two organisms.

Permission from institutional ethical committee was taken (READ-IRB/20/879) and informed consent was taken from the patient. Age, gender, hospital identity and other particulars were recorded on specially designed Performa. All specimens were inoculated on blood agar, MacConkey's agar, Chocolate agar, Sabouraud's agar and anaerobic media culture plates. Culture plates were incubated at 35°C (+2) at ambient air. Anaerobic medium was kept in anaerobic jar. The plates were held for 72 hours before declaring them negative. Pathogen identification was done by colony configuration, Gram staining, biochemical reactions i.e., Catalase test, Coagulase, DNAse, Oxidase, Motility, API (Analytical Profile Index) (20E) and API (Coryne) appropriate for gram positive and negative cocci/rods. McFarland 0.5 suspension of all isolated bacterial pathogens was inoculated on Muller Hinton agar plate. The Kirby-Bauer disc diffusion method was employed to administer antimicrobial sensitivity discs tailored to specific isolated pathogens, following the guidelines specified in the 2018 document from the Clinical Laboratory Standard Institute (CLSI). For Polymixin B susceptibility testing broth microdilution was performed on automated system Vitek 2. *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as control organisms. The analysis of data was conducted utilizing Statistical Package for the Social Sciences (SPSS) version 24.00 and MS Excel 2016 software. For continuous variables, the mean ± standard deviation (SD) was computed, while categorical variables were assessed in terms of frequency and percentage. Chi-square tests and t-tests were employed, with significance set at a p-value ≤ 0.05.

RESULTS

Average age in this research was 31.95 ± 8.95 years.

Most 52 (54.74%) of the patient's age were >30 years old. 64 (67.37%) of 95 patients were males and 31 (32.63%) were females, resulting in males to female's ratio of 2.2:1.

Among the *Staphylococcus* spp., 11 isolates were Coagulase Negative *Staphylococci*, 8 isolates were Methicillin Resistant Coagulase Negative *Staphylococci* and 9 isolates were *Staphylococcus aureus* of which 5 were MRSA. To go in details, mixed infections included *Acinetobacter baumanii* and *Pseudomonas aeruginosa*, Coagulase negative *Staphylococci* and *Staphylococcus aureus*, *Morganella morganii* and *Stenotrophomonas maltophila*, *Escherichia coli* and *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Acinetobacter baumanii*, Coagulase negative *Staphylococci* and *Burkholderia cepacia*. Among *Candida* species one of the isolates was identified as *Candida albicans* and the other as *Candida tropicalis*. Among *Pseudomonas* Spp, 7 isolates were *Pseudomonas aeruginosa* and one was *Pseudomonas stutzeri*. All enterococci were identified as *Enterococcus faecalis*. Ventriculoperitoneal shunts and EVD were used in our patient population.

The antimicrobial susceptibility pattern of various Gram positive and Gram-negative pathogens isolated from central nervous system shunt related infections is shown in Table-2 and 3.

Table-1: Frequency of various pathogens isolated from central nervous system shunt related infections.

Bacterial pathogens	No. of Isolates n (%)
Staphylococcus spp.	28 (29.47)
Escherichia coli	16 (16.84)
Acinetobacter baumanii	13 (13.68)
Klebsiella pneumonia	10 (10.53)
Pseudomonas spp.	08 (8.42)
Enterococcus spp.	07 (7.37)
Morganella morganii	02 (2.11)
Enterobacter cloacae	02 (2.11)
Candida spp.	02 (2.11)
Stenotrophomanas maltophila	01 (1.05)

Table-2: Antibiotic susceptibility pattern of Gram-negative bacteria isolated from central nervous system shunt related infections (n= 60).

Antibiotic	Sensitive	Resistant
Ampicillin	21 (35%)	39 (65%)
Cefipime	33 (55%)	27 (45%)
Ceftriaxone	30 (50%)	30 (50%)
Meropenem	56 (93.33%)	04 (6.67%)
Gentamicin	21 (35%)	39 (65%)
Polymixin	57(95%)	3(5%)

Table-3: Antibiotic susceptibility pattern of Gram-positive bacteria isolated from central nervous system shunt related infections (n= 39).

Antibiotic	Sensitive	Resistant
Penicillin	14 (40%)	21 (60%)
Ampicillin	14 (40%)	21 (60%)
Linezolid	35 (100%)	0 (0%)
Cloxacillin	19 (54.28%)	16 (45.71%)
Vancomycin	35 (100%)	0 (0%)
Gentamicin	12 (34.28%)	23 (65.71%)

DISCUSSION

Central nervous system (CNS) shunt surgery is the most common treatment modality in diagnosed cases of hydrocephalus.[9] One of the most dreadful complications associated with shunt placement, is an infected shunt which is responsible for shunt failure and chronic poor-health.[10] Different figures in literature for CNS shunt infection have been reported, and there are considerable variations internationally. Post-operative infection of cerebrospinal fluid (CSF) shunts ranges from 2% to 27%.[11,12] Shunt infection may be defined as detecting pathogens in shunt fluid, the shunt tube, reservoir, and/or blood culture, in conjunction with clinical manifestations indicative of shunt infection, including fever, peritonitis, meningitis, signs of infection of shunt tract, or nonspecific symptoms like headache, vomiting, or altered consciousness.[13]

Despite of recent developments in technology and availability of new antibacterial agents, Infection is still another very common and serious side effects of CSF shunts.[12] Shunt infections develops via colonization of shunt with skin flora and organisms are usually introduced during perioperative period.[10] Coagulase-negative *Staphylococci* (CoNS), most important being *Staphylococcus epidermidis* followed by *Staphylococcus aureus* are taken to be the most common etiological pathogens responsible for shunt infection.[14–17] These studies demonstrate around 50% of all shunt infections are due to Coagulase Negative Staphylococci and 25% are due to *Staphylococcus aureus* with increasing prevalence of Methicillin Resistance *Staphylococci*.[18,19] A retrospective study in Royal hospital for sick children in York hill demonstrated that out of 308 shunt operations 28 episodes of infection were noted making incidence of infection to be 9%. The primary source of infection was Coagulase-negative *Staphylococci*, constituting 44%. Notably, there was a low sensitivity to flucloxacillin and gentamicin among Gram-positive strains, at 22% and 14%, respectively. Resistance to gentamicin was observed in 87% of coagulase-negative *Staphylococci*, while 81% exhibited resistance to flucloxacillin.[17]

A study conducted in China by Xing Wu *et al* showed a trend shift to gram negatives with *Acinetobacter baumanii* as emerging pathogen in neurosurgery units followed by *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Gram-negative rods, repeatedly isolated pathogens responsible for 58% of infective episodes, followed by *S.aureus*, *S. epidermidis* and *Staphylococcus haemolyticus*. Shunt removal and intravenous antibiotics therapy resulted in improved survival of patients to 82%. The large percentage of the isolated bacteria exhibited vancomycin and carbapenem sensitivity. In two other experiments, Gram-negative bacteria caused 7–24% of all ventriculoperitoneal shunt infestations.[22,23] A study in Kenya showed that *Staphylococcus* species were the source of 51% of infections, whereas in 40% of cases, the[20] infection was attributable to gram-negative bacilli. Cefazolin demonstrated susceptibility against each *Staphylococcus aureus* infection and 79% of other *Staphylococcus* infections.[21]

In our study the main etiology of shunt infections are gram-negative rods. This is in contrast to other studies, which reported the most common pathogens to be gram-positive bacteria. [16,17,19] The elevated Gram-negative bacterial infection percentage in this research can be speculated as most device related infections are caused by nosocomial Gram-negative rods and they colonized patient population contributing to CSF shunt infections in Neurosurgery units.[22] Most of Gram-negative bacilli exhibited resistant to three or more categories of antibacterial agents, and were sensitive to Polymixin B and meropenem.[9] Considering the resistance pattern of these isolates in our setup, a combination potent antibiotic such as Vancomycin and meropenem or cefipime is highly recommended, as these antimicrobials will have adequate gram-negative and gram-positive coverage.[20]

Limitations of this study are that prevention of shunt related infections was not addressed. Moreover, risk factors associated with shunt infections were not taken in to consideration.

The eradication of CSF shunt infections has always been a challenge to the treating Neurosurgeon. Outcome can be improved by early recognition of CSF shunt infection along with appropriate use of antimicrobials to achieve CSF sterility. The paradigm shifts in pattern of organisms causing shunt infections is significant and more studies must be taken to achieve better understanding.

CONCLUSION

The study concludes high proportion of Gram-negative infections in our setup which is different from western data. Our study established that the most frequent Gram-negative bacteria isolated from infections related to central nervous system shunts were *Escherichia coli*, *Acinetobacter baumannii*, *Klebsiella pneumoniae, and Pseudomonas aeruginosa*, with Meropenem and Vancomycin being appropriate coverage for treatment of Gram-negative and Gram-positive isolates respectively. So, we recommend that proper causative organism should be identified in these particular patients before starting any antimicrobial therapy in an effort to lower death and disability.

AUTHOR CONTRIBUTION:

Rafia Irfan: Conception, research analysis and manuscript drafting.

Gohar Zaman: Data collection, research supervision.

Irfan Ali Mirza: Research analysis and final approval of the draft.

Uzma Qamar: Research analysis and final approval of the version to be published.

Zunera Sajjad: Research supervision and analysis

Azka Zulfiqar: Data interpretation and drafting.

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ORIGINAL ARTICLE

EXTENSIVELY DRUG RESISTANT (XDR) *SALMONELLA TYPHI* RED FLAG FOR CLINICIANS AT KARACHI

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ABSTRACT

Background: *Salmonella typhi* cause enteric fever which is a grave community health problem with increasing antibiotic resistance. *Salmonella typhi* is responsible for 10.9 million morbidities and 116800 mortalities worldwide with the greatest burden falling on third world countries. It is common among children with incidence rate of typhoid fever 573.2 per 100,000 in aged 2-5years and 451.7 cases per 100,000 children per year among 2-15years old in Pakistan. Early detection of *Salmonella typhi* and selection of appropriate antibiotics are important to mitigate the morbidity and fatality rate related to these kinds of infections. We have conducted this study to assess the extensively drug resistance in isolates of *Salmonella typhi* in Karachi, Pakistan.

Material and Methods: This cross-sectional study was done at private set up, Karachi from 2019-2021. This study was ethically approved from hospital management. Data related to phenotypic detection of antimicrobial susceptibility was obtained from LIMS (Laboratory Information Management System). According to record both in-door and out-door patients were included in the study. The collected data was scrutinized by using descriptive statistics performed with SPSS version 24. Chi-square was used to test for statistical significance ($P \leq 0.05$).

Results: Total 10564 samples were extracted from data, out of which 4582 samples were found positive for growth of different microorganisms. *Salmonella typhi* were positive in 1500 cases and only 412 samples were exhibiting XDR *Salmonella typhi*. Maximum cases of XDR *Salmonella typhi* were detected in age group 5-10 years with 38.8%. There were 61% male and 39% female.

Conclusion: Our study explains that cases of enteric fever as a result of *Salmonella typhi* are swarming in all cities of Pakistan and particularly at Karachi. XDR is not restricted to Karachi but it is the tip of the iceberg. There is an urgent need to detect these cases timely and start appropriate treatment to overcome complications.

Keywords: *Salmonella typhi*, XDR, Antimicrobial susceptibility, Ceftriaxone, Azithromycin.

BACKGROUND

Salmonella typhi isolates exhibit extensively drug-resistance is alarming situation in the city of Karachi. *S. typhi* bacteria are known as extensively drug resistant (XDR) *S. typhi* when they confer resistance to ampicillin, chloramphenicol, cephtriaxone, fluoroquinolones, trimethoprim sulfamethoxazole.[1]

Typhoid (enteric fever) is global issue and 14.3 million patients of typhoid were documented in 2017, followed by 135.9 thousand deaths with a considerable pressure(69.6%) in South Asia.[2] Typhoid fever is endemic in third world countries of South Asia, like Pakistan, as a result of poor hygiene, lack of clean drinking water and well established sewerage system.[3] This is critical illness presented with severe complications especially in children, with rate of 451.7 cases per one lakh children aged 2-15 years.[4] In Pakistan pure and safe water can be provided only 20% of the population and the left over are compelled to utilize impure and dirty water.[5] However, in the 1980s, extensive spread of IncH1 plasmids in *Salmonella* led to immediate resistance to all these antibiotics, known as multidrug-resistant (MDR) *Salmonella*. Firstly, the ceftriaxone resistant isolates of *Salmonella* was reported as endemic in Hyderabad in the year of 2016.[6] According to the report of World Health Organization (WHO), the Provincial Disease Surveillance and Response Unit (PDSRU), 5274 XDR *S. typhi* cases were present in 14 districts of Sindh from 2016 to

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This article can be cited as: Bakhat S, Nadeem S, Hussain A, Sarwar S, Hussain B, Faheem F. Extensively drug resistant (XDR) *Salmonella typhi* red flag for clinicians at Karachi. Infect Dis J Pak. 2023; 32(4): 138-142. DOI: 10.61529/idjp.v32i4.253

Receiving date: **26 Sep 2023** Acceptance Date: **23 Nov 2023**
Revision date: **03 Nov 2023** Publication Date: **29 Dec 2023**

2018. These revealed 4% cases from different districts of Sindh, 76% cases from Karachi city, and 27% cases from Hyderabad district.[7] Seven patients were diagnosed with XDR *Salmonella typhi* in UK, USA and Canada, all had a visit to Pakistan.[8, 9] The Government of Pakistan and disease controlling institutions have tried to control the situation but still marked rise in detected cases of XDR of *Salmonella typhi* from 2017 to 2021.[10]

Frequency of extensively resistant cases of enteric fever is high but still limited data regarding this infection is available. This study was designed to detect these blood culture-proven XDR *S. typhi* cases along with their age overview and therapy options. It also showed the resistance pattern of *S. typhi*, suggesting the contribution burden made by XDR *S. typhi* isolates, at private hospital of Karachi. We can determine the frequency of XDR *Salmonella* cases. Situational alertness of the XDR *Salmonella typhi* will facilitate physicians to choose appropriate antibiotics in order to overcome the dissemination of resistant pathogen. Furthermore, the study is likely emphasizing the seriousness of this waterborne/food borne communicable disease and motivate community toward preventive actions like personal hygiene, proper sewerage system, clean water supply, and also vaccination campaigns by government.

This study will raise and detect the XDR typhoid cases for future outlook of antibiotic stewardship and maintenance of surveillance program. This study will facilitate to over-come resistant cases before it is no longer manageable and leads back to pre-antibiotic era.

MATERIAL AND METHODS

This cross-sectional study was conducted at private set up, Karachi from 2019-2021. This study was ethically approved from hospital management. Data related to phenotypic detection of antimicrobial susceptibility was obtained from LIMS (Laboratory Information Management System). According to record both in-door and out-door patients were included in the study. Non-probability convenient data collection technique was used. Typhoid patients with blood culture confirmation for *S. Typhi* and resistance to the five classes of antibiotics (ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, fluoroquinolone, and 3rd generation cephalosporin (ceftriaxone or cefixime) were eligible to be included in this study.

Those patients who were on antibiotics for last two weeks were excluded from study. Patients with incomplete medical records especially missing information on antimicrobial treatment, duration of treatment, and treatment failure were excluded from the study.

After processing of blood samples in BACTEC automated blood culture system, gram staining was done in order to differentiate gram positive from gram negative. Biochemical identification was done by API 20E (Biomerieux) in order to interpret species of *Salmonella typhi*. Serological tests were done in order to differentiate species. Lawning was done on Muller Hinton agar plated with antibiotic discs and antimicrobial susceptibility was performed by using the Kirby-Bauer disk diffusion method. Ampicillin 30µg (oxoid), ceftriaxone 30 µg (oxoid), ciprofloxacin 5 µg (oxoid), trimethroprim sulphamathoxazole 30 µg (oxoid) and chloramphenicol (30µg) were used and antibiotic susceptibility interpretation was checked by the Clinical and Laboratory Standards Institute (CLSI) M100 criteria.[11] The data collected was analyzed using descriptive statistics performed with Statistical Package for the social sciences (SPSS) version 24:00. Chi-square was used to compare with p-value, which is considered statistically significant as ($P \leq 0.05$).

RESULTS

Out of 10564 samples of patients were extracted from data, 4582 samples were found positive for growth of different microorganisms. *Salmonella typhi* were positive for 1500 cases and only 412 samples were exhibiting XDR *Salmonella typhi* from June 2019 to June 2021 at Karachi.

Maximum cases of XDR *Salmonella typhi* were detected in age group 5-10 years with 38.8%. There were 61% male and 39% female. Women have strong immune system and show powerful immune response to all antigens than males.[12]

Table-1: Age distribution with XDR *Salmonella typhi* (n=412).

Age	Frequency	Percent (%)
≤ 5years	60	14.6
5-10 years	160	38.8
11-20years	100	24.3
21-40years	92	22.3
Total	**412**	**100.0**

Table-2: *Chi-square test was applied for significance at p-value ≤0.05AMP (Ampicilline), SXT (trimethoprim sulphamethoxazole), C (Chloramphenicol), CRO (ceftriaxone), CIP (ciprofloxacin), (MEM) meropenem, IMP (Imipenem), AZI (Azithromycin)

		Age				
		≤ 5years (n=60)	**5-10 years (n=160)**	**11-20years (n=100)**	**21-40years (n=92)**	**P-value**
AMP	Sensitive	0	0	0	0	0.000
		0.0%	0.0%	0%	0	
	Resistant	60	160	100	92	
		100.0%	100.0%	100 %	92%	
SXT	Sensitive	0	0	0	0	0.000
		0.0%	0%	0%	0%	
	Resistant	60	160	100	92	
		100.0%	100.0%	100%	92%	
C	Sensitive	0	0	0	0	0.000
		0.0%	0%	100.0%	0%	
	Resistant	60	160	100	100	
		100.0%	100%	100%	100%	
CRO	Sensitive	0	0	0	0	0.000
		0.0%	0%	0%	0%	
	Resistant	60	160	100	100	
		100.0%	100%	100.0%	100%	
CIP	Sensitive	0	0	0	0	0.000
		0.0%	0.0%	0.0%	0%	
	Resistant	60	160	100	92	
		100.0%	100.0%	100.0%	92%	
MEM	Sensitive	138	137	137	100%	0.00
	Resistant	0	0	0	0	
AZI	Sensitive	117	200	95	100%	0.00%
	Resistance	0	0	0	0	
IMP	Sensitive	112	25	265	100%	0.000
	Resistance	0	0	0	0	

Table-3: Frequency of XDR *Salmonella typhi*.

Total number of *Salmonella typhi*	MDR	Percentage (%)	XDR	Percentage (%)
1500	500	33.33	412	27.46

DISCUSSION

We report phenotypically detected XDR *Salmonella typhi*. *Salmonella typhi* strain exhibits resistance towards penicillin, cloramphenicol, cephalosporin, ciprofloxacin and co-trimethoprim.

Typhoid fever is alarming threat in Karachi, Pakistan, with the maximum rate in children less than 15 years, which is in accordance to preceding studies from India and Bangladesh.[13,14] Situation was aggravated during pandemic of COVID-19, with highest rate in children is similar to our study.[15] Local surveillance program was activated after detection of the XDR *S. typhi* from Hyderabad, and multiple cases were reported in the province of Sindh.[16] Antibiotic susceptibility pattern was detected in this study, similar situation is faced by our neighbor country like India.[17] From other parts of Pakistan, similar alarming condition of antibiotic resistance in isolates of *Salmonella typhi* was reported.[18] Similar cases of XDR *Salmonella typhi* were detected in study conducted at Aga Khan university.[19] Decades of rampant and inappropriate use of antibiotics has obsessed the emergence extensively drug-resistant (XDR) *Salmonella typhi* (*S. Typhi*) strains in Beijing.[20] Our study is also supported by another study conducted in India and reported XDR *Salmonella typhi*.[21] One study was conducted at Peshawar which showed a case of extensively resistant *Salmonella typhi* with multiple abscesses in spleen.[22] Both azithomycin and meropenem are last hope to save lives of patients, another study conducted in Pakistan in association with our results.[23] Azithromycin resistance was also reported by Bangladesh at molecular level.[24] Even people travelled from Pakistan to other countries like United Kingdom and United States, were diagnosed as XDR typhoid and responsive only to two drugs as meropenem and azithromycin.[25] Azithromycin is now considered as the only oral antibiotic option. The potential emergence of azithromycin resistance is superfluous threat; if attained by the XDR strain of *S. Typhi*. Even patients will be killed with typhoid because it may become practically terminal. We as a nation facing XDR *Salmonella* are in red zone because India suffered from spontaneous emergence of azithromycin resistance.[26] On other hand, meropenem exhibits poor response as monotherapy, showed red flag for all physicians and patients.[27] We have to face some limitations like patients already started antibiotics before the antibiotic susceptibility test. These people have started either self-medication at home or prescribed by general physicians. This is facilitating the dissemination of antibiotic resistance encoding genes.

Our research showed XDR *Salmonella typhi* susceptibility and resistance pattern, for guidance of physician and establishment of antibiotic stewardship. This also emphasizes the use of TCV vaccine both for children and adults.

CONCLUSION

It is concluded that our country is in red zone in case of XDR *Salmonella typhi*. We need to catch our flaws and established epidemiological data as well as awareness of vaccine.

CONFLICT OF INTEREST

None

AUTHOR CONTRIBUTION:

Shaista Bakhat: Original idea, Literature search, Manuscript write up, final layout
Saman Nadeem: Data collection, Data entry
Ashfaq Hussain: Data entry
Saba Sarwar: Data collection
Beenish Hussain: Coordination
Faisal Faheem: Statistical analysis

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ORIGINAL ARTICLE

IMPACT OF QUALITY IMPROVING INTERVENTIONS ON BLOOD CULTURE CONTAMINATION RATE- A PROSPECTIVE OBSERVATIONAL STUDY

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ABSTRACT

Background: Blood cultures (BC) is the gold standard for diagnosis of bacteremia but false positive blood cultures due to specimen contamination can lead to increased usage of unnecessary antibiotics and have negative impact on antimicrobial stewardship program. Overgrowth of contaminants can also obscure growth of genuine pathogens and hamper accurate diagnosis. The aim of this study is to evaluate effectiveness of Quality improving (QI) interventions to reduce the blood culture contamination rate

Material and Methods: Prospective observational study, conducted at a public sector hospital, for a total duration of 6 months. A taskforce developed and implemented QI interventions to reduce blood culture contamination, and evaluated effectiveness of these interventions by comparing contamination rates prior to and after implementation of the intervention.

Results: The post-intervention average contamination rate was significantly lower with the mean contamination rate of 4.7 ± 1.5% as compared to baseline BC contamination rate which was 10.6 ± 1.8% (p=0.013)

Conclusion: Quality improving (QI) interventions significantly reduced the blood culture contamination rate.

Keywords: Quality improvement, Interventions, Blood culture, Contamination.

BACKGROUND

Blood cultures (BC) remain the gold standard for diagnosis of bacteremia in both hospitalized and non-hospitalized patients.[1] Prompt and accurate identification of the causative bacteria is crucial for the commencement of appropriate treatment and hence, lifesaving. However false positive blood cultures, due to specimen contamination is a commonly encountered issue which creates difficulty in reaching to an accurate diagnosis. According to The Clinical and Laboratory Standards Institute, institutions should limit blood culture contamination rate to maximum of 3%.[2] Blood culture contamination (BCC) is a significant problem in tertiary-care hospitals and may lead to excess patient morbidity by excessive use of antibiotics, unnecessary diagnostic testing and replacement of intravenous catheters. Resultantly not only laboratory expense shoots up but also increases overall financial burden hospital and may have harmful effects on the organizations' antimicrobial stewardship efforts.[3, 4, 5]

There can be many causes of specimen contamination including ignorance among healthcare staff regarding importance of aseptic sample collection, unavailability of chlorhexidine for skin disinfection, improper skin or bottle disinfection technique, poor transportation conditions and improper sample processing technique in the laboratory. However, introduction of several quality improvement (QI) methods such as using standardized sterile technique for sample collection, ensuring availability of disinfectant products and equipped blood culture collection kits, training sessions for phlebotomists, regular monitoring of contamination rates, audits and assessment of collection methods and reinforcement of correct practices has shown promising results in reducing BCC rates.[6,7] In this study, we developed a task force to introduce quality improvement (QI) interventions to reduce the BCC rate across the hospital and evaluated the effectiveness of these QI interventions by calculating BCC rate before and after implementation of QI interventions. The task force included an infectious diseases physician, microbiology laboratory personnel and infection control personnel.

MATERIAL AND METHODS

This was an observational study conducted at a 500-bed tertiary care public sector hospital in Karachi Pakistan, from July 2021 to December 2021. Blood culture processing and identification was performed at the

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This article can be cited as: Fatima T, Rezwan F, Ali F, Rustum M, Nadeem M. Impact of quality improving interventions on blood culture contamination rate - A prospective observational study. Infect Dis J Pak. 2023; 32(4): 143-146. DOI: 10.61529/idjp.v32i4.180

Receiving date:	**16 Mar 2023**	Acceptance Date:	**07 Sep 2023**
Revision date:	**28 Aug 2023**	Publication Date:	**29 Dec 2023**

microbiology section of laboratory. An average of 6000 blood cultures are received annually in the department. All blood culture collected from the emergency department; general, surgical wards were included in the study. After getting approval from the institutional ethical review committee the 06 months study was divided into pre-intervention and intervention periods. The blood culture contamination data from July 2021- September 2021 was taken as baseline pre-intervention period. The BCC data from October 2021- Dec 2021 was taken as intervention period. The QI interventions introduced during this period consisted of

1. Educating and training phlebotomists and distribution of pictorial education material
    a. Using standardized aseptic sterile technique
    b. Optimizing volume of blood culture
    c. Labeling of blood culture bottles with the initials of phlebotomists who drew them, followed by individualized feedback on contamination rates
    d. Ensure prompt transportation of collected blood cultures to microbiology laboratory (within 2- 4 hours)
2. Ensuring availability of skin disinfectant (2% chlorhexidine, alcohol swabs) and other products (gloves, hand sanitizer)
3. Audits and assessment of collection methods
4. Regular monitoring of contamination rates and reinforcements of correct practices by providing feedback
5. Cleaning of blood culture bottle with chlorhexidine before processing and sub culturing in microbiology laboratory

Single blood culture positive for skin flora or environmental organism's i.e. *Coagulase negative staphylococcus (CONs), Viridans group Streptococcus, Corynebacterium species, Bacillus species, Pseudomonas Stutzeri,* and *Micrococcus species* were considered contaminants. However, multiple blood cultures of a patient or blood cultures collected through central lines with isolation of above-mentioned organisms were considered pathogen and clinically significant and therefore not counted as contaminants.[8] BCC data was regularly monitored and summed up at the end of every month to calculate the contamination rate each month. Percentage contamination rate was calculated as total number of contaminants divided by total number of blood culture collected by venipuncture. Data was entered and analyzed in SPSS 28.0.1

RESULTS

From July 2021- September 2021 total 1,191 blood cultures were received. The average contamination rate in these 3 months was taken as baseline BCC rate. During the intervention period which was October 2021- December 2021, total 1,834 blood cultures were received. The post-intervention average contamination rate was significantly lower with the mean contamination rate of 4.7 ± 1.5% as compared to baseline BCC rate which was 10.6 ± 1.8% (p=0.013).

Table-1: Trends of BCC from July 2021- Dec 2021.

Month	Total number of blood cultures received	Total number of contaminated blood cultures	Percentage
July	400	40	10%
August	378	48	12.7%
September	413	38	9.2%
October	520	31	5.9%
November	620	32	5.1%
December	694	21	3.02%

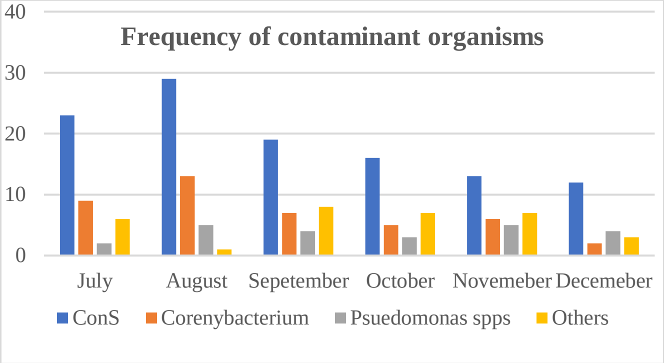

Figure-1: Trends of BCC from July 2021- Dec 2021

DISCUSSION

This study set out with the aim of assessing the importance of quality improvement (QI) interventions in decreasing the blood culture contamination during sample collection. And it was hypothesized that the proper implementation of QI interventions will bring down the detection of contaminants considerably. The most obvious finding to emerge from the analysis is the significant decline in contamination rate from approximately 10.6% during pre-intervention phase to 4.6% in post-intervention period. Another important finding was that there is increased number of total blood cultures received; 1834 during post intervention period as compared to 1191 in July-September. These findings may explain the relatively good correlation between interventions and detection of contaminant organisms. Moreover, this also shows the strong impact of quality interventions despite increased number of blood cultures.

Another important clinically relevant finding was the substantial reduction in isolation of *Coagulase negative staphylococcus species* (CoNS) and *Corynebacterium species* on a monthly basis from approximately 25% to 12%. The observed decrease in isolation could be attributed to strong quality interventions focusing on antisepsis for sample collection skin site hence leading to reduction of isolation of *CoNs* and *Corynebacterium species* which are part of skin flora. On the other end pseudomonas species isolation does not show any steep decline rather shows steady detection rate. Similarly other category of microorganisms which mostly comprises of *Bacillus species*, which is a part of environment, has not shown any significant decline. A note of caution is due here since this also needs to come down with focus on environment cleaning and disinfection with suitable working conditions.

A strong relationship between QI interventions and decrease in blood culture contamination has been reported in the literature globally.[9, 10, 11, 12, 13, 14, 15] It is encouraging to compare our study findings with that found by Self *et al* conducted in a single emergency department at a tertiary care adult hospital in the United States who noticed the marked reduction of blood culture contamination from 4.3% to 1.7% with QI assessment and intervention.[6]

Furthermore, our study results are in accord with other studies indicating the impact and outcome of QI measures. Another study by Feghaly *et al* showed the decrease of contamination rate from 2.85% to 1.54% by implementing best practice for blood culture collection.[16] Likewise, a study from Australia by Allen *et al* showed that decrease in blood culture contamination rate from 2.0% to 1.0% by applying same strategies for sample collection.[17]

In reviewing the national literature, strong association was found between QI interventions and reduction in BCC rate. An interventional study carried out by Shaheen *et al* 2020 at a tertiary care hospital Karachi observed a decline of BCC from 8% to 3.9% within a span of 3 years through continuous QI efforts.[18] Another study has also highlighted the issues faced by healthcare staff during the working hours at hospital including stress, overcrowded places and hot and humid weather which hampers the appropriate sample collection.[19] Similar issues, especially limited number of phlebotomist dealing increased number of patients hampers proper sample collection.

This combination of findings provides some support for the conceptual premise that the strong education and training of phlebotomists along with continuous audit and monitoring of culture contamination rate will surely help the hospitals in bringing down the contamination rate and its resulting consequences. In public sector hospital with limited resources, the placement of trained phlebotomist in different units will definitely help in reducing the contamination rates. As isolation of contaminating organism will pose problems for clinicians in evaluating its significance and sometimes lead to the start of inappropriate antibiotics contributing to increased antimicrobial resistance. Hence ideal sample collection not only helps in isolating the true pathogens and commencement of appropriate antibiotic which also benefits our antimicrobial stewardship plan.

There is abundant room for further progress in determining and alleviating the cause of contamination in blood culture due to inadequate sample collection technique or limitations in provision of appropriate equipment and disinfectants. However, these findings contribute in several ways to our understanding of QI interventions, training of staff with provision of proper sample collection kits and provide a basis for decrease in blood culture contamination rates.

CONCLUSION

Quality improving (QI) interventions significantly reduced the blood culture contamination rate

ACKNOWLEDGEMENTS

Authors are obliged to contribution made by Mr. Samson Boota, Mrs. Wajeeha Babar, and Mr Muhammad Mohsin for their service and assistance in microbiology laboratory.

CONFLICT OF INTEREST

None

AUTHOR CONTRIBUTION:

Tazeen Fatima: Conception, Design, Execution, Manuscript Writing, final drafting

Faiza Rezwan: Manuscript writing
Farheen Ali: Concept, design, manuscript review
Muhammad Rustum: Execution
Muhammad Nadeem: Manuscript review

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ORIGINAL ARTICLE

THE CURRENT SPECTRUM OF INFECTIONS IN CANCER PATIENTS WITH CHEMOTHERAPY RELATED NEUTROPENIA

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ABSTRACT

Background: New cancer treatments have improved patient survival but also bring side effects like weakened immunity and higher infection risk. The present study aimed to assess the pattern of blood stream infections and antimicrobial resistance in blood isolates of neutropenic patients.

Material and Methods: This Cross-sectional, observational study was conducted at Department of Oncology, Jinnah Postgraduate Medical Centre, Karachi, Pakistan between August 2019 to August 2020. Malignant patients who underwent chemotherapy and presented with fever, neutropenia, and positive bacterial blood culture were enrolled in the study. Patients with a recent history of blood transfusion were excluded. The spectrum of bacterial infections and the antimicrobial resistance patterns of causative organisms isolated from blood were studied. The CLSI standard antimicrobial panel was used for each microorganism. Findings were presented in tabular form.

Results: During the study period 95 organisms were isolated from the blood cultures. Strains of *E. Coli*, *Klebsiella*, *Pseudomonas*, *S. Aureus*, *S. Pneumoniae*, were found in 27 (28.4%), 18 (18.9%), 13 (13.7%), 17 (17.9%), and 2 (2.1%) patients, respectively. 44.0% of *E. coli*, 68.8% of *Klebsiella*, and 38.5% of *Pseudomonas* were resistant to tazobactam. 92.6% of *E. coli*, 88.2% of *Klebsiella*, 100% of *Pseudomonas* were resistant to Co-amoxiclav. 52.9% of *Staph aureus* were resistant to methicillin. 23 (92.0%) strains of *E. coli*, 15 (83.3%) strains of *Klebsiella*, 6 (46.2%) strains of *Pseudomonas*, and 11 (68.8%) of *Staph aureus* were resistant to ciprofloxacin.

Conclusion: Resistance to the majority of the commonly used antimicrobial drugs was noted. Continuous antimicrobial resistance surveillance of the everchanging spectrum of causative organisms and their characterization is very important to treat the bloodstream infections (BSI) in cancer patients with chemotherapy induced neutropenia.

Keywords: Neutropenia Infection, Fever, Antimicrobial resistance, Chemotherapy.

BACKGROUND

With the advancement in cancer treatment, many novel chemotherapeutic drugs have been introduced which have significantly improved the survival rate of cancer patients.[1] However, these potent cytotoxic chemotherapeutic drugs can result in several side effects including substantial immunosuppression thereby, an increased risk of infection.[2] Neutropenia is defined as neutrophil concentration of less than or equal to 1500 of the total leukocytes (WBC Count). Patients with neutropenia are highly susceptible to infections which are associated with significant morbidity and mortality.[3] Chemotherapy-induced neutropenia is one of the most common and dangerous complications seen in cancer patients all over the world.[4]

Neutropenia related to chemotherapy is a serious hematologic toxicity which limits the doses and duration of treatment that can be tolerated by the patient.[4,5] Infections in cancer patients with neutropenia related to chemotherapy is associated with a poor patient outcome if not timely treated with appropriate antibiotics.

Even though the overall cancer survival rate has improved in the last decade owing to the adoption of a more aggressive approach particularly the use of monoclonal antibodies against cancer cells, the risk for

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This article can be cited as: Ashfaq M, Haider G, Ather T, Ahsan M, Khan ME, Seher S. The current spectrum of infections in cancer patients with chemotherapy related neutropenia. Infect Dis J Pak. 2023; 32(4): 147-153. DOI: 10.61529/idjp.v32i4.265

Receiving date: **31 Oct 2023** Acceptance Date: **29 Nov 2023**
Revision date: **23 Nov 2023** Publication Date: **29 Dec 2023**

neutropenia among patients has elated significantly. Hence, it has become more difficult to treat and manage patients with chemotherapy related neutropenia especially if the patients are elderly or have multiple comorbidities.[6]

Gram negative *bacilli* and *staphylococcus* aureus or else are the most frequently isolated organisms from neutropenic cancer patients. Age of patient, type of chemotherapeutic medicine being used, and its duration are some of the risk factors associated with the infections among these patients.[7-9]

A local study by Saghir *et al*., reported *pseudomonas aeruginosa* to be the most frequently isolated bacterial strain with a rate of 38%, followed by *E. coli* with a frequency of 25%, *Klebsiella*, *Proteus*, and *Shigella* with a frequency of 20%, 10%, and 7%, respectively.[9]

Recent trends have shown an increased antimicrobial resistance in the gram-negative bacteria. A review of literature showed that resistance to amoxicillin was present in 44% cases of *E. coli* in 2004 which increased to 56% a year later (P=0.01). Similarly, the rate of *E. coli* resistance to piperacillin was 11 percent by 2004 and increased to 38% in 2005 (P<0.001).[7]

Pseudomonas isolates were resistant to several antibiotics including cefepime (60%), ciprofloxacin (80%), meropenem (13%), ceftriaxone (67%), cefoperazone (90%), tobramycin (40%), and imipenem (10%).

However, the local literature is more than a decade old and outdated. Due to the ever-changing spectrum of infection in cancer patients and the rise in multiple drug resistant bacteria, it is of the utmost importance to perform regular antimicrobial resistance surveillance studies and assess the change in characterization of the microbes. Studies have shown that empirical antibiotic therapy is associated with reduced morbidity and mortality among cancer patients with systemic infection. Therefore, it is vital to have an updated knowledge about the common most infectious agents in our community so that empirical as well as therapeutic antibiotic doses can be administered.[8,9]

Unfortunately, there is a scarcity of local literature on the subject. Therefore, the current study was undertaken. The goal of the study was to evaluate the spectrum of bloodstream infections (BSI) and study the antimicrobial resistance in cancer patients with chemotherapy induced neutropenia.

MATERIAL AND METHODS

A cross-sectional, observational study was conducted at the oncology department, Jinnah Postgraduate Medical Centre, Karachi, Pakistan between August 2019 to August 2020. The study was approved by the ethical committee board and an institutional review board (IRB) letter with reference number NO. F-81-IRB/2019-GENL/32729/JPMC was issued prior to the study.

Cancer patients who underwent chemotherapy (inpatient or day care) within the last 15 days and presented with a documented fever, neutropenia (ANC $\leq$ 1500) and a subsequent positive bacterial blood culture were enrolled in the study. Patients receiving chemo >15 days back, had a drug reaction, had a recent history of blood transfusion were excluded. Urine, sputum, and pus cultures were excluded. Positive fungal/viral cultures were also excluded. Blood cultures were sent according to standard lab protocols.

Fever due to non-infectious causes was ruled out before enrolling participants in the study. An informed written consent was obtained from all patients. A non-probability convenience sampling technique was applied to select participants for the research.

All sociodemographic parameters including age, gender, occupation, comorbidities, and residence were recorded using a predefined structured pro forma. Data regarding duration of symptoms, blood cell counts (including WBC and ANC), chemotherapy regimen, and type of malignancy was recorded. A normal white blood cell count was defined as a concentration between 4.5 to 11.0 X 10^9/L.[10] Neutropenia was classified as mild, moderate, or severe, based on the absolute neutrophil count (ANC). Mild neutropenia was defined as ANC between 1000-1500 cells/µL, moderate neutropenia was labeled as ANC of 500-1000/µL, while severe neutropenia was tagged as ANC of lower than 500 cells/µL.[11] Fever was defined as a single oral or axillary temperature of $\geq$ 38.30 Centigrade for more than one hour.[12]

The antimicrobial resistance patterns of causative organisms were studied. Various culture media including nutrient broth, nutrient agar, blood agar, etc.

were prepared to isolate and grow the causative agent. Blood samples were taken and sent to the laboratory using standard lab protocols.

A total of 95 microorganisms including both gram negative and positive bacteria were isolated and studied for their susceptibility pattern. 5 ml of blood was immediately added to brain heart infusion (BHI) broth and 8–9 ml of thioglycolate broth for anaerobes following collection. The blood culture vials were cultured at 37 C for seven days. A blood culture was deemed positive if an organism developed in at least one dish. The isolates were identified using Gram staining and common biochemical testing. The modified Kirby-Bauer disc diffusion method was used for assessing the susceptibility of microbes to various antibiotics. All standard biochemical tests were performed according to the Manual of clinical microbiology to detect and identify the bacterial isolates (as discussed with the microbiology team).

To study the antimicrobial sensitivity several groups of antibiotics including the Penicillins, Carbapenems, Cephalosporins, and Aminoglycosides were used. The results of antimicrobial sensitivity were recorded after 24 hours of incubation at 37ºC. The data was entered and analyzed using SPSS (Statistical Package for the Social Sciences). The results were presented using mean and frequency or percentages. The continuous variables were presented as mean and standard deviation like age of the patients. Categorical variables like gender, residence, etc were presented as frequency and proportions.

RESULTS

The mean age with standard deviation of patients was 48 ± 27.5 years. There was a total of 95 patients who fulfilled the eligibility criteria. 43 (45.3%) were male while 52 (54.7%) were female patients and mostly n=65 (68.4%) belonged to the urban area. The majority of the female participants were home makers. The median duration of symptoms before presenting to the hospital was 3.41 weeks. The most common presenting symptom of infection in cancer patients was fever which was documented in 95 (100%) patients. Cough was found in 20 (21.1%) patients while diarrhea and shortness of breath were found in 14 (14.7%) and 9 (9.5%) patients, respectively. The mean white blood cell count was 2.235 × 10^9/L.

The frequency of isolated strains of bacteria are presented in Table-1. Strains of *E. Coli*, *Klebsiella*, *Pseudomonas*, *S. Aureus*, *S. Pneumoniae*, were found in 27 (28.4%), 18 (18.9%), 13 (13.7%), 17 (17.9%), and 2 (2.1%) patients, respectively.

Table-2 demonstrates the pattern of antimicrobial resistance of the isolated agents in our study. 11 (44.0%) *E. coli*, 11 (68.8%) *Klebsiella*, and 5 (38.5%) *pseudomonas* were resistant to tazobactam. 25 (92.6%) *E. coli* and 15 (88.2%) of *Klebsiella*, were resistant to Co-amoxiclav. 23 (92.0%) strains of *E. coli*, 15 (83.3%) strains of *Klebsiella*, 6 (46.2%) strains of *Pseudomonas*, and 11 (68.8%) of *staph aureus* were resistant to ciprofloxacin. Carbapenem resistance was most seen in *Klebsiella*, followed by E. coli and Pseudo. MRSA was isolated from 9 (52.9%) isolates. Most isolates were resistant to amoxiclav followed by cephalosporins and quinolone.

Cyclophosphamide plus doxorubicin (AC) and Docetaxel, cisplatin and fluorouracil (TPF) were the most common regimes causing neutropenia with a frequency of 12 (12.63%) patients in each regime. 33 (34.7%) patients were admitted to ward for the management of neutropenia related to chemotherapy. The mean hospital stay of patients was two days. 62 (65.2%) were managed in the outpatient's department.

Table-1: The sociodemographic characteristics of study participants (n=95).

Mean Age (± SD) ***in years***	**48.0 (±27.5)**
Gender	
Male	43 (45.3%)
Female	52 (54.7%)
Residence	
Rural	30 (31.6%)

Urban	65 (68.4%)
Occupation	
Homemaker	39 (41.1%)
Labourers	14 (14.7%)
Farmers	7 (7.4%)
Others	27 (28.4%)
Unemployed or retired	8 (8.4%)
Presenting symptoms	
Fever	95 (100%)
Cough	20 (21.1%)
Shortness of breath	9 (9.5%)
Diarrhea	14 (14.7%)
Isolated Bacterial Strains	
E. Coli (gram -ve)	27 (28.4%)
Klebsiella (gram -ve)	18 (18.9%)
Pseudomonas (gram -ve)	13 (13.7%)
S. Aureus (gram +ve)	17 (17.9%)
S.Pneumoniae (gram +ve)	2 (2.1%)
Others	18 (18.9%)
Severity of neutropenia	
Mild	24 (25.3%)
Moderate	50 (52.6%)
Severe	21 (22.1%)
Organ of Malignancy	
Breast	14 (14.7%)
Gastrointestinal tract	6 (6.3%)
Genitourinary tract	10 (10.5%)
Hematological malignancy	39 (41.1%)
Others	26 (27.4%)

Table-2: Pattern of antimicrobial resistance of the isolated organisms in the study.

Antimicrobial Drug	**Isolated Microorganism**			
	E.coli	***Klebsiella***	***Pseudomonas***	***S. Aureus***
Tazobactam	11 (44.0%)	11 (68.8%)	5 (38.5%)	NA
Co-amoxiclav	25 (92.6%)	15 (88.2%)	NA	NA
Methicillin	NA	0 (0.0%)	NA	9 (52.9%)
Ciprofloxacin	23 (92.0%)	15 (83.3%)	6 (46.2%)	11 (68.8%)
Amikacin	5 (20.8%)	3 (21.4%)	3 (23.1%)	0 (0.0%)
Imipenem	7 (26.9%)	11 (64.7%)	5 (38.5%)	NA
Cefixime	23 (92.0%)	16 (94.1%)	NA	NA
Ceftriaxone	23 (92.0%)	14 (87.5%)	NA	NA
Fosfomycin	1 (6.3%)	2 (16.7%)	NA	NA
Cotrimoxazole	14 (73.7%)	13 (86.7%)	NA	1 (9.1%)
Tetracycline	8 (66.7%)	6 (66.7%)	NA	6 (42.9%)

DISCUSSION

Despite the continuous advancement in the management of cancer patients with introduction of newer chemotherapeutic drugs, neutropenia is still considered as one of the most dreadful adverse effects of cytotoxic chemotherapy.[13] Febrile neutropenia is a serious medical condition which can complicate the disease course of cancer patients and is associated with considerable financial burden. Since 1980, the spectrum of bacterial infections has drastically changed from gram-negative to gram-positive organisms owing to the fluoroquinolone prophylaxis being administered in most settings.[14]

However, more recently a further change in the spectrum of organisms causing the bacterial infections in neutropenic patients is being observed. In a study by Carvalho *et al*., a trend towards gram-negative bacteria was observed.[15] Similarly, another local study by Jamal *et al*., reported a trend towards gram-negative bacteria with a frequency of 79% in patients with febrile neutropenia. *E. coli* and *Staphylococcus aureus* were the most commonly reported causative agents.[16]

We have also reported a higher frequency of gram-negative isolates in our study, *E. coli* being the most common, findings which are in accordance with the study quoted above. These findings further consolidate the notion that there is indeed a shift from gram positive bacteremia towards multidrug resistant gram-negative bacterial infections in neutropenic patients with malignancy.

We identified numerous strains of bacteria that were multidrug resistant. The increasing trend towards multidrug resistant gram-negative bacterial infections can be attributed to the injudicious and inadequate use of antibiotics.[17]

A study conducted to evaluate the incidence of bloodstream infections in 4,200 cancer patients revealed that inadequate empirical antimicrobial therapy in the first twenty-four hours of chemotherapy may lead to an increased morbidity and mortality.[18] It was concluded that the wrong antibacterial management in the critical 24 hours' time was linked with a higher number of deaths.

In a local 2013 study by Fayyaz *et al*., it was found that the majority of the isolates were gram positive (47.3%) and out of the *staphylococcus spp.* 67 percent were methicillin resistant.[19] In our study however, 52 percent were methicillin resistant. The difference between the rate of resistance could be because of the study population. The current study only included patients who had undergone chemotherapy whereas, the former study included all patients with bone marrow transplant, kidney transplant in addition to malignancy.

Unnecessary and rampant use of antimicrobial drugs like methicillin and vancomycin have led to resistant strains of bacteria including MRSA (methicillin resistant *Staphylococcus aureus*) and VRSA (vancomycin-resistant *Staphylococcus aureus*), respectively.[20] A study by Jaksic *et al*., reported Linezolid to be effective against all MRSA organisms.[21] Similarly in our study 100% MRSA were sensitive to Linezolid.

Failure to identify the causative organism and irrational use of antibiotics is a global challenge. Over fifty percent of all antibiotics which are prescribed or administered are unnecessary or inappropriate. Whereas the other fifty percent of patients are either non-compliant or are not adhering to correct use of antibiotics.[22] Some of the factors affecting this irrational and inappropriate use of antimicrobials include socio-economic status, doctors' knowledge and educational training, patient burden, misdiagnosis, unavailability of adequate antimicrobial guidelines, and unrestricted pharmaceutical marketing.[23] In Pakistan, it is unfortunately a very common practice to administer and prescribe antibiotic therapy without a culture sensitivity report hence, patients are given unnecessary doses of antibiotics which eventually lead to bacterial resistance.[24] In a local qualitative study by Saleem *et al*., it was found that the majority of the practicing physicians did not have adequate knowledge on the current spectrum of infection, appropriate use of antimicrobials and were unaware about the ongoing antimicrobial resistance patterns in their local settings.[24]

The current data indicate that the initial empiric coverage, even in low risk, febrile neutropenic patients, needs to be broad spectrum until the blood cultures become available. Potent gram-negative coverage is essential particularly the coverage against *P. aeruginosa* considering the latest trend towards these gram-negative organisms.

It is important to realize the challenging situation that we are currently dealing with before we run out of options. With the changing infection spectrum and multidrug resistant organisms on the verge, it is time to formulate strict regulations, promote awareness campaigns, arrange training sessions for practitioners and develop a proper local guide on antimicrobial use.

CONCLUSION

The study highlights the most common bacterial strains isolated from neutropenic cancer patients undergoing chemotherapy. Gram negative bacteria were more frequently isolated as compared to the gram positives bacteria. Resistance to the majority of the commonly used antimicrobial drugs was noted. The emergence of CRE isolates and increase in MRSA is very concerning. Therefore, continuous antimicrobial resistance surveillance of the ever-changing spectrum of causative organisms and their characterization is very important to treat the bloodstream infections (BSI) in cancer patients with chemotherapy induced neutropenia.

CONFLICT OF INTEREST

None

AUTHOR CONTRIBUTION:

Maliha Ashfaq: Conceptualized, drafted the article, **Ghulam Haider:** Provided supervision, developed study design, conducted critical appraisal

Tooba Ather and Mahnoor Ahsan: Contributed in data acquisition and drafting of manuscript

Mohammad Ejaz Khan and Sana Seher: Contributed in drafting of manuscript and proofreading

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ORIGINAL ARTICLE

COMPARISON OF BETA-LACTAM/ BETA-LACTAMASE INHIBITORS WITH CARBAPENEM FOR URINARY BACTEREMIA CAUSED BY CEFTRIAXONE RESISTANT *ESCHERICHIA COLI* AND *KLEBSIELLA SPECIES*

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ABSTRACT

Background: The debate, whether to treat ceftriaxone resistant (CROr) gram negative urinary tract infection with bacteremia (bUTI) with Beta-lactam/beta lactamase inhibitors (BL/BLI) or Carbapenem is going on. Carbapenem are expensive in our part of the world. Our aim was to see the efficacy of BL/BLI in the treatment of bUTI caused by CROr *Escherichia coli* (E-coli) or *Klebsiella species* (spp.) To compare microbiological clearance, clinical failure, new bacteremia and mortality between patients with bUTI who receive BL/BLI or Carbapenem.

Material and Methods: A retrospective chart review was done at a tertiary care hospital of kidney diseases from October 2021 till June 2022. We included all adult patients with bUTI caused by CROr E-coli or *Klebsiella spp*, and they were excluded if bacteremia with no clear source or received antibiotics other than BL/BLI or carbapenems or received for less than 48 hours duration. Demographic characteristics, comorbidities, and clinical outcome were compared between BL/BLI and carbapenem groups.

Results: A total of 41 files were reviewed. The demographics, risk factors and clinical features were comparable. We did not find statistically significant difference in microbiological clearance, clinical failure, and mortality between BL/BLI and Carbapenem groups.

Conclusion: BL/BLI combinations are as efficacious as Carbapenems in CROr bUTI in terms of microbiological clearance. The clinical success and mortality were same in both groups.

Keywords: bUTI, *Klebsiella spp*, Carbapenems, BL/BLI, Mortality, Disease severity, Microbiological clearance.

BACKGROUND

Urinary tract infection (UTI) is one of the most common infections in community as well as in hospital setting. and clinical presentation ranges from simple cystitis to severe pyelonephritis.[1] It has been estimated that around 20 %-30% of patients with complicated UTI or pyelonephritis have bacteremia at the time of presentation.[2] Various risk factors have been identified for bacteremic UTI including diabetes mellitus with poor glycemic control, acute pyelonephritis, solid organ malignancy, elevated neutrophil count or c reactive protein and presence of pyuria.[3,4] *Escherichia coli* and *Klebsiella species*, the members of Enterobacteriaceae family are the predominant pathogens causing UTI.[5] Extended spectrum beta lactamase (ESBLs) and plasmid mediated AmpC beta lactamases production is the most important mechanism of resistance in these pathogens noted worldwide.[6] To treat to ESBL-producing gram negative infections the Carbapenems are the preferred antibiotics of clinicians. MERINO trial has not shown non-inferiority in 30 days mortality with piperacillin-Tazobactam compared to meropenem.[7] The authors recommend against use of piperacillin-Tazobactam for ESBL producing blood stream infection. However, Rodriguez-Bano *et al* in a post MERINO trial commentary concluded that piperacillin-Tazobactam should be considered as an alternative to avoid excessive use

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This article can be cited as: Rani B, Babar ZU, Dodani SK, Nasim A, Badlani S. Comparison of beta-lactam/beta-lactamase inhibitors with carbapenem for urinary bacteremia caused by ceftriaxone resistant *Escherichia coli* and *Klebsiella species*. Infect Dis J Pak. 2023; 32(4): 154-158. DOI: 10.61529/idjp.v32i4.239

Receiving date: **31 Oct 2023** Acceptance Date: **29 Nov 2023**
Revision date: **23 Nov 2023** Publication Date: **29 Dec 2023**

of Carbapenems in urinary tract infection or non-severe infection.[8] In a retrospective cohort study from Pakistan comparing carbapenem versus BL/BLI for ceftriaxone resistant E.coli bacteremia there was no difference was observed in mortality between two groups and urinary tract infection was found as a protective factor for mortality.[9]

Based on the previous evidence on significantly high mortality in CRO resistance E.coli and *Klebsiella spp* bacteremia other than non-severe infections, we designed our study to see any difference in mortality and clinical response in BL/BLI versus Carbapenems for treatment of ceftriaxone resistant E.coli and *Klebsiella spp* bacteremic urinary tract infections.

MATERIAL AND METHODS

This retrospective cohort study was conducted at Sindh Institute of Urology and Transplantation (SIUT) Karachi Pakistan the data was collected from October 2021 till June 2022. All adult patients with ceftriaxone resistant *E.coli* and *Klebsiella species (spp.)* bacteremia secondary to urinary tract infection were included in analysis. Positive blood cultures of above-mentioned pathogens during this period were collected from microbiology laboratory and medical record was reviewed of those patients who were admitted due to bacteremia originating from urinary source (bUTI) defined below and received either BL/BLI or carbapenem. Only one episode of bacteremia per person was included. Polymicrobial bacteremia and patients who received less than 48 hours of antibiotics were excluded.

Data was collected retrospectively from patient's file for baseline characteristics, clinical features and laboratory results, any recent antibiotics use, ICU stay, Charlson co morbidity index and Pitt bacteremia score were calculated for all patients at baseline.

Follow up on day 3 was recorded for bacterial clearance and clinical failure (Persistence of fever, leukocytosis and hypotension). New episode of bacteremia and all-cause mortality was noticed on day 14 and day 28.

The primary end point was all-cause mortality at day 14 and 28. The secondary end point were bacteriological clearance, clinical failure and new episode of bacteremia. Patients were divided into two groups. Those who received imipenem, meropenem or ertapenem were kept in Carbapenem group and who received piperacillin-Tazobactam, amoxicillin-clavulanate or cefoperazone-salbactum as BL/BLI group.

All blood cultures were performed at microbiological laboratory of SIUT, as per CLSI protocols. Susceptibility test was conducted by disc diffusion method. The results were analyzed as per CLSI break points.[10]

Bacteremia originating from urinary source (bUTI) is defined as blood culture showing growth of ceftriaxone resistant *E coli* or *Klebsiella spp* and symptoms/signs suggestive of UTI with or without positive urine culture of same organism and sensitivity.

If the patient presented with sign and symptoms of lower urinary tract (frequency, urgency, dysuria, decrease urine output) or with pyelonephritis (fever, vomiting, flank pain) or any radiological evidence of renal, perirenal collection or emphysematous pyelonephritis with or without renal failure and or requirement of hemodialysis.

All the data was entered and analyzed using SPSS version 22 for the results. The data was reported as mean and standard deviation for continuous variables if data were normally distributed while reported as median (IQR) if it was not normally distributed. Their mean difference or median were compared using unpaired "t" or Mann Whitney U test as appropriate. Categorical variables were reported as count while percentages and proportion difference were compared using Chi-squire or Fisher' Exact tests. P-value 0.05 was considered as statistically significant.

RESULTS

Among all files total 41 patients were found eligible for having *E.coli* and *Klebsiella spp* bacteremia originating from urinary tract and were analyzed. Of those 41 patients, 16 were received Carbapenem and 25 BL/BLI combinations. Mean age (SD) of study population was 51 ± 14.61 versus 43.76 ± 17.19 (Carbapenem and BL/BLI groups respectively) A total of 12 (29.2%) patients were diabetic and 25(60.9%) had renal failure in both groups. Clinical parameters at the onset of bacteremia including fever, leukocytosis, hypotension and altered mental status were comparable between groups (Table-1). We have found overall 28-day mortality of 14.6% (6.3% in Carbapenem and 11% in BL/BLI group) with no statistically significant difference between two groups (*p*=value 0.228). None of patient after 14 days of onset of bacteremia died within Carbapenem group, in contrast 5(11%) patients died in comparative group although did not reached statistical difference. Repeat blood cultures after 72 hours showed microbiological clearance 91.6% in Carbapenem group versus 88% in BL/BLI group (*p*=value 0.570). Other secondary outcomes (clinical failure and new episode of bacteremia) were also found to be comparable between the groups (Table-2).

Table-1: Demographics and clinical characteristics of bUTI due to ceftriaxone resistant *E.coli* and *Klebsiella spp* in Carbapenem vs BL/BLI group

Demographics Characteristics	**Carbapenem n= 16(%)**	**BL/BLI n= 25(%)**	**P Value**
Age Mean ± SD	51 ± 14.61	43.76 ± 17.19	0.172
Female	8 (50)	15 (60)	0.529
Diabetes Mellitus	6 (37.5)	6 (24)	0.281*
Renal Failure	9 (56.3)	16 (64)	0.620
On Hemodialysis	3 (18.8)	6 (24)	0.646
Transplant recipient	-	2 (8)	0.366*
Charlson Comorbidity Index>3	2 (12.5)	5 (11)	0.431*
Risk Factors			
Foleys	12 (75)	18 (72)	0.544*
PCNs	4 (50)	4 (16)	0.374*
Central Line	1 (6.3)	2(8)	0.666*
Recent antibiotics usage in last 1 month	5 (31.3)	10 (40)	0.375
Clinical and Lab Parameters			
Fever	8 (50)	16 (64)	0.375
Leukocytosis	10 (62.5)	12 (48)	0.364
Hypotension	2 (12.5)	5 (11)	0.431*
Altered mental status	1 (6.3)	1(4)	0.341*
ICU stay(n=6)	3 (18.8)	3 (12)	0.434*
Mechanical ventilation	1 (6.3)	1 (4)	0.634*
Pitt's≥ 4	-	2 (8)	0.366*

*-Fischer exact Test

Table 2: Comparison of primary and secondary Outcome between Carbapenem and BL/BLI groups.

Outcomes	**Carbapenem N= 16 (%)**	**BL/BLI N= 25 (%)**	**P-value**
Primary Objective			
14 Day mortality	-	5 (11)	0.071*
28-day Mortality	1 (6.31)	5 (11)	0.228*
Secondary Objective			
Microbiological clearance	11/12 (91.6)	15/17 (88)	0.570
Clinical Failure	4 (25)	7 (28)	0.564*
New Bacteremia in 28 days	2 (12.5)	2 (8)	0.512*

*-Fischer exact Test

DISCUSSION

Carbapenem use for ESBL producing Enterobacteriaceae has been subject of discussion for a long time because of risk of developing Carbapenem resistance.[12] In this cohort of ceftriaxone resistant *E.coli* and *Klebsiella spp* bacteremia originating from urinary source have shown no significant difference in terms of mortality, clinical failure and microbiological clearance.

Our findings are similar to post hoc analysis done by Rodriguez-Bano *et al* where no difference was found in mortality and length of hospital stay in Carbapenem or BL/BLI group. The authors therefore recommended using amoxicillin-clavulanate or piperacillin-Tazobactam as suitable alternatives to Carbapenems with blood stream infection due to ESBL Enterobacteriaceae.[13] Our findings are in concordance with Nasir *et al* showing urinary tract infection as protective effect on mortality in contrast to higher Pitt bacteremia score with BL/BLI combination for ceftriaxone resistant *E.coli* bacteremia.[9]

We have found overall mortality of 14.6% at day 28 with no difference between Carbapenem or BL/BLI group. Mortality caused by urosepsis has shown variable results. Tocut *et al* in a retrospective cohort of urosepsis have shown mortality of 6.3% - 12.7% in sensitive and resistant *E.coli* strain respectively.[14] Another retrospective study done by Tal *et al* in elderly patients with bacteremic UTI found in hospital mortality of 33%.[15] There is scarce data to the best of our knowledge on head to head comparison of bacteremic versus non bacteremic UTI in terms of mortality. This study is adding evidence to controversy about use of BL/BLI as an acceptable Carbapenem sparing option in ceftriaxone resistant *E.coli* and *Klebsiella* bacteremia of urinary origin. Our study has several caveats. Firstly, due to rare presentation of disease and included only one medical centre, we could able to obtain limited and small sample size. Secondly, due to retrospective data collection clinical information was limited to physician's note. Lastly this data cannot be generalized to other non-Enterobacteriaceae organisms.

CONCLUSION

In conclusion, our study shows no difference in mortality, clinical failure and microbiological clearance treated with either BL/BLI combination or Carbapenem. We recommend randomized controlled trial addressing use of BL/BLI and Carbapenems for bacteremia of urinary source.

CONFLICT OF INTEREST

None

AUTHOR CONTRIBUTION:

Beena Rani: Study design, data collection, data interpretation, data analysis, literature search

Zaheer Udin Babar: Literature search, questionnaire design and research writing.

Sunil Kumar Dodani: Data interpretation analysis methodology study design questionnaire design

Asma Nasim: Study design, literature search data analysis

Sanjay Badlani: Participate in data analysis, literature search methodology questionnaire format.

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REVIEW ARTICLE

THE MUCORMYCOSIS CRISIS: AN EPIDEMIC WITHIN A PANDEMIC

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ABSTRACT

With the second wave of COVID-19 in the April of 2021, the deadly mucormycosis outbreak surfaced in South Asia. Mucormycosis infection is an uncommon yet perilous angio-invasive fungal infection with a fatality rate of 54% globally. Immunocompromised COVID-19 patients with underlying comorbidities like diabetes mellitus and who were on prolonged steroid medications were the most vulnerable to develop this infection. Regionally, a highly disproportionate increase in COVID-19 associated mucormycosis (CAM) was observed. While there has been vigorous attention on the pathological risk factors, little attention has been paid to the environmental factors specific to different regions which may explain the grotesque increase in CAM cases in some regions while ordinary increase in the other. Recognizing the interplay between the two factors will aid epidemiologists in understanding the spread of the disease and help prevent future epidemics. This review analyzes the current literature on CAM to update clinicians on circumstances that lead to outbreak.

Keywords: Covid-19 Associated Mucormycosis, Mucormycosis, South Asia.

BACKGROUND

Along with the second wave of COVID-19 in April of 2021, the deadly mucormycosis surfaced both in Pakistan and India.[1] With the already collapsing healthcare systems due to the COVID-19 pandemic, both countries faced great challenges. Although, the situation in Pakistan was statistically better, it might possibly be due to insufficient surveys and an indeterminate number of unreported cases.[2,3]

Mucormycosis is an uncommon yet perilous, primarily angio-invasive fungal infection belonging to the order Mucorales and class Zygomycetes.[4] It is reported to be the third most common invasive mycosis infection after candidiasis and aspergillosis in immunocompromised patients.[5] Since most human infections from Zygomycetes class are caused by Mucorales, the terms mucormycosis and zygomycosis are often used synonymously. Mucorales are heat-resistant molds widely present in the environment, found in soil, decaying organic matter like rotten fruits and vegetables, compost piles, and animal excreta. However, its ubiquitous spread is still under study. It enters the body through ingestion, inhalation of spores or percutaneous route like inoculation into disrupted or abraded skin. The spores have no pathologic significance and are harmless in immunocompetent people.[6] Once inside the body of predisposed individuals, they germinate into hyphae and invades the blood vessels penetrating and eventually clogging them causing tissue necrosis that is characteristic black and dark in color and hence the name blank fungus is attributed to mucormycosis.[6,7] Depending upon the location of the invasion, six types of mucormycosis have been described, which are: (1) cutaneous, (2) pulmonary, (3) rhinocerberal, (4) gastrointestinal, (5) disseminated infection, and (6) uncommon presentations.[5,8] The most common occurring type is the rhinocerberal infection.[8]

It is a highly lethal infection and a fatality rate of 54% globally.[9]It has as rapid progression, a poor prognosis[8] and has been described as a 'time-sensitive' disease. If left untreated for merely six days, it can lead to a double-fold increase in the mortality rate. [10]The median time interval between the COVID-19 infection and the first appearance of the sign of mucormycosis was estimated to be fifteen days.[11]

MATERIAL AND METHODS

A comprehensive literature search was conducted from May to December 2021 using the following search terms: "COVID-19", "Covid-19 associated mucormycosis", "mucormycosis", and "South Asia" and BOOLEAN operators "AND" and "OR" were applied. The search terms were used as keywords and in combination as MeSH terms. We identified a total of (n=1,148) articles by searching databases, including PubMed (n=183), Scopus (n=207), and Google Scholar

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***This article can be cited as:* Amir A**, Hanif F. The mucormycosis crisis: An epidemic within a pandemic. Infect Dis J Pak. 2023; 32(4):159-164.
DOI: 10.61529/idjp.v32i4.240

Receiving date: **31 Jul 2022** Acceptance Date: **05 Dec 2023**
Revision date: **11 Nov 2023** Publication Date: **29 Dec 2023**

(n=758). After removing duplicates, screening and assessment for eligibility, (n=28) studies were included in the final review. These included original and review articles, brief communications, and published guidelines. In addition, a manual search of articles and other relevant studies was conducted and was included in the review that were missed in initial search. Articles in language other than English and articles regarding Apergillosis, Candidaisis and other opportunistic fungal infections were excluded.

DISCUSSION

Burden of CAM in Pakistan: Before the spread of COVID-19, Mucormycosis was mostly prevalent in immunocompromised patients with blood dyscrasias, malignancies and individuals who had undergone organ transplants. [7] While there is a paucity of data regarding the precise burden of overall fungal diseases in Pakistan, reports indicate high rates of mucormycosis infections even prior to the onset of the COVID-19 pandemic.[12] A study from 2017 estimated the rate of Mucormycosis infection in Pakistan to be around 14 per 100,000 (Non-Covid Associated Mucormycosis cases).[3] Nonetheless, a rapid increase in the prevalence of this infection was observed during the rise of COVID-19 cases in 2020-21, especially in India, where its prevalence was 80 times higher than the developed countries, estimated at 140 per million population.[7,13] Similarly, CAM rampantly became a cause of concern in different regions of Pakistan, as highlighted by the aforementioned studies. A multi-center, retrospective observational study conducted in Pakistan during the third wave of COVID-19 in 2021 aimed to observe the frequency and survival of CAM patients across three tertiary care hospitals in Punjab. Among the 43 documented cases of mucormycosis, 22 had a prior history of COVID-19 and diabetes mellitus was reported to be the most common co-morbid (88.4%).[14] Additionally, a case series highlighted the association between mucormycosis and COVID-19 infection in Pakistan, emphasizing the need for optimal glycemic management and early detection of mucormycosis to decrease morbidity and mortality associated with the disease.[15] These studies collectively underscore the significance of CAM in Pakistan and further emphasize the need for further research and surveillance to understand the specific epidemiological tendencies in the region.

Circumstance – what went down?: The circumstances that lead to a rise in COVID-19 associated mucormycosis (CAM) cases are multifactorial and may be attributed to many causes. When demands surpass the capacity of hospitals, reckless healthcare crisis becomes inevitable. Scarcity of medical equipment, supply shortages, burnout medical staff, and overcrowded hospitals and intensive care units due to the COVID -19 pandemic only exacerbated the situation. [16]

For instance, the high rise in mucormycosis infection could be associated with the inadequate supply of sterilized water for oxygen humidification in hospitals. Unsterilized water has a high possibility of being infected with fungal spores, which might be associated with an increased risk of mucormycosis transmission.[11]

A study of three immunocompetent non-diabetic patients with high steroid intake, who were on supportive oxygen ventilation reported with rhino-cerbero-orbital mucormycosis (RCOM) which might be indicative of mucormycosis infection secondary to exposure of unsterilized water for oxygen humidification.[14]

Diabetes mellitus (DM) is an important underlying risk factor in the pathogenesis of mucormycosis.[17–19] Therefore, in order to understand the epidemiology of CAM, it is important to pay attention to DM cases regionally. According to the International Diabetes Federation, in 2021 India has the second-highest rate of DM cases (74.2 million) in the world after China (140.9 million), whereas Pakistan has the third highest cases (33 million) and an estimated 26.9% of adults in Pakistan are living with undiagnosed DM. [17]Moreover, the comparative prevalence rate for Pakistan in 2021 is estimated to be around 30.8%, highest in the world. A retrospective observational multi-centric analysis of 18 cases of RCOM illustrated a strong correlation between immunocompromised SARS-CoV-2 patients, diabetes mellitus, and steroid intake.[20] Another cross-sectional multi-centric study in Iran comprising of 15 cases of CAM depicted a high incidence of DM (87% cases), of which 46% were being treated with systemic corticosteroids, and 60% had nasal oxygen supplementation.[19]

Moreover, the administration of systemic corticosteroids for treating symptoms of COVID-19, as recommended by the World Health Organization (WHO), did mitigate the effects of COVID-19 symptomatically but also caused major drawback, i.e. the injudicious and unnecessary use of systemic corticosteroids. [21] A study in Pakistan revealed that the majority of the population in the second wave of COVID-19 consumed corticosteroids like dexamethasone as an early treatment of SARS-CoV-2 which resulted in delayed viral clearance.[22] This may also explain the CAM outbreak in the country following the second wave.[12]

Another plausible factor is that mucormycosis is complex to diagnose in its early stages and requires aggressive diagnostic strategies, posing great challenges for physicians.[2,8] It presents with non-specific symptoms like fever, headache, nasal and sinus congestion.[2,9]The diagnosis primarily lays on traditional methods like microscopic examination and culture tests for detection of mucorales which are time and labor intensive and require tissue biopsy specimens. The study of new, standardized, molecular and genetic based test like PCR assays is still being investigated. [8,23] In a developing country like Pakistan, unavailability of diagnostic laboratory facilities and anti-fungal susceptibility tests and lack of expertise in the area of antimicrobial examination imposed even greater challenge in the early identification of this disease.[2] As the CAM cases increased, cost of amphotericin B, the first-line antifungal drug for the treatment of mucormycosis infection also skyrocketed.[2,10] Research suggests that one infected person requires 20 vials of the liposomal amphotericin-B injection for treatment. Its non-availability further aggravated the situation.[21,24]

Regional Factors – A "missing link"?: Depending upon the individual perceptions and understanding, combined with a heavy influence of political norms and religious biases, different communities reacted differently to cope with the stresses of the COVID-19 pandemic, its treatment and prevention.[25–27] As described earlier, the two main factors that increase the risk of CAM were a great many DM cases and an unwarranted use of corticosteroids. However, the disproportionate increase of CAM cases in India as compared to other South Asian countries like Pakistan where DM is as prevalent[12] and where corticosteroid use was also reported to be high,[16] remains unclear. However, there have been hypotheses formulated which may explain the "missing link" in the vehement burden of CAM cases, and which may be attributed to a unique interplay of environmental and socio-cultural factors specific to India.[25] These are as follows: Firstly, use and burning of cow-excreta including dung and urine. This included burning cow dung cakes, drinking cow urine and other practices. States where cow slaughter was banned and use of cow excreta was less, like the state of Kerala showed a relatively smaller number of CAM cases as compared to areas like Tamil Nadu that faced highest load of CAM. Secondly, crowding in religious festivals including community bathing in holy river waters, for instance, the *Ganga* river. Thirdly, due to the increased COVID related deaths, burning of the corpses at the river embankments escalated in parallel. This significantly increased the water borne spread of mucroles.[25] In addition, converting and reconstructing hospitals into infectious disease control wards to in order to receive and a large inflow of COVID-19 patients is also related to increase risk of airborne spread of fungal spores.[24] On the other hand, in Pakistan these sociocultural factors were absent which may explain a comparatively small caseload of CAM in the country. Regionally, the major challenge in Pakistan was an underdeveloped, fragile health care system which was second to the widespread culture of denial amongst the general public and renunciation of the COVID-19 pandemic itself; dismissing it as a "Western conspiracy" or a "political game". This led to a jeopardized state of the COVID-19 standard operating procedure (SOP) and required informed public healthcare measures to be strictly implemented. [26,27]

Pathophysiological mechanism: Several pathophysiological mechanisms of SARS-CoV-2 increase the likelihood of an individual to opportunistic bacterial and fungal infections. Firstly, the cytokine storm syndrome in severe COVID-19 infection; a hyper-inflammatory state. SARS-CoV-2 is associated with increased production of pro-inflammatory cytokines, such as IL-6, IL-2 and TNF Alpha, that mediate inflammatory cascades resulting in cytokine burst and immune dysregulation. If not controlled, it can cause acute respiratory distress syndrome (ARDS), disseminated intravascular coagulation (DIC) or multiple organ failure.[28] Hence, to alleviate the effects, clinicians used immunosuppressive drugs which proved to be helpful. However, due to suppressed immune system, patients had a higher risk of catching opportunistic infections.[20]Additionally, there is increased likelihood of developing Insulin resistance and beta-cell dysfunction as SARS-CoV-2 can infect the islets cells of pancreas causing decreased production of insulin. In addition, increased levels of IL-6, as seen in COVID-19, are also linked to insulin resistance.[11]

Furthermore, COVID-19 is associated with the development of ketoacidosis, a potentially fatal metabolic complication, whether the patient has DM or not previously. Ketoacidosis or ketosis causes the pH of the blood to fall and become acidic. The acidic pH permits the germination of spores into filamentous hyphae structures that latch onto the blood vessels, blocks them and causing ischemia and tissue necrosis. This markedly hinders the chemotaxis of polymorphonuclear cells and also deters the phagocytic activity of the T-cells resulting in immune dysregulation, therefore, increasing the susceptibility of an individual to acquiring opportunistic infections.[8,11]

Moreover, a recent study suggested a strong association between iron overload and CAM pathogenesis.[29] In fact, Severe COVID-19 infection is believed to be a hyper-ferretinemic state.[11] After entering the host, the virus attacks the hemoglobin in the RBCs causing the release of free iron in the circulation. To compensate for this increase, the production of ferritin; a protein that stores iron is stimulated. Excess ferritin levels cause death of the hepatocytes and subsequent release of iron from ferritin, leading to further increase of free iron in the blood.[30] In addition, acidosis and raised IL-6 levels associated with covid-19 are also linked to intracellular iron overload.[11,31] This is problematic because of two reasons. Firstly, excess free unbound iron plays a role in producing reactive oxygen species (ROS) and is linked to ferroptosis and hypercoagulative state observed in severe forms of COVID-19.[30] Secondly, pathogens in the host require a source of exogenous iron for growth and multiplication.[21] All these factors provide a perfect environment for mucormycosis to nourish and thrive in. Therapy with deferoxamine (DFO), an iron chelator used for the treatment of DKA and kidney failure patients who undergo routine dialysis is contraindicated in CAM and can reportedly cause a twofold risk of mucormycosis in immunocompromised COVID-19 patients.[3]

Additional factors include usage of certain drugs. For instance, overconsumption of steroid drugs like dexamethasone proved to be one of the leading causes as it is associated with lymphocytopenia, hyperglycemia and subsequently weakened immunity.[21] Moreover, the use of anti-IL-6 drug like Tocilizumab, prescribed for limiting the pro-inflammatory cytokine release in treating severe forms of COVID-19 infections like ARDS, also hampers the immune system rendering the body weak.[7,21] All these factors have been shown to ramp up the pathogenesis of CAM.

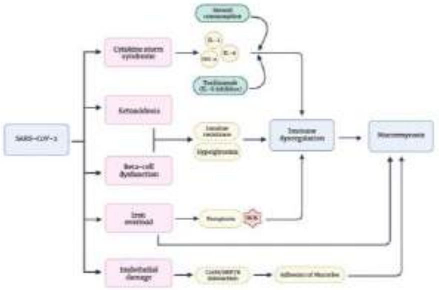

Figure-1: Illustrating the Pathophysiological Factors of Covid-19 Associated Mucormycosis (CAM).

Management and Treatment Approaches: Considering the fatal outcome of CAM and the rate with which the cases were surging, the European Confederation of Medical Mycology (ECMM) and the International Society for Human and Animal Mycology (ISHAM) composed standardized diagnostic criteria and a comprehensive management plan, including treatment approaches and preventive measures specifically devised for low- and middle-income countries.[32]

Global ECMM-MSG guidelines stated for CAM management are much alike the guidelines for non-COVID-19 mucormycosis patients.[31] For effective management of the disease, timely diagnosis using clinical, histopathological and radiological components remains as an indispensable factor.[7,32,33] Drug therapy with amphotericin B, and in case of its unavailability, treatment with other antifungal drugs have been stated clearly in the ECMM/ISHAM guidelines. One study has shown the survival rate in RCOM patients to be doubled (61%) if treatment with anti-fungal medication like amphotericin-B is started within the first twelve days of appearance of symptoms and it drops down to 33%, if started after thirteen days. Therefore, prompt anti-fungal treatment is crucial for the containment of the disease.[34]

However, due to unavailability or intolerance, Posaconazole and Isavuconazole may also be used. But these are generally recommended as prophylaxis of mucormycosis infections.[35] In case of unavailability of Isavuconazole and posaconazole, Itrocanzole; another azole derivative may be recommended for mucormycosis management. However, its efficacy against Mucorales requires further trials and research.[32]

Iron chelators as adjunct therapy have also been effective in patients with elevated serum iron levels.[7]

Moreover, timely surgical intervention for local containment of the infection is crucial. A recent study showed that CAM patients subjected to surgical therapy in adjunct to antifungal treatment had a survival rate of 64.96%.[7] Resection and debridement of the necrotic tissue for RCOM and affected lobe or lung in pulmonary mucormycosis are recommended. Surgical resection of RCOM requires a team of specialists like otolaryngologists, ophthalmologists and neurosurgeons.[32]

Therefore, a multidisciplinary approach involving microbiology and pathology specialists, critical care, ophthalmology, neurosurgery, and maxillofacial surgery have proved to be imperative in decreasing the morbidity and mortality rate as well as evading possible complications.[4,21,32]

Preventative measures were also stated in the ECMM/ ISHAM guidelines. For instance, stringent checks on the glycemic control of diabetic patients, evidence-based use of systemic corticoids, use of surgical masks, and a high index of suspicion for signs of mucormycosis such as, facial ache, nasal blockage, swollen gums and loose teeth were suggested.[32]

CONCLUSION

Given the urgency of the circumstances, a global response was needed to curb the outbreak. This epidemic has made us learn that fungal infections cannot be ignored as a co- or super-infection in COVID-19 patients. Healthcare providers need to be vigilant and well aware, especially in high-risk patients. There is still limited research on the sequence of molecular mechanisms and pathogenesis of CAM. Moreover, the role of diagnostic imaging, medicinal and surgical interventions in the prognosis of the disease is under study. The solution demands a collaborative approach to better understand the epidemiology and prognostic importance of the disease, its multifaceted nature during the pandemic, and potential preventive methods to avoid future mucormycosis outbreaks.

CONFLICT OF INTEREST

None

AUTHOR CONTRIBUTION:

Asma Aamir: Study conception and design, Analysis and Interpretation of results

Faisal Hanif: Study conception and design, draft manuscript preparation

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SYSTEMATIC REVIEW

Prevalence of human papilloma virus 16 and 18 in oral squamous cell carcinoma patients: A systematic review

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ABSTRACT

Background: HPV 16 and 18 are the two strains of Human Papillomavirus that have been associated with the development of OSCC. The aim of this study was to assess the prevalence of HPV 16 and18 in Oral Squamous Cell Carcinoma patients in comparison to controls.

Data Sources: Literature search was conducted on PubMed, Scopus, and Google Scholar. All relevant studies till May 2025 were included. The search strategy combined Medical Subject Headings and relevant keywords. PRISMA guidelines were followed.

Study Selection: Studies conducted on OSCC patients in which prevalence of HPV 16 and 18 was studied in comparison to controls were included. Studies that assessed the prevalence of HPV 16 and 18 in HNSCC, pre-malignant lesions, any other carcinoma and or strain of HPV other than 16 and 18 were excluded.

Data Extraction: Data included publication title, year, authors, study design, sample size, prevalence of HPV 16 & 18 in cases and controls, number of cases and controls and detection methods. Quality assessment was done using Newcastle Ottawa scale.

Data Synthesis: HPV 16 had higher prevalence in OSCC patients as compared to controls. Both the virus strains were seldom found in healthy controls.

Conclusion: HPV 16 can have a significant role to play in OSCC development. HPV 16 (and HPV 18 to a lesser extent) is more prevalent in OSCC. Therefore, it may not be the primary cause but it has role to play in the development of OSCC.

Keywords: Oral Squamous Cell Carcinoma, Human Papilloma virus, Polymerase chain reaction

BACKGROUND

Oral squamous cell carcinoma is the sixteenth most common type of malignancy across the globe.[1] Not only that, it also accounts for 95% of all head and neck malignancies.[2] Despite advances in cancer related treatment modalities, OSCC is associated with severe disease and treatment related morbidity, poor disease-free survival and with high recurrence rates.[3,4] OSCC usually results from exposure of tobacco associated carcinogens that amass a series of genetic and epigenetic alterations resulting in oncogene activation and inactivation of tumor suppressor gene.[5,6] The resultant genetic changes impart proliferative and survival adaptations to the mutated cells characterized by cell division independent of growth factors, resistance to apoptotic signaling and an increased ability to invade adjacent structures by moving through the tissues of the extracellular matrix.[5,7,8]

But even in the absence of these established risk factors of tobacco and alcohol 15% to 20% of patients still develop OSCC(9). Also younger patients have a short exposure time to these risk factors so their role in these patients is yet not fully understood.[9,10] Keeping this in view it has been suggested that there might be other causes of OSCC such as genetics, diet and viral agents.[9] Exposure to Human papilloma virus or HPV is an established risk factor for anogenital and oropharyngeal squamous cell carcinoma.[9] But its role in the pathogenesis of OSCC remains controversial.[9]

HPV is a double stranded DNA virus that exhibits tropism for squamous epithelium. Till date 202 subtypes of HPV have been identified.[11] Based on its

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This article can be cited as: Tariq AB, Huda NU, Aman M, Butt H, Shumyle DE, Khan NR. Prevalence of human papilloma virus 16 and 18 in oral squamous cell carcinoma patients: A systematic review. Infect Dis J Pak. 2023; 32(4):165-172.

DOI: 10.61529/idjp.v32i4.408

Receiving date:	**01 Jun 2023**	Acceptance Date:	**10 Dec 2023**
Revision date:	**11 Sep 2023**	Publication Date:	**29 Dec 2023**

oncogenic potential it is subcategorized into high risk (hr) or low risk (lr) types. High risk HPV which includes subtypes 16, 18 and 33 is associated with malignant proliferation.[11] While low risk HPV including subtypes 6, 11 and 32 is associated with benign mucosal lesions.[11] The mechanism through which high risk HPV induces malignancy is primarily by viral integration whereby the virus integrates its genome into that of the host.[12] The specify fragments to be inserted are E6 and E7.[12] This insertion leads to the inhibition of tumor suppressor gene functions resulting in unregulated cell proliferation, apoptosis and genetic stability.[12]

HPV 16 and 18 has been associated with and established as an etiological factor of cervical cancer.[13] Their pathogenesis includes integration of viral DNA into epithelial cells of host.[14] This leads to the disruption of tumor suppressor genes. These genes include p53 and Rb.[14] If dysplasia caused by these viral strains are left undetected or un-treated, it can progress to neoplasia of the cervical intra-epithelial cells which culminates in invasion of the carcinoma.[15]

Studies have shown the presence of human papilloma virus DNA in oral squamous cell carcinoma, nevertheless these studies have shown great variability in viral prevalence that is explained by several factors.[9] There can be discrepancies in the sample collection and preservation methods, the sensitivity of tests conducted to detect the virus, and there is little proof of the actual oncogenic potential of HPV since the presence of HPV DNA alone is insufficient evidence for an association.[9] This review was conducted to assess the prevalence of HPV 16 and 18 in OSCC patients and healthy controls. The objective of this systematic review was to assess the prevalence of HPV 16 and18 in Oral Squamous Cell Carcinoma patients in comparison to health controls.

MATERIAL AND METHODS

1. Data sources: An extensive search of literature was conducted across the databases of PubMed, Scopus, and Google Scholar. A search of grey literature was carried out through ProQuest. No language, publication year and geographical restrictions were applied. The search strategy combined Medical Subject Headings (MeSH) and relevant keywords including: "Oral Squamous Cell Carcinoma (OSCC)," "Human Papillomavirus," "HPV 16," "HPV 18," and "Polymerase Chain Reaction (PCR)." Boolean operators such as AND and OR were applied to refine the search. Reference lists from relevant articles and previously conducted reviews were manually screened. The review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Figure 1 outlines the PRISMA flow diagram used for study selection. All database searches, article screening, and data extraction were independently performed by two reviewers to ensure methodological rigor and minimize bias.

2. Study selection: The process of search was conducted by two reviewers who were independent. The preliminary search was carried out based on titles and abstracts. This was done in order to select articles for full text review. Full text articles were re-assessed based on the inclusion criteria and the selected articles were then subjected to quality assessment.

Inclusion criteria: The inclusion criteria were:

1. Studies conducted on diagnosed cases of Oral Squamous Cell Carcinoma patients
2. Studies in which prevalence of HPV 16 and 18 was studied in Oral Squamous cell carcinoma patients in comparison to healthy controls.

Exclusion criteria: The exclusion criteria were:

1. Studies that assessed the prevalence of HPV 16 and 18 in Head and Neck Squamous cell carcinoma and not specifically on Oral Squamous Cell Carcinoma patients.
2. Studies that assessed the prevalence of HPV 16 and 18 in oral potentially malignant lesions or pre-malignant lesions.
3. Studies that included any strain of HPV other than 16 and 18.
4. Studies that assessed the prevalence of HPV 16and 18 in any other cancer

3. Data extraction: An extensive search of literature was conducted across the databases of PubMed, Scopus, and Google Scholar. A search of grey literature was carried out through ProQuest. The key terms used for searching data were Oral Squamous Cell Carcinoma (OSCC), Human Papilloma virus 16 and 18 and polymerase chain reaction (PCR). The retrieved data from studies including title of publication, year of publication, authors, study design, sample size, prevalence of HPV 16 in cases and controls, HPV 18 prevalence in cases and controls, number of cases and controls and HPV detection methods.

4. Quality Assessment: New castle Ottawa scale (NOS) was the scale used for quality assessment of the studies included in this systematic review. Based on this scale the studies were assessed on the points of comparability (2 points), selection (4 points) and exposure (3 points). The studies are then classified as high, moderate and low quality based on the points. Studies with a score of 7-9 are considered high quality, 4-6 are moderate while <4 are low quality studies.

All the studies included in this systematic review were high quality studies. They were assessed on the New Castle Ottawa scale and it was seen that they fulfilled all three criteria. The criteria of selection were fulfilled as the recruitment and selection of cases and controls was done using the appropriate methodology. By controlling the confounding factors of age, gender and tobacco use, the criteria of comparability were also addressed. Lastly, the exposure assessment was appropriately carried out as PCR technique was used to detect Human Papilloma virus. The details of scoring of the included study based on NOS has been shown in Table-I.

5. Data Synthesis: Table-II shows a comparison of the prevalence of HPV 16 and 18 in patients with Oral Squamous Cell Carcinoma and healthy controls. These studies assess if these two strains of HPV are more prevalent in OSCC patients as compared to healthy individuals without OSCC.

It was seen that HPV 16 had high prevalence in OSCC patients as compared to controls. The overall prevalence of HPV 18 was less as compared to HPV 16 except in a study conducted by Purwanto et al[17] in Indonesia where the prevalence of HPV 18 was higher than HPV 16 in OSCC patients as compared to controls. A similar trend was seen in the study conducted by Rubab et.al.[19] Both the virus strains were seldom found in healthy controls. While the occurrence can be quoted as a less frequent, it cannot be reported as zero. This low prevalence of the viral strains in controls and higher prevalence in OSCC patients suggests that they do have a role to play in etiology of OSCC. The analysis of these studies revealed that the prevalence of HPV 16 and 18 across all the countries. The prevalence was much higher in countries like Iran and China.[16,20] The prevalence was much lower in Pakistan and Indonesia among OSCC patients.[17,19] While majority of the studies used PCR, the most sensitive detection technique for HPV, some also employed the techniques of hybridization and DNA sequencing to confirm the presence of the virus.

Excluded Studies: A total of 20 studies were excluded from the systematic review. The list of these studies and the reason for exclusion have been mentioned in the Table-III.

Table-I: Quality assessment of the included study based on New Castle Ottawa scale

S #	Study (Author, Year)	Country	Selection (total score 4)	Comparability (total score 2)	Exposure (total score 3)	Total Stars (total score 9)	Quality Rating	Reference
1.	**Tabatabai *et al.*, 2015**	Iran	4	2	3	9	High	(18)
2.	**Purwanto *et al*, 2020**	Indonesia	4	2	3	9	High	(20)
3.	**Chowdary *et al, 2018***	India	3	0	2	5	Moderate	(21)
4.	**Baig *et al, 2018***	Pakistan	4	1	2	7	Good	(22)
5.	**Gan *et al,2014***	China	4	1	2	7	Good	(23)
6.	**Phu Singha *et al*, 2017**	Thailand	4	2	3	9	High	(24)

Table-II: Comparative analysis of the included studies.

Sr #	**Study (Author, Year)**	**Country**	**Sample Size (OSCC/Control)**		**Detection Method**	**HPV-16+ only in OSCC (%)**	**HPV-16+ only in Controls (%)**	**HPV-18+ only in OSCC (%)**	**HPV-18+ only in Controls (%)**	**HPV 16 & 18 co-infection**		**Key Findings**	**Reference**
			OSCC cases	**Controls**						**OSCC**	**Controls**		
1.	**Tabatabai et al., 2015**	Iran	39	27	PCR	12 (30.7%)	0 (0%)	0 (0%)	0 (0%)	5 (12.8%)	0 (0%)	HPV-16 and -18 present in OSCC, none found in controls	(18)
2.	**Purwanto et al., 2020**	Indonesia	78	79	PCR	1 (1.3%)	3 (3.8%)	12 (15.4%)	0 (0%)	1 (1.3%)	0(0%)	HPV-18 more common in OSCC; HPV-16 more in controls	(20)
3.	**Chowdary *et al,2018***	India	20	20	PCR	6 (30%)	3 (15%)	3 (15%)	1 (5%)	2 (10%)	2(10%)	Prevalence of HPV 16& 18 higher in OSCC cases	(21)
4.	**Baig *et al,2018***	Pakistan	100	200	PCR	4 (4%)	2 (1%)	5 (5%)	4 (2%)	23 (23%)	3(1.5%)	Relatively higher prevalence of both strains in OSCC cases. Co-infection of HPV 16 and 18 was highly prevalent.	(22)
5.	**Gan *et al, 2014***	China	200	68	PCR	39(19.5 %)	0 (0%)	15(7.5%)	2(2.9%)	0(0%)	0(0%)	Both viral strains prevalent in OSCC cases more than the controls. HPV 16&18 co-infection was not seen	(23)
6.	**Phu Singha *et al,* 2017**	Thailand	80	100	PCR + Hybridization	13 (16.3%)	11 (11%)	1(1.3%)	0(0%)	0(0%)	0(0%)	HPV-16 higher in OSCC as compared to controls.	(24)

Table-III: Record of the studies excluded from the systematic review.

Sr #	Article excluded	Reason for exclusion	Reference
1.	Kulkarni *et al*	Study was conducted on carcinoma of the cervix and not only on OSCC	(8)
2.	Kaewmaneenuan et. al	Oral potentially malignant lesion	(25)
3.	Jalouli *et al*	Oral potentially malignant lesion	(26)
4.	Xhang *et al*	Oral potentially malignant lesion	(27)
5.	Chang *et al*	• HPV strains other than 16 and 18 were studied • Study did not include healthy controls	(28)
6.	Laco *et al*	• HPV strains other than 16 and 18 were studied • The study included oropharyngeal Squamous cell carcinoma as well.	(29)
7.	Popvi`c *et al*	Study did not include healthy controls	(30)
8.	Shroyer *et al*	Oral potentially malignant lesions were included	(31)
9.	Menezes *et al*	Study did not include healthy controls	(32)
10.	Rajesh *et al*	Study did not include healthy controls	(33)
11.	Shaikh *et al*	Study did not include healthy controls	(34)
12.	Kreimer *et al*	Study was conducted on Head and Neck Squamous cell carcinoma and not OSCC in particular	(36)
13.	Quintero *et al*	Study was conducted on Head and Neck Squamous cell carcinoma and not OSCC in particular	(37)
14.	Kabagenyi *et al*	Study was conducted on Head and Neck Squamous cell carcinoma and not OSCC in particular	(39)
15.	Azhar *et al*	Study did not include healthy controls	(41)
16.	Sri *et al*	Oral potentially malignant lesions were included	(42)
17.	Yang *et al*	Oral potentially malignant lesions were included	(43)
18.	Khasawneh et. al	Study was conducted on Head and Neck Squamous cell carcinoma and not OSCC in particular	(44)
19.	Parshad et. al, 2015	Study did not include healthy controls	(45)
20.	De Abreu *et al*, 2018	Study did not include healthy controls	(46)

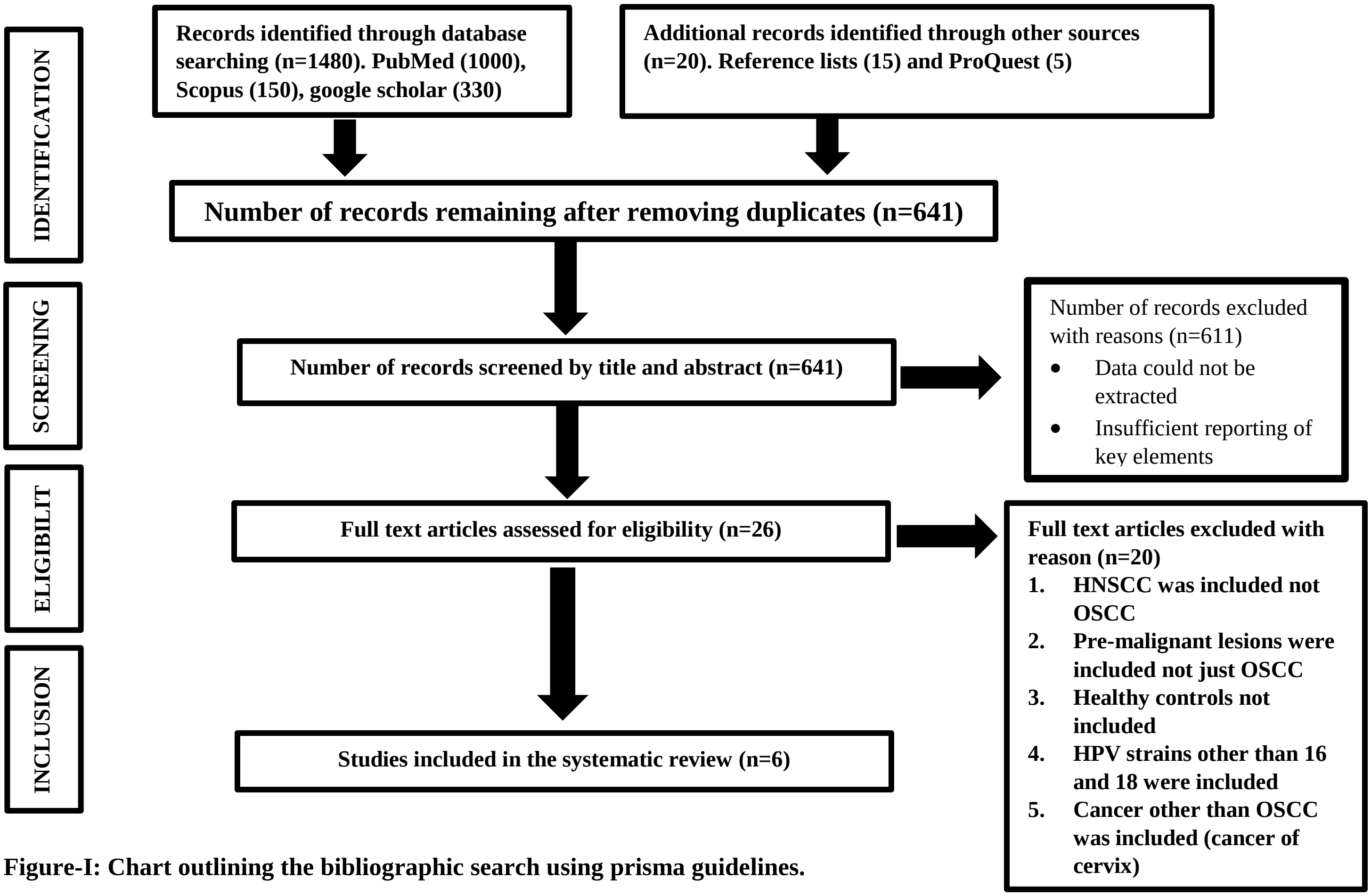

Figure-I: Chart outlining the bibliographic search using prisma guidelines.

DISCUSSION

In this systematic review most of the studies reported that the prevalence of HPV 16 was much higher in OSCC patients as compared to controls. For example, according to a study conducted in Iran by Tabatabai et al, the prevalence of HPV 16 in OSCC patients was 15% as compared to 3.7% controls.[16] Similarly, Purwanto et al reported a similar trend with a 23% prevalence of HPV 16 in cases as compared to 1.5% controls.[17] This indicates a very strong association between occurrence of this virus strain and OSCC. These findings are similar to those reported by a metanalysis, where the HPV 16 prevalence was 20.1% as compared to a very negligible prevalence in controls further solidifying the evidence that there may be an association between OSCC pathogenesis and HPV 16.[41]

Regarding the prevalence of HPV 18, it was seen that in this systematic review, studies revealed variable results. While one study conducted by Purwanto et al reported a higher prevalence of HPV 18 (15.4%) in OSCC patients as compared to control, where there was no detection of the virus.[17] Many studies have reported findings similar to these. A study conducted in China reported 19.5% prevalence of HPV 16 and 7.5% of HPV 18 in OSCC patients as compared to controls (2.9%).[20] The high prevalence of HPV 18 in OSCC cases indicates that there can be a role of this virus in the pathogenesis of OSCC.

The prevalence of HPV is influenced by geographical variation as well.[42] Among our included studies, it was seen that in studies included form China[20] and Iran[16], the prevalence of both viral strains was substantially higher in patients with OSCC. On the contrary a study conducted in Indonesia by Parwanto et al reported a higher prevalence of HPV 16 in controls as compared to OSCC cases.[17] These differences in prevalence due geographical variations can be attributed to the varying risk factors. These include a genetic predisposition to development of disease, oral health and hygiene, sexual practices and use of tobacco.[43]

Polymerase chain reaction is a standardized detection test used for HPV.[11] The sensitivity and specificity of this test has long been established and is widely used for detection purposes.[11] In this systematic review also, PCR technique was the most widely used technique. While this is true, some studies also used DNA sequencing for confirmation. One such study was that conducted by Lima et al in China.[12] Employing various detection techniques can also be responsible for the variation in results across our included studies.

The association of HPV 16 with OSCC across different studies highlights the important issue of HPV vaccination.[44-47] The high prevalence of HPV 16 and comparatively variable prevalence of HPV 18 indicates their potential role in the pathogenies of OSCC and underscores the importance of development of a vaccine for this virus which could lead to substantially reducing the incidence of OSCC.

CONCLUSION

The comparative analysis of our selected studies with additional literature revealed that HPV 16 can have a significant role to play in OSCC development, with HPV-18 also playing a role, albeit with variable prevalence. HPV 16 (and HPV 18 to a lesser extent) is more prevalent in OSCC. This supports the hypothesis that while it may not be the primary cause but it has role to play in the development of OSCC. Additionally, the variation in prevalence of both virus strains in cases and controls can be attributed to geographical variations and detection methods. Two main factors imparted strength to this systematic review. Firstly, the inclusion of healthy controls in studies allowed us to draw more meaningful information regarding role of HPV in OSCC. It allowed for a comparison of the prevalence of virus in OSCC cases in comparison to healthy controls. Secondly, studies from various geographical regions were included which enabled us to get an insight in prevalence of and association of HPV with OSCC across different regions. One weakness of this study was that since this was not a meta-analysis, due to the lack of quantitative analysis the findings of this systematic review is not statistically supported and therefore, the results and conclusion are mainly descriptive.

CONFLICT OF INTEREST

None

AUTHOR CONTRIBUTION:

Ahmer Bilal Tariq: Concept and design, literature review, manuscript write-up

Noor ul Huda: Literature review and manuscript write-up

Muhammad Aman: Literature review and manuscript write-up

Hira Butt: Concept & design, literature review, manuscript write-up, supervision, critical revision and final approval

Dur E Shumyle: Literature review and manuscript write-up

Nauman Rauf Khan: Concept& design, literature review, supervision and final approval

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