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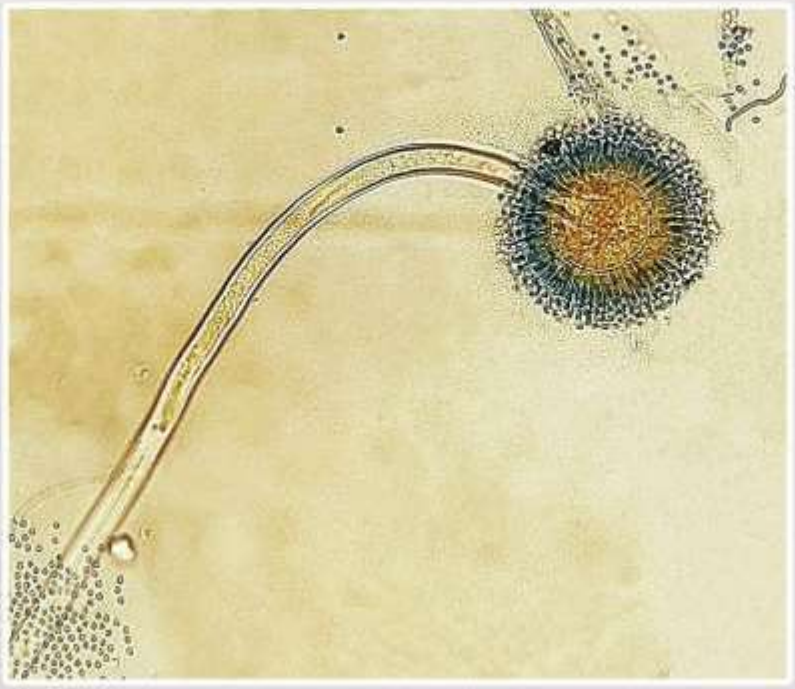

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CONTENTS

ORIGINAL ARTICLE

Clinical outcomes of remdesivir in patients with COVID-19 infection: An observational study from a tertiary care hospital in Pakistan

Sher Muhammad Sethi[1], Memoona Irshad[2], Roodaba Iqbal[1]

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ABSTRACT

Background: The primary objective was to assess the change in oxygenation status of patients after remdesivir treatment.

Material and Methods: This retrospective cross-sectional study was conducted at the Aga Khan University Hospital, Karachi from September to December 2020. All patients aged >18 years admitted with a positive reverse transcriptase-polymerase chain reaction for COVID-19 were included. Infection severity was subcategorized into two groups (moderate-to-severe and critical). We compared oxygenation status and assisted ventilation before and after remdesivir treatment. An analysis of outcomes between the groups was conducted using chi squares and the student t-test at a significance level of <0.05.

Results: We had 213 COVID-19 patients, of whom 114 (53.5%) received remdesivir during their hospital stay. 69 (60.5%) patients were male and the mean age was 52±12 years. 56 patients (49.1%) had moderate-to-severe infections. 21 out of 56 patients (37.5%) with moderate-to-severe COVID-19 infection while 47 out of 58 patients (81%) with critical COVID-19 required oxygen following remdesivir treatment (p-value: <0.001). Out of 58 critical COVID-19 patients, 46 patients (79.3%) were on non-invasive ventilation and 12 patients (20.7%) were on invasive ventilation prior to remdesivir therapy. 22 out of 46 patients (47.8%) recovered from non-invasive ventilation after remdesivir treatment (p-value: <0.001). 15 patients had mortality (13.1%) while the mean length of hospital stay with moderate-to-severe COVID-19 infection was 6.8 days and with critical COVID-19 infection was 11.3 days.

Conclusion: The study emphasizes that remdesivir was found to be clinically beneficial in patients with moderate-to-severe COVID-19 infection. Besides improving oxygenation status, it also reduced mortality and shortened hospital stays.

Keywords: Antiviral drug, COVID-19, Hypoxia, Noninvasive ventilation, Severe acute respiratory distress syndrome

BACKGROUND

Coronavirus disease of 2019 (COVID-19) is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and has had a significant impact on the world.[1] The first case was reported from Wuhan, China in December 2019 and since then it has spread rapidly throughout other parts of the world.[2] The World Health Organization declared it a global pandemic on March 11, 2020.[3] The COVID-19 pandemic brought unexpected challenges to treatment and created great stress for treating physicians. As the pandemic worsens, so do the efforts to find the best treatment. A variety of medications, including antimalarials, antibiotics, antivirals, plasma, corticosteroids, and anti-interleukin 6 inhibitors, have been used to treat the virus.[4]

Remdesivir is a nucleotide analogue that inhibits viral replication and was used against Ebola virus infection.[5] In vitro trials have shown that remdesivir inhibits SARS-CoV-2.[6] There are various ongoing trials and cohort studies on the role of remdesivir in COVID-19 disease. The preliminary report from a small cohort study demonstrated various positive findings that shows improved clinical outcomes in severe COVID-19 disease.[7] Due to the favorable outcomes reported in the literature, the U.S. Food and Drug Administration (FDA) granted remdesivir Emergency Use Authorization for the treatment of COVID-19 on May 1, 2020.[8]

Despite this, the clinical impact of remdesivir on COVID-19 disease is uncertain.[9] There is consensus that remdesivir use in the early stages of the disease had a more fruitful outcome. But its clinical efficacy, side

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effects and tolerability need to be evaluated further for a better understanding of this drug.[10]

As a result of the above considerations, we are conducting this study to identify the clinical effects of remdesivir in a close surveillance environment at a tertiary care hospital in Karachi, Pakistan. The primary objective was to assess the change in oxygenation status of patients after remdesivir treatment. We also compared mortality, re-admission and length of hospital stay in moderate-to-severe and critical COVID-19 patients treated with remdesivir.

MATERIAL AND METHODS

This is an investigator initiated retrospective cross-sectional study conducted in our tertiary care hospital from September to December 2020 to identify the effectiveness and clinical outcomes of remdesivir. Ethical review committee (ERC) of the institute reviewed the study proposal and exemption was granted (ERC Number: 2020-5206-11731). The study was conducted in accordance with Strobe's guidelines. International disease classification version 9.1 was used to derive records of patients admitted to our hospital with COVID-19 infection.

All patients aged >18 years admitted to the hospital with a positive reverse transcriptase-polymerase chain reaction for SARS-CoV-2 were included. Patients with suspected COVID-19 and those diagnosed on computed tomography of the chest were excluded from the study. The patients were recommended to use remdesivir after consulting with an infectious disease specialist.

All patients who received intravenous remdesivir (200mg on day 1 followed by 100mg once daily infusion for 4 more days) were further studied. After enrollment, their demographic information, co-morbid conditions, clinical presentation, management, oxygen and assisted ventilation requirements, and clinical outcomes were recorded. Severity of COVID-19 infection was subcategorized into two groups i.e. group 1 was moderate-to-severe in which patients were symptomatic and hypoxic that required supplemental oxygen only and group 2 was labelled as critical in which patients had hypoxic respiratory failure and with supplemental oxygen required assisted ventilation.[(11)]

Patients were further classified into pre- and post-remdesivir categories. The oxygenation status and assisted ventilation prior to remdesivir administration were called "pre-remdesivir", and on day six (after completing a 5-day course of remdesivir) were labelled as "post-remdesivir". The primary outcome was to compare oxygenation status and assisted ventilation after remdesivir treatment. Secondary outcomes were mortality, re-admission, and length of hospital stay in both groups of patients.

Clinical data was filled out with designed pro-forma and entered into the system software. Statistical Package for the Social Sciences (SPSS) Version 23, IBM, Chicago, USA was applied for data analysis. Number and percentages of categorical variables were compared between groups using chi square with a level of significance of <0.05. Mean and standard deviation of continuous variables were compared between groups using an independent student t-test with 95% confidence interval.

RESULTS

A total of 213 patients were admitted to our tertiary care hospital from September to December 2020 with COVID-19 infection. Out of these 213 patients, 114 (53.5%) patients received remdesivir during their hospital stay. 69 (60.5%) of the patients were male and the mean age was 59±12 years. The main co-morbidities in these individuals were hypertension in 69 patients (60.5%), diabetes in 57 patients (50%) and ischemic heart disease in 17 patients (14.9%). Fever was the major presenting complaint in 77 (67.5%), followed by cough in 62 (54.4%) and dyspnea in 61 (53.5%). 56 patients (49.1%) had moderate to severe COVID-19 infection. All patients (100.0%) required supplemental oxygen therapy during their hospital stay. 46 patients (40.3%) used non-invasive ventilation while 12 patients (10.5%) required invasive ventilation for hypoxic respiratory failure due to COVID-19. 15 patients (13.2%) had mortality from COVID-19 infections. Table-I shows the detailed demographics, clinical characteristics and outcomes of these patients.

All patients received supplemental oxygen prior to hospital admission. At day 6 following a five-day course of remdesivir, 21 out of 56 patients (37.5%) with moderate-to-severe COVID-19 infection still used supplemental oxygen. Based on this, 47 out of 58 patients (81%) with critical COVID-19 required oxygen after treatment with remdesivir (p-value <0.001). Figure-I shows the comparison of the proportion of oxygenation improvement after remdesivir treatment in both groups.

From 58 Critical COVID-19 patients, 46 patients (79.3%) were on non-invasive ventilation and 12 patients (20.7%) were on invasive ventilation prior to remdesivir therapy. 22 patients (47.9%) recovered from non-invasive ventilation after completing remdesivir treatment (p-value: <0.001). Figure 2 compares the ventilation status of patients before and after treatment with remdesivir.

Our study found that 15 patients (25.8%) died in the critical COVID-19 group. The causes of mortality were septic shock with multi-organ failure in 9 patients (60%) and hypoxic respiratory failure in 6 patients (40%). We also identified various complications with COVID-19 infection. 2 patients with moderate-to-severe COVID-19 infection had pulmonary embolisms. Pneumothorax and/or pneumomediastinum was identified in 1 patient with moderate-to-severe and 6 patients with critical COVID-19 infection. 99 patients were discharged from the hospital and 5 patients (5%) were re-admitted to the hospital again in a one-week period. The re-admission reasons were hospital acquired pneumonia in 2 patients, super-added fungal pneumonia in 2 patients and pulmonary embolism in 1 patient. The mean length of hospital stay with moderate-to-severe COVID-19 infection was 6.8 days and with critical COVID-19 infection was 11.3 days. Table-II demonstrates secondary outcomes among patients with moderate-to-severe and critical COVID-19 infection.

Table-I: Demographics, Clinical Characteristics & Outcomes of COVID-19 patients (N: 114)

		N (%)
Age		
	Mean ± S.D.	59 ± 12
	Range	29 – 84 Years
Gender		
	Male	69 (60.5%)
	Female	45 (39.5%)
Co-Morbid		
	Diabetes	57 (50.0%)
	Hypertension	69 (60.5%)
	Ischemic heart disease	17 (14.9%)
	Chronic kidney disease	2 (1.8%)
	Chronic obstructive pulmonary disease	8 (7.0%)
Clinical Symptoms		
	Fever	77 (67.5%)
	Cough	62 (54.4%)
	Dyspnea	61 (53.5%)
	Sore Throat	26 (22.8%)
	Myalgia	23 (20.2%)
Severity		
	Moderate to Severe	56 (49.1%)
	Critical	58 (50.9%)
Management		
	Steroids	113 (99.1%)
	Tocilizumab	46 (40.3%)
Outcomes		
	Supplemental oxygen	114 (100.0%)
	Non-invasive ventilation	46 (40.3%)
	Invasive ventilation	12 (10.5%)
	Mortality	15 (13.2%)
	Length of hospital stay (mean ± S.D.)	9 ± 4 days

Table-II: Outcomes of patients with COVID-19 infection received remdesivir.

	Moderate - Severe	Critical	p-value
Mortality – N (%) *	0	15 (100)	<0.001
Re-admission – N (%) *	2 (40)	3 (60)	0.676
Length of Hospital Stay (days) – mean ± S.D. **	6.8 ± 3.2	11.3 ± 4.8	<0.001

***Chi-square tests were used with a level of significance at p-value <0.05, ** Independent student t-test was used with a level of significance at p-value <0.05**

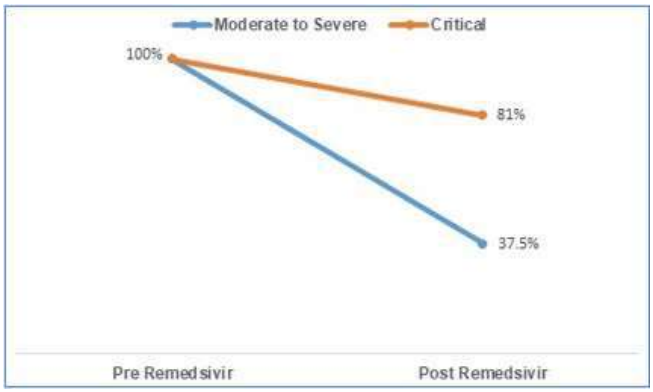

Figure-1: Improvement in oxygenation status after remdesivir treatment.

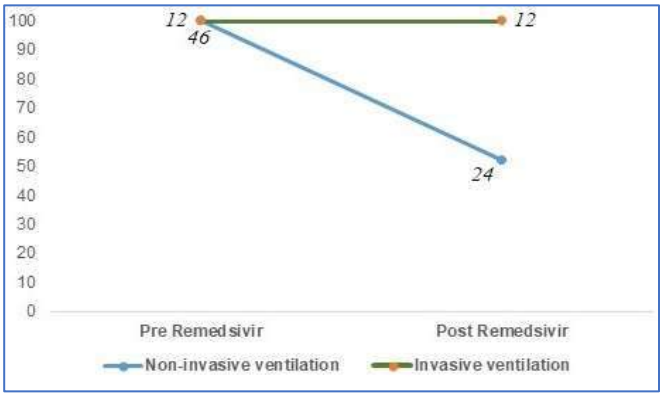

Figure-II: Ventilation status of patients before and after remdesivir treatment.

DISCUSSION

Our study highlights that remdesivir significantly improves oxygenation status in patients with moderate-to-severe COVID-19 infection. In nearly half of our study participants, non-invasive ventilation was also discontinued after a complete course of remdesivir treatment in critically infected COVID-19 patients. Despite remdesivir treatment, mortality was high in patients critically ill due to COVID-19 infection. The duration of hospital stay was also much lower in the moderate-to-severe group compared to the critical group.

Our findings are consistent with the current and recent COVID-19 guidelines.[12] Two major studies SOLIDARITY and ACTT-1 both concluded that remdesivir reduces the time to clinical recovery, resulting in earlier discharge and a shorter hospital stay.[13-15] In our study, remdesivir showed significant clinical improvement when used earlier in the course of illness. Supplemental oxygen was successfully removed from 62.5% in moderate-to-severe and 19% in critical COVID-19 disease. Non-invasive ventilation was discontinued in 47.8% of our study population with critical infections. Patients who were started on remdesivir earlier also had reduced oxygen demand and could therefore be discharged earlier on room air.

Remdesivir shortens the length of hospital stay in patients with mild to moderate disease.[16, 17] Our study found that patients with critical illness had a longer hospital stay (11.3 days versus 6.8 days, p = 0.000). Prolong hospital stay is one of the many risk factors for acquiring nosocomial infections thus patients who were discharged earlier have less chance of acquiring these infections. This also reduces the financial burden on patients, hospital resources and stabilizes the current economic situation.

The significance of our study lies in its conformity to recent guidelines. However, our study was conducted in Pakistan, an Asian country. The study helps understand the impact of racial characteristics on COVD-19 management. It is reassuring and satisfying to know that remdesivir lowers oxygen demand in patients requiring either non-invasive or invasive ventilation. A study in India and Egypt also depicted similar results with remdesivir usage in their population when used in moderate-to-severe disease.[18,19] The ultimate goal of our study was to understand the role of remdesivir in our population. It clearly demonstrates in our study that patient on remdesivir had an early clinical recovery and reduce hospital stay.

In a cohort study by Diaz *et al*. they reported a lower mortality in the group of patients who received remdesivir.[20] In a cross-sectional study, it is not possible to predict the mortality rate. Additionally, we observed all of the mortality in patients with critical infections. Only 6 patients (5.2%) died due to hypoxic respiratory failure. Remdesivir given in the initial stages of illness (when the patient had a mild disease) drastically decreased hospital re-admissions.[21] This shows that remdesivir reduced disease progression. There was no significant difference in re-admission rates between the two groups. However, these re-admissions are primarily due to complications related to health care.

COVID infection can result in pneumomediastinum and pneumothorax.[22] We also identified in this study, more cases of pneumothorax in patients with critical COVID infection compared to those who had moderate-to-severe COVID infection. Thromboembolism is another entity associated with COVID infection.[23] In our study, only two patients had a pulmonary embolism.

Our study has some limitations. The retrospective nature of the study with a small sample size cannot be generalized to the whole population. The cause/reason of disease progression, risk factors for superadded infections, and impact of concurrent other COVID management strategies were not included in our study.

The strength of our study is that it compares clinical effects on oxygenation and ventilation status before and after remdesivir treatment. The severity of COVID illness was also compared to better identify remdesivir's usefulness in the illness phase. Besides this, we also report mortality and re-admission rates in these patients after remdesivir treatment. Ideally the role of remdesivir in early stages of disease in all patients including those with low to no risk of disease progression should be studied. The dosage and duration of remdesivir according to the stage of illness should be studied especially in resource-limited countries like Pakistan.

CONCLUSION

The study emphasizes that remdesivir was found to be clinically beneficial in patients with moderate-to-severe COVID-19 infection. Besides improving oxygenation status, it also reduced mortality and shortened hospital stays.

CONFLICT OF INTEREST

None

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHOR CONTRIBUTION

Sher Muhammad Sethi: Concept and study design; data analysis; initial manuscript writing

Memoona Irshad: Concept and study design; proof reading and reviewing

Rodaba Iqbal: Data collection, manuscript reviewing

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ORIGINAL ARTICLE

Antibiogram and susceptibility pattern of bacterial isolates of urinary tract infection among children and adolescents at a tertiary care hospital

Salman Arshad, Shahid Rasheed, Mudassar Hussain, Saqib Munir, Nadia Qamar, Abdul Sattar

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

ABSTRACT

Background: UTI is one of the major causes of morbidity among children and adolescents in developing countries like Pakistan, where precise knowledge of causative micro-organisms and their antimicrobial susceptibility patterns remains inadequate. To identify common organisms involved in urinary tract infection and their antimicrobial sensitivity patterns among children & adolescents.

Material and Methods: This retrospective descriptive study was conducted on urine cultures reports of children less than 18 year presented with suspected UTI in Allama Iqbal Memorial Teaching hospital between the periods of January 2020 to December 2022 were taken. Urine sample cultured on CLED agar plates, micro-organism were identified by performing their morphological & biochemical tests.

Results: Urine culture reports of 500 patients of age <18 years with suspected UTI were collected, out of which 102 were positive. The most common age for UTI was 5-11 years. The ratio of boys and girls was 30 and 72 respectively. Both Gram-negative and Gram-positive bacterial species were recovered. The ratio of gram negative to gram positive was 94 and 8 respectively. Among Gram-negative bacterial species, *E. coli* was the most common organism. Antimicrobial susceptibility pattern of *E. coli* showed that susceptibility to commonly prescribed drugs as follows Gentamicin (80%), co- amoxiclav (52%), Trimethoprim- Sulfamethoxazole (19%), Cephalosporin's (8%), and Amoxicillin (5%). Susceptibility to Nitrofurantoin & Fosfomycin was 80% and 63% respectively.

Conclusion: *E.coli* was most common organism Isolated. Co-amoxiclav, Nitrofurantoin and Fosfomycin have good susceptibility profile among the oral drugs and Gentamicin has high susceptibility among injectables.

Keywords: Urinary tract infection, Antibiotic sensitivity, Urine culture

BACKGROUND

Urinary tract infection is the presence of bacteria (> 10^5) in the urine.[1] Urinary Tract Infection is one of the most common infections around the world that can affect both the upper and lower urinary tract. It can be acquired from the hospital or community at any age.[2] The term UTI is applied to variety of clinical conditions ranging from the asymptomatic bacteruria to severe infection of the kidney leading to sepsis. If diagnosis in not made in time it can result in long term complications in the form of hypertension, failure to thrive and end stage renal disease[3]. The overall prevalence of UTI is approximately seven percent in febrile infants and young children but it differs according to age, sex and circumcision status. It is more common in un-circumcised males, especially who are younger than three years old. Girls have two-to-four-time increased risk of UTI than circumcised Boys [4]. females are more prone to UTI due to short urethra, absence of prostatic secretion, pregnancy and fecal contamination of Urinary tract.[1]

Worldwide about 150 million people are diagnosed with UTI annually, costing almost more than 6 billion dollars.[5] In the developing countries various studies have been conducted which showed that about 10% of children who presents with febrile illness are suffering from UTI. This can be increased to 8-35% if the children are suffering from malnutrition.[3] UTI can involve both upper and lower urinary tract system so the clinical presentation depends on the area of involvement. Lower urinary tract symptoms include dysuria, urgency increased urinary frequency and suprapubic tenderness.[6] In childhood the risk of developing UTI is 3-10% in girls and 1-3% in males.[7] The most common organism causing UTI is *E.coli* followed by *Klebsiella Pneumonia, Pseudomonas aeruginosa, Staphylococcus saprophyticus,* and *Enterbobacter species.* with variability in sequence of their prevalence.[8] However, the organisms and

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antimicrobial susceptibility in pediatric UTI differ from one place to another place and even within the same place between various geographical areas.[7] Thus, precise knowledge about the causing organisms and its susceptibility pattern is necessary.[9]

The increasing antibiotic resistance in the management of UTI is a serious public health concern, particularly in developing countries where other than poverty, ignorance and poor hygienic practices, there is prevalence of substandard drugs in the market. Urinary Pathogens having strong ability for invasion, adhesion and virulence along with emerging increased antibiotic resistance is becoming a great concern throughout the world.[10] Although very few studies have shown the status of UTI in general population, larger studies are limited towards specific target population such as HIV patients, diabetic patients, pregnant woman, children and students.[1] Similar situation applies to Pakistan and limited data on UTIs, risk factors, and drug resistance profiles are available from the general population.[1] Information about causative organisms and its sensitivity to the available drug is paramount for judicious selection of antibiotics and to develop suitable prescribing policies in the institute.[5] Commonly Prescribed empirical antibiotics in the treatment of UTI are cephalosporin, Gentamicin, ciprofloxacin, Co-amoxiclav, Trimethoprim-sulphmethoxazole, nitro-furantoin and cephalexin.[11-12] This study was carried out to see the microbial pattern of UTI as well as build the current antibiogram that will help the local practioners to use effective antibiotics in the treatment of UTI in children.

MATERIAL AND METHODS

This was a retrospective descriptive study conducted at Allama Iqbal Memorial Teaching Hospital (AIMTH). Children less than 18 years old, whose urine cultures were sent between January 2020 to December 2022, were included in the study. Cultures of both out door and hospitalized patients were included. Children who were on steroid therapy, taken previous antibiotics, were excluded from the study. Urine specimen was collected by 3 methods depending on the age i.e.2-24 month of age - urinary catheterization, suprapubic aspiration method, and toilet trained - mid-stream clean catch method. After collecting the urine sample in the urine container, they were labeled with patient registration number and samples were sent to the lab. The total samples collected were 500. Urine sample were cultured on CLED agar plates. The samples showing no growth on CLED agar were also excluded. The significant growth of organism considered only if there were up to two organisms' growth in significant number of colonies. Significant bacteruria was identified as more than 100,000 CFU/ml in mid-stream clean catch specimen, or > 50,000 CFU/ml in sample collected by urethral catheterization, or any number of colonies if sample collected by suprapubic aspiration. Probably significant bacteruria was defined as 1000-100,000CFU/ml in a mid-stream specimen [7].

Colonies which appeared on CLED agar plates were characterized on the basis of color and colony. From the CLED agar plates a total of 102 bacterial strains were identified. A total of 102 bacterial isolates, which were isolated were further screened for confirmation of organism species by performing their morphological and biochemical tests. Purification of bacterial isolates was done on several types of CLED-inhibited colonies. A total of 102 bacterial strains were designated names accordingly. The morphological characterization of 102 strains obtained from samples. Gram-staining was performed. All strains were tested for oxidase activity, catalase activity, triple sugar iron test, gas production, urease activity, and citrate utilization. Indole test was also performed. The disk diffusion method was used to assess the in.vitro susceptibility of the positive samples to the most commonly used antimicrobial drugs for UTI treatment including ceftriaxone (30ug), ceftazidime (30ug), cefixime (5ug), cefoperazone/ sulbactam (105/ 30ug), cefipime (20ug), cefotaxime (30ug), cefoperazone (105ug), amoxicillin (10ug), amoxicillin-clavulanic acid (20/10ug), ciprofloxacin (5ug), fosfomycin (200ug), nitrofurantoin (300ug), trimethoprim-sulamethoxazole (5/250ug), amikacin (30ug), gentamicin (10ug), imepenem (10ug), meropenem (10ug), piperacillin-tazobactam (30ug) and pipemidic acid (20ug).

Culture and sensitivity reports were obtained from laboratory of Allama Iqbal Memorial Teaching Hospital. Data were presented as descriptive statistics (frequency tables, charts and percentages). Data analysis was carried out using Microsoft Excel 2023 version 16.72. Approval for the conduct of this research was obtained from ethical review board of Allama Iqbal Memorial Teaching Hospital, Sialkot.

RESULTS

Urine culture of 500 patients of age <18 years with suspected UTI were collected, out of which

102(20.4%) showed significant bacterial growth. (Figure-I).

The most prevalent age group for UTI was 5-11 years (39.2%) followed by 11-18 (25.4%), 2-5 (19.6%) and < 2year of age (15.6%). The ratio of boys and girls was 30(29%) and 72(71%) respectively (Table-I).

Both Gram-negative and Gram-positive bacterial species were recovered. The ratio of gram negative to gram positive was 94/102 (93%) and 8/102 (7%) respectively. Among Gram-negative bacterial species, *E. coli* (70%) was the most frequently isolated bacteria followed by the *Providencia species* (9%) and *Enterobacter cloacae* (8%). Among the gram-positive species there were only eight isolates six of them were *Enterococcus fecalis* while only two were *Staph aureus.* Antimicrobial susceptibility pattern of *E. coli* showed that it was 100% sensitive to meropenem and imipenem, followed by amikacin (94%), piperacillin-tazobactam (86%). Sensitivity to gentamicin, cefoperazone-sulbactam, and nitrofurantoin was 80%, followed by fosfomycin (63%) and then co-amoxiclav (52%). Cephalosporins (cefepime, cefixime, ceftriaxone, cefoperazone, cefotaxime, and ceftazidime) and trimethoprim- sulphmethoxazole had sensitivity of only 8% and 19% respectively. Amoxicillin showed very high resistance with susceptibility of only 5% (Table-II).

Providencia was the second most common isolated organism which showed that resistant strains to carbapenem are emerging with susceptibility of 80%. The susceptibility to amikacin was (80%), followed by 60% to piperacillin-tazobactam, and cefoperazone-sulbactam while only 40% towards Gentamicin (Table-II). *Enterobacter cloacae* was the most resistant among the isolates with uniformly low susceptibility i.e. 75% to meropenem, imipenem, piperacillin-tazobactam, gentamycin, cefoperazone-sulbactam and nitrofurantoin. The susceptibility to cephalosporins is 25%. All strains were resistant to amoxicillin (Table-II).

Table-I: Demographic feature of children & frequency of bacteria isolated in urine culture.

Patient Characteristics	*E. coli n=72 (70%)*	*Providencia species 10 (9%)*	*Enterobacter Cloacae 8 (8%)*	*Enterococcus faecalis 6 (5%)*	*Klebsiella Pneumonia 2 (2%)*	*Staph Aureus 2 (2%)*	*Enterobactor species 2 (2%)*	Total n 102 (%)
Age								
<2years	12	-	2	-	2	-	-	16(15.6%)
2–5years	16	4	-	-	-	-	-	20(19.6%)
5–11years	22	6	4	4	-	2	2	40(39.2%)
11-18 years	22	-	2	2	-	-	-	26(25.4%)
Gender								
Male	20	4	2	2	2	-	-	30 (29%)
Female	52	6	6	4	-	2	2	72(71%)

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

Antibiotics	*E.coli* (72)	*Providencia species* (10)	*Enterobacter cloacae* (8)	Overall sensitivity
Meropenem	100%	80%	75%	85%
Imipenem	100%	80%	75%	85%
Amikacin	94%	80%	0%	58%
Piperacillin-tazobactam	86%	60%	75%	73%
Gentamicin	80%	40%	75%	65%
cefoperazone-sulbactam	80%	60%	75%	71%
Nitrofurantoin	80%	0%	75%	77%
Fosfomycin	63%	0%	0%	63%
Co-amoxiclav	52%	0%	0%	52%
Ciprofloxacin (quinolones)	33%	0%	0%	11%
Pipemidic acid	27%	0%	0%	9%
trimethoprim-sulphmethoxazole	19%	0%	0%	6%
Ceftriaxone	8%	0%	25%	11%
Cefipime	8%	0%	25%	11%
Cefotaxime	8%	0%	25%	11%
Cefixime	8%	0%	0%	2.6%
Ceftazidime	8%	0%	25%	11%
Cefoperazone	8%	0%	25%	11%
Amoxicillin	5%	0%	0%	1.6%
Cefradine	0%	0%	0%	0%

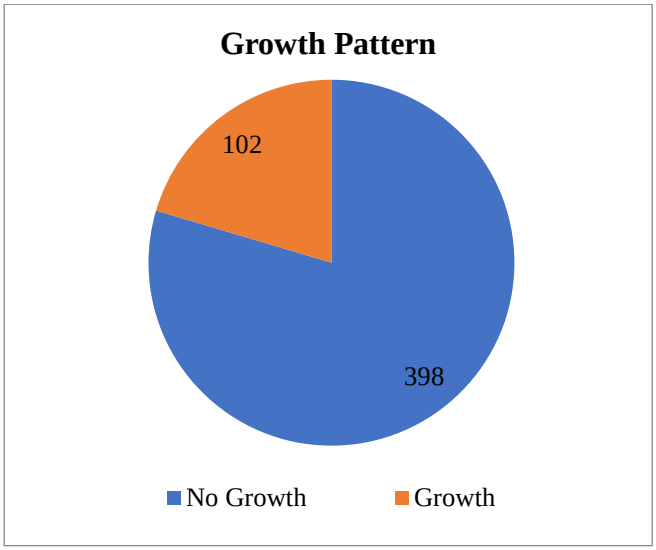

Figure-I: Growth pattern of organisms (n=500).

DISCUSSION

Urine culture yield is variable and depends upon laboratory methods, geographical location and countries. In our study urine culture yield was 20.4% which is much higher than the study conducted at Mexico (16.9%)[13], Ethiopia 16.7%[3], and Kuwait 13.7%.[9] However, it was much lower than the previous studies conducted in Pakistan 32.8%[2], 65.1%[1] and 66.5%.[18] Similarly, a study in south India showed slightly higher yield 21.2%.[7] There was one study in northern India[8] which showed highest yield of around 77%, but they included patients of all age groups. Furthermore, one study in Iran showed bacterial growth of only 6.7% probably because they had taken large sample size of 21604.[15]

Our study shows UTI was more prevalent among 5-11 year of age which was similar to study conducted in Kuwait and Ethiopia.[9,3] However, studies in Mexico and Tanzania shows that UTI was more prevalent among age less than 5 year.[13,14] One study conducted in south India that included children less than 15 years of age found that UTI was more prevalent in children less than 1 year age group.[7] Few studies conducted in Pakistan in which they include all age group patients they found UTI was more common among patient of age 26 to 35year.[1,2]

Our study shows that UTI was more common in females than males and this gender predilection is similar in studies conducted at Pakistan India, Mexico, Ethiopia, Iran, Kuwait and Nigeria. [2-1-10-18,6,13,3,15,9, 5] However, one study conducted in south India shows no gender difference[7], whereas a study conducted in Northern India where they included all age patients found that after the age of 80-year UTI was more prevalent in males than females.

Our study shows that gram negative organisms are more common than gram positive in causing UTI. Among gram negative organisms *E. coil* was most the most common isolate. These results were similar to all the studies conducted previously in Pakistan, Nigeria, Kuwait, Ethiopia, India, Uganda and Tanzania. [18,1,5,2,9,3,6,16,14]

In our study *E. coli* was 100% sensitive to imipenem and meropenem. Previous studies conducted in Pakistan shows 98%[2], 85.3%[18], 76%[1], and 72%[10] sensitivities to meropenem and imipenem. Previous studies in other countries also showed the similar results 100%[7,9,13], 97%[3], 98%[9], 94%[16] and 83%.[18] In our study *E. coli* was 94% sensitive to amikacin whereas in other studies sensitivity to amikacin were 89.5%[19], 87%[7], 81%,[2] 95.5%[9], 40.4%,[1] 51%[18], and 63%.[10] In our study sensitivity to cefoperazone-sulbactam, piperacillin-tazobactam and Nitrofurantoin was >80%. Other studies in the past also shows the similar results for nitrofurantoin 98%, 85.3%, 82%, 97%, 88%, 83%, and 82%.[9,19,2,13,16,9,3] However, one study showed only 43% sensitivity to nitrofurantoin.[5] Surprisingly one study in Pakistan shows 0% sensitivity toward nitrofurantoin [1]. Sensitivity to piperacillin-tazobactam in previous studies was 98%, 85%, 87%, 75%, 75% and 50%.[9,13,16,7,2,1]

Sensitivity to Fosfomycin in our study was 63%, whereas in previous studies it was 90%[16] and 85.3%.[18,1] Sensitivity to co-amoxiclav in our study was 53% whereas in previous studies it was 71%[9], 52%[7], 35%[16], 26.5%[18], 18%[2], 18.5%[1] and 18%[3]. Sensitivity to trimethoprim- sulphmethoxazole in our study was 19% only, while in previous studies it was 97 %[16], 70%[19], 63%[9], 50%[2], 42%[3], 39%[13], 26%[7], and only 6%.[10] Sensitivity to cephalosporin in our study was only 8% which is qui*et al*arming situation for our geographic area because in previous studies sensitivity to cephalosporins was quite good, i.e., it was 85% to cefoxitin, 82% to cefotaxime, and 75% to ceftazidime in one study[3] while in other studies it was 93 % to ceftriaxone [16], 74% to cefotaxime[9] and 19.8% to cefixime.[1] Similarly studies conducted in Pakistan previously which shows sensitivity of 32.3%, 39%, 21.3% and only 10% to ceftriaxone[18,1,19,10] whereas sensitivity to cefotaxime 23.8% and 22.5%.[18,19] The

greatest resistance was noted to Amoxicillin which has only 5% sensitivity which differs from one study conducted in Pakistan[1] which shows 25% sensitivity towards amoxicillin. However, our results were similar to Previous Study[17] in which only 15.2% and in other studies[18] conducted in Pakistan shows 5%, sensitivity to Amoxicillin found. Whereas, surprisingly in study[10] all strains were resistant to amoxicillin.

CONCLUSION

Our study concludes that *E. coli* was the most common isolate, has higher susceptibility to carbapenems, which needs to be preserved. The sensitivity to trimethoprim- sulphmethoxazole, third generation cephalosporin and amoxicillin was very low. These drugs should not be used as first line keeping in view the higher resistance. The sensitivity to the commonly used antibiotics like Gentamicin, co-amoxiclav, nitrofurantoin and Fosfomycin is good, so they are better choice to be used as first line.

CONFLICT OF INTEREST

None

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHOR CONTRIBUTION

Salman Arshad: Study design, methodology, manuscript writing

Shahid Rasheed: Data collection, statistical analysis, results interpretation

Mudassar Hussain: Literature review

Saqib Munir: Data collection, statistical analysis, results interpretation

Nadia Qamar and Abdul Sattar: Data collection

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DOI: https://doi.org/10.9734/jpri/2021/v33i33A31779

ORIGINAL ARTICLE

Epidemiology and antifungal susceptibility patterns of invasive candidiasis: A single-center study

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ABSTRACT

Background: Invasive candidiasis remains a leading cause of morbidity and mortality. Despite the availability of various antifungal treatments, global reports continue to highlight resistance, treatment ineffectiveness, and outbreaks. This study aimed to analyze the epidemiology of various *Candida* species isolated from invasive samples and assess their antifungal susceptibility profiles.

Material and Methods: The study identified and evaluated a total of 200 *Candida* isolates, recovered over a 6-month period from various clinical specimens. These were assessed against various antifungals according to recommendations.

Results: Among the samples, blood (79%) and pus (12%) were the most commonly encountered sources of *Candida* isolation. *Candida* isolates comprised 14.5% (30) *C. albicans* and 85.5% (170) non-albicans species. Non-albicans species included 15% (30) *C. guilliermondii,* 14.5% (29) *C. lusitaniae,* 11.5% (22) *C. tropicalis*, 2.3% (4) *C. auris,* and 8.5% (17) *C. parapsilosis*. Overall, the strains showed 74% and 91% sensitivity to Fluconazole and Voriconazole, respectively, and 100% sensitivity to Caspofungin and Amphotericin B, with the exception of *C. lusitaniae.*

Conclusion: The susceptibility profile of antifungals is evolving. This study demonstrated a low rate of resistance to four antifungals in invasive candidiasis in Pakistan. None of the isolates were resistant to more than one drug. Early and prompt treatment through the implementation of an antifungal stewardship program and strict infection control is crucial.

Keywords: Invasive candidiasis (IC), Antifungal susceptibility profile, Fluconazole, Candidemia

BACKGROUND

Candida species account for approximately 20% of all microbiological infections in critical patients.[1] Recent studies have reported higher minimum inhibitory concentrations (MICs) to commonly used antifungals against both *C. albicans* and *non-albicans*.[1] The increase in fungal infections corresponds to factors such as advanced age, prolonged length of ICU stays, use of steroids or immunosuppressive drugs, diabetes mellitus (DM), multiple invasive procedures, renal replacement therapy (RRT), and the wide use of invasive devices such as central venous catheters (CVCs) and biofilm formation. Colonization may also lead to invasive disease in 3-25% of cases. The spectrum of invasive candidiasis is broad, including fungemia, intra-abdominal infections, septic arthritis, iatrogenic and neonatal meningitis.[1] Significant geographic variation has been observed in invasive candidiasis, with a poor mortality rate of 40–55%. In a pan-European ICU cohort study (2015–16), *C. albicans* (57%) was the dominant species, followed by *C. glabrata* and *C. parapsilosis*.[2-6] A descriptive epidemiological data from Pakistan in 2012, for invasive *Candida* isolates among adults and neonates revealed *Candida tropicalis* (38% and 36 %, respectively) as the most common species.[7] Despite the availability of antifungals, therapeutic failure may occur due to inadequate responses to common treatments.[8] The Infectious Diseases Society of America (IDSA) guidelines recommend the empiric use of Echinocandins, as it is associated with better survival. Echinocandins have also been found to have limited adverse effects and minimal drug–drug interactions.[8,9] The objective of this cross-sectional study is to observe the frequency of different *Candida* species and determine the susceptibility to antifungals in clinical isolates during a 6-month period (April - September 2023) in the microbiology department of Chughtai Institute of Pathology, Lahore, Pakistan.

MATERIAL AND METHODS

This study included sterile and invasive samples (such as blood, ascitic fluid, pus aspirates, deep or debrided tissue, CVP tips, and CSF) with yeast growth, encompassing both genders across all age

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groups. CVP tips with blood culture positive with the same pathogen were included in this study. All duplicate isolates and non-invasive samples were excluded.

A cross-sectional observational study was conducted over a 6-month period at Chughtai Institute of Pathology (CIP) in the Microbiology, BSL-2 laboratory, under the assigned IRB number. The study focused on the analysis of various *Candida* species isolated from clinical invasive samples, alongside their antifungal susceptibility profiles, from April to September 2023.

Two hundred yeast isolates from invasive samples were included, processed for identification using MALDI-TOF, VITEK MS, and their antifungal susceptibility profiles were determined. *Candida* isolates that couldn't be identified to the species level using VITEK MS were labeled as "*Candida* species." Antifungal susceptibilities were determined by the minimum inhibitory concentration (MICs) using E-strip for Fluconazole, Voriconazole, Amphotericin, and Caspofungin, as guided by CLSI M60. However, Amphotericin was not tested for *C. lusitaniae,* and Fluconazole was not tested for *C. krusei.* Quality control strains were also employed to standardize the testing. Antifungal breakpoints were species-specific, and the results were reported as sensitive (S), resistant (R), or susceptible dose-dependent (SDD). Patient privacy was maintained by using case numbers exclusively. Statistical Analysis: Descriptive analysis was performed, calculating the frequencies of *C. albicans* and non-albicans isolation, gender, age groups, and the MIC 50 and 90 percentile of antifungals using SPSS 21.

RESULTS

A total of 200 yeasts from various invasive samples were studied, with the majority originating from Punjab (82.5%). *Candida* isolates were most commonly obtained from blood (79%), followed by pus (12%), ascitic fluid (3%), CSF (2.5%), tissue (2%), and CVP tips (1.5%). The study revealed the isolation of 14.5% *C. albicans* and 85.5% *non-albicans* species. Among the *non-albicans* species, *C. guilliermondii* was the most frequent, accounting for 15% of cases. Additionally, 14.5% of *C. lusitaniae,* 11.5% of *C. tropicalis*, 8.5% of *C. parapsilosis*, and 2.3% of *C. auris* were isolated from clinical samples, with other *Candida* species accounting for 18.5% of cases. Antifungal sensitivities were also assessed. Amphotericin B and Caspofungin were found to be 100% effective in all isolates, both *C. albicans* and *non-albicans*. Of the 193 (96%) isolates tested for fluconazole, 143 (74%) were found to be sensitive, 17 (8.8%) were resistant, and 33 (17%) showed susceptible dose-dependent results. Total of fifteen Fluconazole-resistant isolates of *C. lusitaniae and C. guilliermondii* were observed to have MIC > 64ug/ml. Similarly, isolates were tested for voriconazole, with 182 (91%) found to be sensitive, 16 (8%, *C. lusitaniae, C. auris*) resistant, and 2 (1%) showing susceptible dose-dependent results. Fifteen isolates of *C. guilliermondii* and *C. lusitaniae* exhibited high voriconazole MIC (> 32 ug/ml). Amphotericin B and Caspofungin displayed 100% sensitivity.

Table-I: Demographic data from provinces of pakistan

Province	Frequency	Percent
AJK	2	1.0
Baluchistan	2	1.0
KPK	25	12.5
Punjab	165	82.5
Sindh	6	3.0
Total	**200**	**100.0**

Table-II: Over all percentile of MICs of *Candida* species against different antifungals

Species(n)	Antifungal agents	MIC90	MIC50
Candida albicans (n=29)	Amphotericin B	0.6	0.125
	Caspofungin	0.25	0.25
	Fluconazole	0.925	0.315
	Voriconazole	0.25	0.12
Candida guilliermondii (n=30)	Amphotericin B	0.5	0.1575
	Caspofungin	1.05	0.75
	Fluconazole	32	24
	Voriconazole	0.44	0.19
Candida lusitaniae (n=29)	Amphotericin B	NT	NT
	Caspofungin	1.5	1
	Fluconazole	64	28
	Voriconazole	0.625	0.22
Candida parapsilosis (n=17)	Amphotericin B	0.5	0.1875
	Caspofungin	1	0.75
	Fluconazole	1.3	0.38
	Voriconazole	0.198	0.12
Candida tropicalis (n=22)	Amphotericin B	0.38	0.125
	Caspofungin	0.25	0.25
	Fluconazole	0.788	0.25
	Voriconazole	0.952	0.12
Candida spp. (n=73)	Amphotericin B	0.75	0.25
	Caspofungin	0.75	0.38
	Fluconazole	32	3.5
	Voriconazole	0.75	0.125

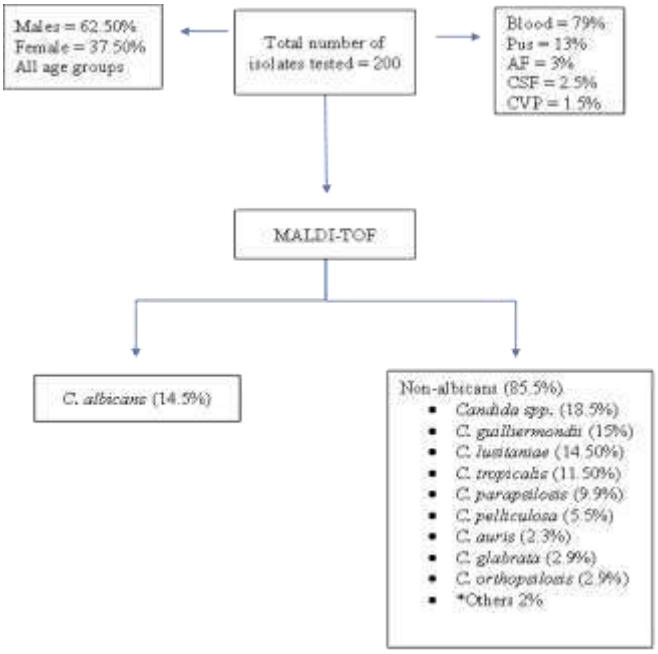

Figure-I: Work flow chart showing the categorization of samples and of *Candida* isolates.
***Others 2% of the total isolates of *C. lipolyica, C. blankii, C. boidinii, C. rugosa* and *C. metapsilosis* were also identified.**

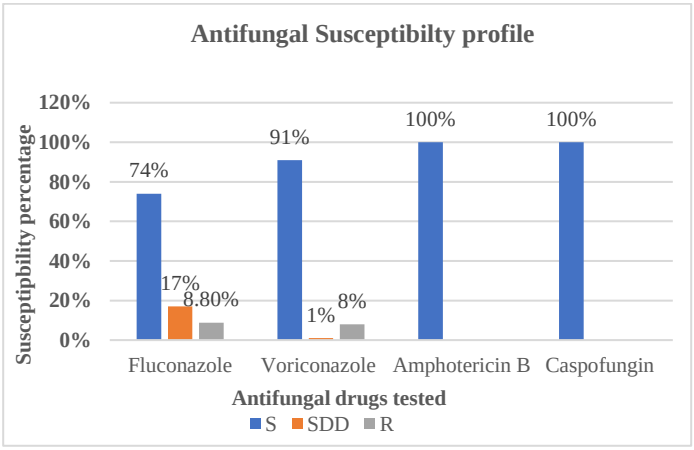

Figure-II: Showing antifungal susceptibilty

DISCUSSION

Candida species, which are the most common cause of invasive candidiasis (IC) across all age groups, present extensive disease manifestations with high mortality rates.[10] This study provides a concise overview of the distribution of *Candida* species and their antifungal susceptibilities in invasive samples from various geographical regions in Pakistan, mainly from Punjab (82.5%), as the laboratory is based in Lahore, the capital city of the province (Table-I).

The distribution of *non-albicans* species, as documented by Papas *et al*., particularly in ICU patients, has been observed globally. It was proposed that *non-albicans* species, including *C. lusitaniae, C. guilliermondii*, and *C. parapsilosis*, were the leading cause of IC, especially in patients with prior exposure to antibiotics, a central venous catheter, recent abdominal surgery or colonization (5-30%).[10] Some studies have suggested that colonization can be used as an indication to initiate prophylaxis and reduce IC.[11,12,13] However, this has not shown any benefits in reducing mortality. Similarly, a study in Switzerland by Egiimann revealed that the shift in ICU fungal distribution resulted from empirical treatment with Caspofungin and Azoles. This led to increased *C. glabrata* without reducing invasive candidiasis-related mortality or prevalence.[14] Our study also demonstrated that *non-albicans* species were the major cause of IC (85.5%). Another study published in 2021 by Ratani *et al*. on the "Spectrum and antifungal resistance in *Candida* species isolated from blood culture of neonates" similarly indicated non-albicans species (86.7%) as the major cause of IC.[15] In contrast to our study, they noted *C. tropicalis* as the most frequently isolated pathogen, while *C. guilliermondii* (15%) was the dominant isolate in our study. This finding might be attributed to the different sample types and age groups included. Our results also differ from the study in Iran on blood cultures from the Pediatric Intensive Care Unit (PICU), which reported 53.6% *C. albicans*, 24.7% *C. parapsilosis*, and 8.5% *C. tropicalis*.[16]

The gold standard for diagnosing IC is culture, yet the sensitivity of blood culture and ascitic fluid is reported to be insignificant. This could be due to the common practice of empiric antifungal therapy. Literature review has shown that the sensitivities of blood and ascitic fluid are only 75% and 5-20%, respectively.[17,18] Similarly, the isolation of *Candida* from sterile sites, such as pus from the abdomen, poses challenges and takes 2–3 days.[17] Our study also indicated low frequency in terms of the isolation of *Candida* from blood (79%) and pus (3%).

A worldwide transformation in the antifungal susceptibility profile, especially in Fluconazole, has been observed. This might be attributed to the common practice of frequently using Fluconazole as an empiric treatment. This study shows reduced sensitivity against Fluconazole (74%) compared to Amphotericin B (100%) and Voriconazole (91%) (Figure-1). These calculations did not include Fluconazole susceptibility profiles for *C. krusei* and *C. auris* as they are intrinsically resistant and have high MICs, respectively.

Approximately 8% of susceptible dose-dependent (SDD) isolates were observed for both Fluconazole and Voriconazole. We detected a >64 ug/ml MIC for Fluconazole in total of 12 isolates of *C. lusitaniae*, 2 isolates of *C. guilliermondii*, and 1 *Candida species*. High MICs of Fluconazole against *C. lusitaniae* may be due to mutations, as discussed by Shawn *et al*. Additionally, prior use and persistent candidemia may contribute to high MICs. Similarly, in a "20 years of antifungal surveillance program from 135 medical centers in the USA, Canada, and Europe," an increase in the isolation of *Candida glabrata* and *Candida parapsilosis* and a decrease in *C. albicans* was observed, along with a gradual emergence of resistance to azoles (Fluconazole and Voriconazole).[19] Unlike our findings, Merhendi *et al*. reported 100% sensitivity to Fluconazole and Voriconazole.[20,21]

An observational study in the USA, Texas, and Columbia on the susceptibility profile of *C. auris* noted 33% and 1% resistance to Amphotericin B and Echinocandins, respectively. [22,23] We reported 4 pan-sensitive *C. auris* isolates from blood and one from a tissue sample. The difference in susceptibility profiles might be due to the different clades, the selective pressure of antifungals, and the methodology of antifungal testing. To date, little information is available for treating infections due to pan-resistant isolates. More research, knowledge, and data are required for the accurate diagnosis and treatment of resistant isolates.[24]

The emergence of *non-albicans* species as predominant causes of invasive candidiasis (IC), particularly in intensive care units (ICUs), underscores the urgent need for targeted infection prevention and control measures. Evidence from this study underscores the need for rigorous surveillance mechanisms and guidelines to ensure judicious use of antifungal agents, thereby minimizing the risk of antimicrobial resistance and improving patient safety. Moreover, addressing diagnostic limitations and enhancing laboratory capacities for *Candida* detection are essential components of comprehensive public health strategies against IC. Policymakers should prioritize investments in diagnostic infrastructure and workforce training to facilitate early detection, prompt treatment initiation and effective management. This study underscores the importance of closing knowledge gaps surrounding *Candida* epidemiology and antifungal resistance mechanisms through targeted research and innovation. Policymakers should prioritize research funding and interdisciplinary collaborations to advance our understanding of IC dynamics and inform evidence-based policy responses.[25]

Several limitations exist in this study. We were unable to identify all inpatient units and the sources for pus samples due to a lack of clinical history. Furthermore, the mechanism of antifungal resistance in our community remains unknown. Discrepancies in the frequencies of *C. albicans* and *non-albicans* might be because of the short duration of the study. Due to small sample size, *Candida* species vise, antifungal susceptibility pattern could not be calculated. Differences in antifungal susceptibilities can also be attributed to the variation in testing methodologies, as broth microdilution is the recommended methodology.

CONCLUSION

This study reveals that there is a rise in *non-albicans candida* species. It is also concluded that there were no pan-resistant *Candida* isolates. However molecular-based assays should be employed to detect the mechanisms of antifungal resistance in our community. DNA sequence analysis of FKS genes should be performed to identify hotspot mutations. The use of antifungals should be restricted and improved through antifungal stewardship programs. A multicenter study should be conducted to observe the trends of antifungals and prevalence over a period of 5-10 years in our community.

CONFLICT OF INTEREST

None

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHOR CONTRIBUTION

Amna Younas: Study design. Study performance. Manuscript drafting, revision and final review of manuscript.

Irim Iftikhar: Conception of work. Study design. Critical review for important intellectual content. Final approval of version.

Karam Rasool: Acquisition, analysis and interpretation of data. Final review.

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ORIGINAL ARTICLE

The frightening disease burden of blood culture proven XDR typhoidal isolates in pediatric population in federal territory: Time to implement a holistic approach countrywide

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ABSTRACT

Background: Typhoid fever is a deadly enteric bacterial infection and millions of people all over the globe are endangered of acquiring it. Since the first outbreak of XDR typhoid in Pakistan in 2016, the ever-rising resistance of typhoid has become a major threat faced by the pediatric population of Pakistan. There is a dearth of available data in children harboring this extensively drug resistant pathogen countrywide. The objective of this study is to highlight the alarmingly increasing prevalence of XDR S. typhi in pediatric population in Federal territory in Pakistan.

Material and Methods: This Cross-sectional study was conducted at Microbiology Lab, Children Hospital, PIMS from June 2022 to May 2023. Blood culture samples of children (with strong clinical suspicion of typhoid) ranging one till 12 years (both genders).

Result: Out of a total of 245 typhoid suspected blood culture samples, 153 (62%) samples yielded growth of typhoidal Salmonella isolates with 109 (71 %) isolates proved as XDR *Salmonella Typhi* ,35 (23%) as MDR *Salmonella Typhi* and the rest 09 cases (6 %) were *Salmonella paratyphi A.* There was a male predominance of 96(63%) and the rest 57(37%) were females. Age range most commonly affected was 5-8 years followed by 9-12 yrs. There was 100% susceptibility to Meropenem in XDR typhoid cases and 99% XDR cases were susceptible to Azithromycin.

Conclusion: We concluded that there is widespread existence of MDR/ XDR *S.typhi* in pediatric population in federal territory as well as all over Pakistan but there is scarcity of documented data nationwide. Hence, our study signifies that a holistic approach should be implemented along with continuous ongoing surveillance to combat this frightening MDR/ XDR disease burden in this particular population.

Key Words: *Salmonella Typhi*, Extensively Drug Resistant (XDR), Typhoidal isolates

BACKGROUND

Typhoid fever is a deadly enteric bacterial infection caused by Salmonella enterica serotype typhi.[1] This disease is marked by clinical manifestations such as step-ladder patterned high-grade fever, chills, headache, muscle aches, loss of appetite and gastrointestinal issue (nausea, vomiting, constipation or diarrhea).[2,4] In addition, Paratyphoid fever caused by Salmonella enterica serovar paratyphi A, B, and C (*S. paratyphi*) also depicts a similar clinical picture but is often less severe.[1,2] Salmonella bacterium has a fecal-oral transmission and is usually contracted by consuming this microbe in contaminated food or water. It predominantly proliferates in poverty-stricken areas with poor socioeconomic conditions. Therefore, countries with underdeveloped and weak infrastructure, poor sanitation practices and neglected food safety guidelines are more prone to have a higher burden of disease.[3,4] Millions of people all over the globe are endangered of acquiring typhoid and paratyphoid fever due to exposure to this pathogen which further contribute to the disease complications and even mortality.[5] More fresh reports propose that around 21 million contract typhoid every year, yielding to 161,000 cases ending in fatalness worldwide.[6]

It is alarming that despite of all major advances that have been made in health care and medicine, there is a lack of universal control over typhoid; posing a serious menace to human health due to the emergence of multi-drug resistance in this pathogen over the years through multiple mechanisms.[5] Hence, more powerful strains have emerged with the passage of time, namely Multi-Drug Resistant (MDR) typhoid i.e

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resistant to all first line antibiotics; ampicillin, trimethoprim-sulfamethoxazole and chloramphenicol, and extensively drug resistant (XDR) typhoid i.e resistant to all five antibiotics (including both first & second line anti- typhoidal antibiotics); chloramphenicol, ampicillin, co-trimoxazole, fluoroquinolones, and third- generation cephalosporins. The outbreak of XDR typhoid was perceived by the world for the first time in November 2016 when health care experts in Pakistan started to report cases of extensively drug- resistant (XDR) typhoid fever, originating in Hyderabad, Sindh. Afterwards, a huge inflow of blood-culture proven extensively resistant typhoid Fever (XDR -TF) cases that were noncompliant to customary therapy were reported.[7] During November 2016 till December 2018, 5274 cases of XDR typhoid fever were recorded out of a total of 8188 cases as per WHO reports. A big chunk among these cases belonged to two major cities of Sindh province with Karachi accounting for 69% of all cases of XDR followed by Hyderabad (27%). The residual 4% cases were dispersed amid other provincial districts of Sindh.[8,9]

It is noteworthy that beyond 80% XDR cases that emerged in the Sindh area consists of children less than 15 years and 18% are below 2 years.[10] Hence, the ever-rising resistance of typhoid has become a major threat faced by the pediatric population of Pakistan over the last few years. It is one of the key issue and main danger faced by the pediatric population sparing neither any region nor any of the pediatric age. Moreover, unluckily there is a dearth of available data in children harboring this extensively drug resistant pathogen countrywide.

The purpose of this study is to report the alarming rising trend of blood culture-proven XDR typhoidal isolates along with their antimicrobial susceptibility patterns in pediatric population in the Federal territory of Pakistan, hence signifying the urgent need to implement a holistic approach at national level to combat the disease burden of this resistant isolate in our population.

MATERIAL AND METHODS

This descriptive, cross-sectional study was conducted at Microbiology lab, Children Hospital, PIMS from June 2022 till May 2023 after taking ethical approval. Children Hospital comprises of a 250 bedded tertiary care set-up which accepts patients from Federal territory as well as AJK and northern areas of Pakistan. Blood cultures from patients of age one year till 12 year (both genders) who presented with the history of fever for last 3 days or beyond, lacking any other recognizable site of infection and strong clinical suspicion of typhoid fever were incorporated in the study. Samples were collected from outpatient as well as indoors. Children with a known focus of infection like upper respiratory tract infection, Otitis Media, and Pneumonia etc. or with a negative culture for Salmonella and identical samples of similar patient were omitted from the study. A simple, well-structured proforma was used to collect the relevant information including socio-demographic data of every patient as well as their water & sanitation practices. Blood sample was drawn from the patient for blood culture, inoculated in Versa TREK bottles, labeled and dispatched to pathology department as soon as possible. These bottles were then incubated in Automated microbial detection system i.e Versa TREK blood culture machine (Thermo Fisher Scientific, USA) and observed continuously for growth for five days. On the basis of machine identification, a positive signaled blood culture bottle was sub-cultured on Blood, Chocolate and MacConkey agar media plates. These media plates were incubated overnight and growth characteristics were observed the next day. Colonies suggestive of Salmonellae were identified and further dealt with based on the colony morphology and rapid biochemical tests.

The definitive identification of Salmonella upto species level was done with API 20E (Biomerieux, France). Serological confirmation was done with Salmonella serology kit (Bio-Rad, France). Antimicrobial susceptibility testing was carried out on Muller Hinton (MH) agar by using Kirby Bauer disc diffusion technique. The standard antibiotic disks i.e Ampicillin (10 µg), Co- trimoxazole (1.25/23.75 µg), Chloramphenicol (30 µg), Ciprofloxacin (5 µg), Ceftriaxone (30 µg) Azithromycin (15 µg) and Meropenem (10 µg) were tested and zone sizes were interpreted according to CLSI guidelines 2022. For quality control of antibiotic disks, *Escherichia coli* ATCC 25922 was used. For quality control of anti-sera, *Salmonella Typhi* ATCC 700931 and *Salmonella paratyphi* ATCC 9150 were used. SPSS version 28 was used for data analysis.

RESULTS

Over the study period from June 2022 to May 2023, a total of 245 blood culture samples with provisional diagnosis / strong clinical suspicion of typhoid fever

patients were dealt with in microbiology department. Out of these, 153 (62%) samples yielded growth of typhoidal *Salmonella* isolates and the rest 48% either yielded no growth or some other bacteria was identified. Of the isolated *Salmonella* typhoidal isolates, 109 (71%) isolates proved to be XDR *Salmonella Typhi* on further work up. 35 cases (23%) came out to be MDR *Salmonella Typhi* and the rest 09 cases (6%) were recorded as *Salmonella paratyphi A.* None of these isolates came out as paratyphi *B or C* Table-I. Mostly, age range of patients was 5-8 years followed by 9-12 yrs. According to gender distribution, male children were 96 (63%) and the rest 57 (37%) were females. Table-II. Among XDR typhoidal isolates, the most susceptible antibiotic was meropenem i.e 100% followed by azithromycin with susceptivity of 98%. Among all MDR cases, the susceptibility of ceftriaxone was around 24%, ciprofloxacin susceptibility was only 1%, ampicillin was sensitive in 16%, chloramphenicol in 20% and cotrimoxazle in 15% cases (Table-III).

Table-I: Species breakup along with antimicrobial susceptibility pattern of Blood culture proven typhoidal Salmonella (n= 153).

Species	Total no. of cases (%)	Antimicrobial pattern (no. %)
Salmonella Typhi	144 cases (94 %)	XDR= 109 (76 %) MDR= 35 (24 %)
Salmonella parayphi A	09 cases (06 %)	MDR= 02 (22%) XDR= Nil
Salmonella parayphi B & C	Nil	Nil

Table-II: Age & Gender distribution of Blood culture proven typhoidal Salmonella (n= 153).

Characteristic		no (%)
Age (Years) Group	1 -< 5	23 (15 %)
	5 -< 8	81 (53%)
	8 -< 12	49 (32%)
Gender	Male	96 cases (63 %)
	Female	57 cases (37 %)

Table-III: Pattern of Antimicrobial Susceptibility among typhoidal isolates; *S. typhi & S. paratyphi* (n=153).

	***Salmonella Typhi* (n= 144, 62%)**			***Salmonella paratyphi* (n= 09, 06%)**		
	R	**I**	**S**	**R**	**I**	**S**
AMP	121 (84%)	0 (0%)	23 (16%)	2(22 %)	0 (0%)	7 (77%)
CAP	116 (80%)	0 (0%)	28 (20%)	2(22 %)	0 (0%)	7 (77%)
SXT	123 (85%)	0 (0%)	21 (15%)	4 (44%)	0 (0%)	5 (55%)
CIP	134 (93%)	9 (6%)	01 (1 %)	5 (55%)	2 (22%)	2 (22%)
CRO	109 (76%)	0 (0%)	35 (24%)	0 (0%)	0 (0%)	9 (100%)
AZT	3 (02%)	0 (0%)	141 (98%)	0 (0%)	0 (0%)	9(100%)
MEM	0 (0%)	0 (0%)	144(100%)	0 (0%)	0 (0%)	9 (100%)

NT= Not Tested, S= Susceptible, I= Intermediate, R= Resistant
AMP-Ampicillin, CAP- Chloramphenicol, SXT-Trimethoprim-sulfamethoxazole, CIP-Ciprofloxacin, CRO-Ceftriaxone, AZT-Azithromycin, MEM-Meropenem

DISCUSSION

Currently, Pakistan is among one of the highly prevalent typhoid fever countries of the world.[11] Inhabitants of Sindh and Punjab province have been stated to be endangered most among the 16 countries of Asia where typhoid disease is prevalent[6]. Since then, there has been an upsurge of both MDR and XDR typhoidal isolates in Pakistan. This is mostly attributable to an antimicrobial resistant strain carrying a mutant, endemic H58 gene that harbours a very strong tendency to disperse this XDR clone worldwide, hence elevating the fear of antibiotic treatment failure.[6,11] There have been reports of emerging cases countrywide and elsewhere due to provincial and international travel, however, the degree of spread in rest of Pakistan still remains poorly understood.[12] Reports of XDR typhoid cases occurring in other parts of Pakistan are now being informally reported as well as in individuals travelling to UK and USA.[13] To our dismay, azithromycin is the

only oral antibiotic available for treating uncomplicated XDR S.typhi cases.[14] On the other hand, the only drug available to treat hospitalized and complicated cases is Meropenem, which is an injectable antibiotic.[15] This heightens the dilemma of limited antibiotic options that we are left with to treat typhoid fever. It is noteworthy and alarming that this resistant typhoid isolate is highly prevalent in pediatric population of Pakistan amplifying the fear of treatment failure and grave outcome in this population.

In our study, the predominant age group involved is 5-9 years (53%) mainly because of informal and frequent visits to street sellers, lack of cognizance about food quality and non-adherence to hygienic practices. Comparable observation was reported by Iftikhar *et al* in a tertiary care set up in Lahore in 2018 with 52% of the affected patients aged 5- 10 years.[16] Another study done by Memon *et al* in Karachi in 2019-2020 reported that the age bracket mainly affected was 5-7.5 years which is also similar to our findings.[17]During another study conducted by Fivizia and colleagues in Karachi in 2017-2018, it was observed that the predominant age range in children was 5-6 years.[18] In a study by Gul *et al* in Lahore, most children were between 4-8 years of age i.e 44.8%.[22] On contrary, a study done at Indus hospital Karachi by Saba Shahid and colleagues showed that children below 5 years of age suffered the most with XDR S.Typhi fever which coincides with our findings.[19] Our finding is also in contrast to the study conducted by Saeed N *et al*. in 2019 where only 33% of the registered patients had the age range of five to 10 years.[12]

There was a distinct high proportion of male children in our study i.e. 63%, most probably due to more outdoor exposure, and extra vulnerable and care-free behavior in boys. This finding is comparable with the study done by Khan and Mohammad in 2012.[20] Similar predominance was observed by Fatima *et al* who found out that almost two- thirds (63%) of their patients were males.[21] Study done by Memon *et al* in Karachi also showed a male preponderance of 61.5%.[17] Moreover, there was male predominance with 56.4% boys and 43.6% girls in study done by Gul *et al* in Lahore that also corresponds to our findings.[22] On contrary, Mubashir *et al* reported that 54.9% cases out of all blood culture positive patients were females and 28 (55.1%) were males which is in contrast to our findings.[26]

In our study, out of 245 blood culture samples, 153 (62%) samples yielded growth of typhoidal Salmonella isolates (both MDR & XDR) and the rest 48% either yielded no growth or some other bacteria was isolated. Similar findings were reported in another study done at Department of Pediatrics, Combined Military Hospital, Lahore by Gul *et al* where 55.2% cases were found to have XDR Salmonella on blood cultures, 34.7% had MDR Salmonella and only 43 (10.1%) children were non-resistant to usual antibiotics.[22] In congruence with our study, Aslam *et al*. also found XDR Salmonella in 54% of cases however their data included both children and adults.[23] Other studies by Yousafzai *et al*. and Hussain *et al*. in Karachi documented 60% and 48% XDR cases respectively[9,24] being consistent with our findings. Qamar *et al* also showed that a almost 67% of the isolates displayed multidrug resistance (MDR), besides out of them, 53% were XDR in their study.[25] On contrary, in a study done by Mubashir *et al* in Lahore in 2020-2021, among 246 patients enrolled, blood culture was positive for Salmonella in only 62 (25.2%) patients, and majority cases came out to be negative i.e 184 (74.8%) patients.[26] Another large hospital-based study conducted in Karachi by Yousafzai *et al*, Pakistan showed a culture positive rate for Salmonella of 22% which also coincides with our results.[9]

By March 2018, WHO endorsed the use of typhoid conjugate vaccine in children aged six months and beyond to combat the highly resistant typhoidal isolates in prevalent areas or countries.[14]In 2019, Pakistan turned out to be the leading country in the world to include the World Health Organization (WHO)-recommended typhoid conjugate vaccine (TCV) into its expanded programme immunization schedule. However, under vaccination in Pakistan is a problem in general due to limited supply of vaccine, lack of trust in vaccinations, in awareness, education barrier and casual attitude of healthcare worker for advocacy of vaccines. Therefore, despite of all preventive measures introduced, we are still facing challenges in combating this disease mainly due to lack of commitment and negligence at mass level both administratively and politically. There is a lack of quality control and standardization of laboratories in

Pakistan and rural areas lack facilities where blood cultures are available. A lot of work needs to be done to improve the health care facilities and diagnostic modalities for timely diagnosis and adequate treatment. Moreover, the common trend of prior medications from quacks, frequent use of over-the-counter drugs to treat fever and prevalence of poor health seeking behavior in our population is also an important contributing factor for spread of XDR strain. Considering the limited alternative therapeutic options, timely diagnosis, and judicious use of antibiotics and preventive management of XDR typhoid fever especially in the pediatric population is becoming very important. It is the need of time that to establish antibiograms in all major tertiary care hospitals countrywide to make the clinicians aware about the current trend of disease, choice for empirical therapy as well as for MDR/ XDR typhoid cases. In addition, widespread use of typhoid vaccines, improvements in water supply and sanitation and community awareness campaigns can extensively help overcome this disease burden constituting a vital part of long- term intervention measures. Moreover, issuing guidelines for judicious use of antibiotics, launching informative campaigns and educational sessions for effective implementation of antimicrobial stewardship program in healthcare settings at national level could make the difference, since XDR typhoid is a battle that needs to be fought against collectively by all stakeholders of society i.e healthcare workers, authorities and civil society.

CONCLUSION

This study lays emphasis on the alarmingly increasing prevalence of XDR ***S.typhi*** in pediatric population in Federal territory and keeping in view the other local studies, we can conclude that there is widespread existence of MDR/ XDR *S.typhi* all over Pakistan. Hence, our study signifies that a holistic approach should be implemented along with continuous ongoing surveillance to combat this frightening MDR/ XDR disease burden country wide.

LIMITATIONS OF STUDY

It was a single-center study. More data from other hospitals in federal territory as well as peripheral health care facilities of Islamabad should have been included to deduce more precise conclusions.

CONFLICT OF INTEREST

None

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHOR CONTRIBUTION

Samia Wazir: literature search, study concept, questionnaire design, data analysis, data interpretation, drafting, final approval to be published

Umme Farwa: Conception/design of the work, interpretation of data, revised it critically, final approval to be published

Soffia Khursheed: literature search, study concept, questionnaire design, data analysis, data interpretation, drafting

Mehreen Mushtaq: Conception/ design of the work, interpretation of data, data analysis

Muhammad Shafiq: Data collection, data analysis

Muhammad Moaaz Ali: Conception/ design of the work, interpretation of data, data analysis

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ORIGINAL ARTICLE

Viral suppression with antiretroviral therapy after single drug substitution of efavirenz with dolutegravir at an HIV Centre in Karachi, Pakistan

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ABSTRACT

Background: AIDS was first reported in the 1980s. The HIV prevalence rate among Pakistani adults is 0.2%. The Sindh Center for AIDS Control Programs (SACP) was established in Karachi in 2006. In 2018, the SACP introduced the integrase chain transfer inhibitor dolutegravir (DTG) and lamivudine (3TC) and tenofovir diproxilfumarate (TDF). Patients who developed virological failure on Efavirenz, 3TC, and TDF were switched to DTG/3TC/TDF. As the two nucleoside reverse transcriptase inhibitors remained the same, EFV and DTG were effectively substituted as single agents. We assessed immunological, virological and clinical responses to DTG.

Material and Methods: A retrospective chart review was conducted at the SACP of adults with virological failure on EFV/3TC/TDF who were switched to DTG/3TC/TDF from April 2019 till November 2023.

Results: The 14 patients were switched after a mean of 28.2 months of prior antiretroviral therapy with mean CD4 of 116 cells/mm^3. Mean age was 28.4 years with 7 (50.0%) males.12 were adherent to the DTG regimen; 11 (92 %) achieved viral suppression. The Mean period of suppression was31 months. There was no clinical or immunological failure with upsurge of mean CD4 to 632cells/mm^3.

Conclusion: The patients with virological failure on an efavirenz-based regimen are likely to have viral suppression after switch to a dolutegravir-based routine even if they potentially have NRTI resistance. These results are relevant to HIV programs in resource poor settings where switches between regimens are often implemented without frequent viral load or resistance testing.

Keywords: HIV, Dolutegravir, Efavirenz, Tenofovir, Viral suppression, CD4 count

BACKGROUND

AIDS (Acquired Immune Deficiency Syndrome) was first reported in the 1980s and has affected more than 75 million people worldwide. In 2021, an estimated 3.4 million people worldwide are living with HIV. WHO has identified Pakistan as a country of concentrated epidemic. HIV prevalence among adults aged 15-49 is 0.2%, with the latest estimates of 270,000 adults living with HIV.[1]

As of June 2023, approximately 60,439 HIV cases were registered with the National AIDS Control Program (NACP), and 38,234 were receiving antiretroviral treatment (ART) at 74 HIV centers.[2] The group at the highest risk were the users of injected drugs, who represented 38% of the registered patients.[3,4] In addition, poor infection control practices in healthcare facilities and unsafe blood transfusions have been recognized as major risk factors for infectious diseases.[5] The Sindh AIDS Control Program Centre (SACP) was established at the Ruth KM Pfau Civil Hospital in Karachi in 2006.[6] In 2018, the SACP launched Dolutegravir, a new integrase strand transfer inhibitor (INSTI) that blocks the integration of the HIV genome into host DNA in a single-dose combination (FDC) with lamivudine (3TC) and tenofovir diproxilfumarate (TDF) for the newly diagnosed patients as well as for the treatment experienced patients. The latter included patients on efavirenz (EFV)-based regimens of EFV/3TC/TDF and those who were virologically suppressed, had side effects, or had virological failure.[7] These patients were changed to DTG/3TC/TDF. Since the 2 nucleoside reverse transcriptase inhibitors

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remained the same, this switch amounted to essentially a single drug substitution from EFV to DTG.

However, one of the steadfast principles of antiretroviral therapy has always been that single drug substitution must be avoided as it can lead to the emergence of drug resistance. Resistance testing to guide management is also strongly recommended.[8] Common mutations include development of M184V in a lamivudine and emtricitabine based regimen leading to decreased susceptibility to these agents, although when options are limited, HIV care providers have continued lamivudine or emtricitabine despite the presence of M184V in order to select for a less fit virus.[9] The mutation K65R leads to resistance to tenofovir. The guidelines state that second line ART regimen should have a new drug class or drug to which individuals have been exposed but with no evidence of cross-resistance and must include two or three active ingredients. The guidelines have repeatedly warned that adding only one active agent to a weak regimen is the same as monotherapy and there is a risk of failure.

The Genotypic resistance testing is not routinely available in resource limited settings such as the SACP. Empiric first line regimens initiated by SACP are based on WHO guidelines which have advised HIV programs to utilize a DTG based regimen as preferred first line treatment.[10,11]

Dolutegravir has been shown to be equivalent or superior to current treatment regimens in experienced and naïve patients, including patients who have previously failed raltegravir or elvitegravir.[12] Dolutegravir has a high genetic barrier to resistance and its use in ART regimens has been associated with sustained viral suppression and immunological recovery.[13] In addition to first line therapy, dolutegravir is also recommended as second line therapy, although the efficacy is uncertain when dolutegravir is given with NRTIs that are predicted to be compromised by resistance.[11] Therefore, WHO guidelines recommend changing one of the NRTIs from tenofovir to zidovudine when switching to second line therapy.[11]

However, these principles and guidelines were challenged in the RCT NADIA study conducted in seven sub-Saharan African sites, where tenofovir was continued in the second line regimen and found to be superior than switching to zidovudine in terms of achieving viral suppression, reducing viral rebound, increasing CD4 cell count and diminishing the risk of high level dolutegravir resistance.[14] The NADIA trial also provided evidence that patients, even if they have extensive NRTI resistance, are likely to have viral suppression after a switch to dolutegravir.[14]

A prospective interventional study conducted in Cape Town South Africa evaluated the recycling of tenofovir and lamivudine/emtricitabine with dolutegravir in second line ART and demonstrated that a high proportion of participants achieved viral suppression. [15]

The data about changing ART in this manner as second line treatment in Pakistan is almost absent. We therefore carried this study so as to see the effects in our HIV center where patients failing to an EFV containing regimen were switched to a DTG containing regimen with two NRTIs not changed. This switch was performed without the benefit of earlier genotypic determination of NRTI resistance. Implementing WHO's recommendation of TDF switch to zidovudine is also not possible as that drug was also not in stock. Therefore, we felt that the focus should be on these patients who completely stopped efavirenz intake and started dolutegravir as a single drug substitution.

MATERIAL AND METHODS

This descriptive retrospective cross-sectional study was conducted at The Sindh AIDS Control Program (SACP) center at the Ruth KM Pfau Civil Hospital in Karachi, Sindh, Pakistan.

This study included HIV infected patients who were registered at the SACP and were above the age of 18 years, were not virally suppressed on EFV/3TC/TDF for last six months and were switched to DTG/3TC/TDF for salvage therapy. They were further required to have a minimum of 6 months of follow-up at the SACP center and at least two viral load determinations documented after start of the DTG regimen in order to be included.

Definitions according to the National AIDS Control Program, Pakistan.[15]

Virological failure: Plasma VL above 1000 copies/ml (based on two consecutive viral load measurements within a 3-month interval, with adherence support following the first viral load test, and after at least six months of starting a new ARV regimen.

Immunological failure: CD4 count not rising above 250 cells/mm^3 following clinical failure or persistent CD4 cell count below 100 cells/mm^3.

Clinical failure: New or recurrent clinical event indicating severe immunodeficiency (WHO clinical

stage 4 condition) after six months of effective treatment.

Viral Suppression: Viral load less than 500 copies/ml.

Adherence: Documentation of >95% of scheduled clinic visits and doses as determined by pill counts and self-reports at each visit.

All patients who had been on Dolutegravir treatment from the time the SACP started DTG in 2018 were identified from the records. A chart review of these patients was conducted using manual reviewing and the reason why they changed from a prior ART to DTG containing regimen was taken note of. These patients who met the inclusion criteria of our study also went the additional analysis and their medical charts were reviewed from the time DTG was given till their last visit documented. The period of our study was from the month of April 2019 when the first patient who was not responding to therapy was started on a DTG-based regimen to the month of November 2023, where the data were collected, analysed and the results were represented.

The features of patients, laboratory results, clinical events and the outcome were recorded. The laboratory tests indicated by HIV RNA PCR level, CD4 cell count and complete blood count, as well as liver function tests, were documented twice during the study: at the point of the switch from EFV to DTG and then the last values documented in the study. The progress notes by the HIV care providers in charts were assessed to see if the patients were taking their HIV medication well without side effects, any new disease that developed or whether they had any weight gain or loss. The primary outcomes of the study on the patients that use DTG containing protocol include virological failure, viral suppression, immunological failure or recovery, clinical response or new HIV related clinical event, and death. Other outcomes were patients lost-to-care-or-transfer. The Patients who met the inclusion criteria were identified and data was entered into Microsoft office Excel 2013. The descriptive statistics were calculated for quantitative variables. The distribution of patients by socio-demographic characteristics and other relevant variables in the study were described using descriptive analysis and were presented as mean or grouped into ranges for determination of percentages. The two main variables, CD4 count and viral loads, were studied and their time series was plotted through MS excel using scatter plot to observe the overall trends of the values over time.

RESULTS

33 patients were recognized who were switched from EFV/3TC/TDF to DTG/3TC/TDF. The indications for the switch were in order to comply with new WHO guidelines in 2 (6.06%) patients, due to adverse effects to efavirenz in 16 (48.4%), and virological failure in 15 (45.4%).

Of 15 patients with virological failure, 14 met the study inclusion criteria since one patient was excluded as he was lost to follow-up after only 4 months of starting the new regimen and had only one viral load determination documented. He did, however, achieve viral suppression and was undetectable at 4 months.

Of 14, the mean age was 28.4 years; there were 7 (50.0%) males. Most patients were from Karachi. Of 14, 6 (42.8%) and 2 (14.3%) patients were classified as WHO HIV Clinical Stages III and IV, respectively. The 14 patients had already been on antiretroviral therapy for an average duration of 28.2 months prior to the switch. The mean CD4 was 116 cells/mm^3 prior to the switch to DTG/3TC/TDF regimen.

Of 14 patients, 12 were adherent to their new DTG regimen. Of the two patients that were non-adherent, one was lost to follow-up and one died. For purposes of evaluation of response to the new DTG regimen, we followed only the 12 adherent patients for an average of 31 months. Of 12, 11 (92 %) achieved viral suppression whereas one patient had virological failure. He was suppressed for 20 months and then became detectable. He was switched to Atazanavir/ ritonavir (ATV/r) based regimen when repeat VL at 23 months was still high, but he also failed the protease inhibitor regimen despite documentation of adherence to ART. Mean duration of suppression for 12 patients on DTG regimen was 31 months (range of 20-38). There was no documentation of immunological or clinical failure (Table-II). A steady increase in CD4 cell counts over time on the DTG regimen was documented (Figure-I). Of 11 patients who achieved viral suppression, 9 were reported as undetectable at < 20cps/ml (Figure-II).

Table-I: Baseline characteristics of HIV Infected patients with virological failure on EFV/3TC/TDF (n=14).

Demographic Characteristics	**n (%)**
Mean Age (Years)	28.42 (20-40)
Gender	
Male	7 (50.0)
Female	5 (35.7)
Transgender	2 (14.2)
Occupation	
Housewife	4 (28.5)
Driver	1 (7.1)
Dancer	2 (14.2)
Unskilled worker	3 (21.4)
Hairdresser	1 (7.1)
Office worker/ shopkeeper	2 (14.3)
Domestic Staff	1 (7.1)
City of Residence in Sindh	
Karachi	13 (92.8)
Thatta	1 (7.1)
HIV Stage as per WHO Classification	
Stage I (Asymptomatic)	1 (7.1)
Stage II (Mild Symptoms)	5 (35.7)
Stage III (Advanced Symptoms)	6 (42.8)
Stage IV (Severe Symptoms)	2 (14.3)
Mean Duration of ART months	28.2 (11-70)
Mean CD4 cells/mm3	116
Clinic Disposition	
In HIV Care at SACP	12 (85.7)
Non-adherent and Lost to Care	01 (7.1)
Non-adherent and Died	01 (7.1)

Table-II: Laboratory Parameters and Outcome of 12 Patients Adherent to DTG/3TC/TDF Regimen

Laboratory Parameters	**Baseline (prior to switch)**	**Most recent values on record (after switch)**
MeanCD4 count (cells/mm3)	125	632
Mean HIV RNA PCR (cps/ml)	475157.3	<500 (n=11) 18100 (n =1)
Mean Hemoglobin (g/dL)	8.25 (5-11)	10.91 (7-13)
Mean Creatinine (mg/dl)	0.80 (0.6-1)	0.85 (0.7-1)
Mean AST (u/l)	18.25 (8-28)	15.83 (8-35)
Mean ALT (u/l)	24.25 (10-53)	19.5 (8-35)
Mean Weight(kg)	50 (32-62)	61 (48-79)
Mean Duration of DTG/3TC/TDF (months)	31 months (20– 38)	
Outcome	**n (%)**	
Viral Suppression	11 (91.63)	
Virological Failure	1 (8.3)	
Mean Duration of Sustained Suppression in Months	31 (range 20-38)	
Immunological failure	None	
Clinical Failure	None	
In HIV Care at SACP	12	

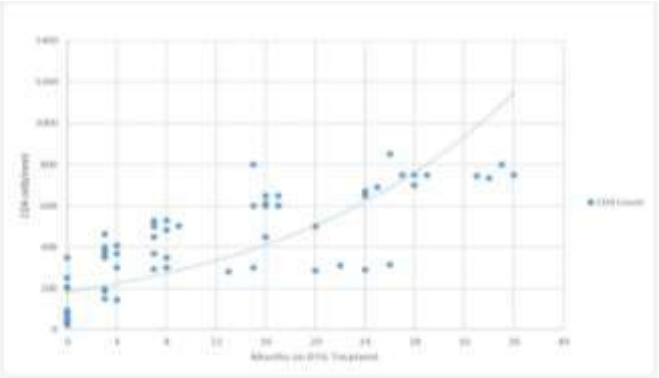

Figure-I: CD4 cell counts in 12 patients adherent to new DTG/3TC/TDF regimen.

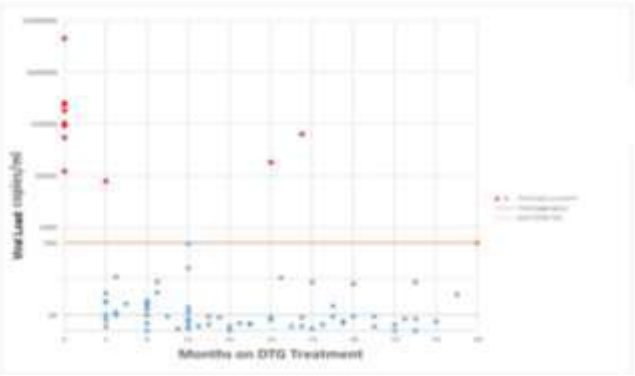

Figure-II: HIV RNA PCR in 12 patients adherent to new DTG/3TC/TDF regimen.

DISCUSSION

Our research revealed that, single drug substitution from efavirenz to dolutegravir while retaining the same two NRTIs among patients with virological failure led to sustained virological suppression in all patients with the exception of one. The regimen using DTG as the backbone was just as successful although the nucleosides coupled to DTG were predicted to lack activity because of drug mutation. Further, the WHO recommendation of using zidovudine instead of tenofovir was disregarded. These results are consistent with the NADIA study which recommends dolutegravir in combination with NRTIs as a treatment for HIV-1 infection, even in those patients with extensive NRTI resistance upon whom no NRTIs are predicted to be active.[14] The reason for preserved NRTI activity is uncertain but it may be due to impairment of viral replicative capacity by NRTI resistance mutations.[16]

Out of 12 patients, we reported that only 1 had virological failure in the DTG based treatment and or the ATV/r-based treatment. He is eligible for resistance screening, but unfortunately this is not offered at the SACP. Dolutegravir resistance is thought to be scarce in the case the drug is co administered with fully potent NRTIs. Reports from the NADIA study of intermediate-to high-level dolutegravir resistance among four patients within 48 weeks present a challenge to public health intervention, particularly in poor settings where frequent viral load monitoring is not permissible and integrase resistance testing is not available or is expensive.[14] The 2023 study, on the other hand, was promising as it evaluated the effectiveness and safety of using only two drugs, dolutegravir/ lamivudine (DTG/3TC), and it showed that most participants had sustained viral suppression, no treatment-emergent resistance, and good safety for more than 48 weeks.[17]

This study demonstrated a substantial immunological response as documented by different studies elsewhere.[18,19] Adverse event to the DTG-based treatment were not reported in our study.

In the context of comparing performance of dolutegravir based second-line treatment vs dolutegravir based treatment as a first regimen, virological suppression percentages are comparable. A 2020 study by Calamy A *et al* comparing dolutegravir based with low-dose efavirenz based regimen as first line treatments for HIV-1 infection found that at week 96 dolutegravir regimen was non-inferior and no dolutegravir resistance had emerged thus making it appropriate for use as first-line antiretroviral regimen. Suppression of viral load was achieved earlier in the dolutegravir trial patients and overweight was more pronounced in this group.[20] A more recent 2023 study conducted in India by Mahale PR *et al* in treatment naïve patients found that viral load suppression was achieved in 55.71% of clients in an efavirenz based treatment group after six months of ART whereas 88.57% of clients achieved viral suppression in the dolutegravir based treatment group, which was highly significant.[18]

The study from India by Mahale *et al* also documented significantly more weight gain at 12 months (mean +6.15 kg) as compared to the EFV-based regimen (mean +1.85 kg).[18] The findings from India are similar to ours where we proved that there was statistically significant weight gain which was a marker of the patients' return to health. However, a study by Brennan *et al* from South Africa disputed in the sense that the benefit of weight increases with dolutegravir and other integrase strand inhibitors should not be obvious in the light of the on-going increasing obesity rates all over the world which in turn increase the risk of metabolic and cardiovascular diseases.[21]

Although number of patients were small, this study is the first significant analysis of practical experience of a single drug substitution in Pakistan. The findings of the study are useful not only to programs of this sort but also to other existing HIV programs situated in resource-deprived settings where switches are often performed in the absence of viral load testing and resistance testing. A resistance test for those patients who had achieved virological suppression or with virological failure in case of such patient's availability would have been defined as a future study direction. Globally, HIV care providers are switching to universal dolutegravir treatment, a combination that will be used by all patients. Those who are on second and third-line drug regimens and are NRTI-resistant are also likely to have viral suppression after the switch to dolutegravir. Some limitations of the research method employed in this study include the use of retrospective study. It is a single center study with a rather small sample size. This study demonstrated that the switch from efavirenz based treatment to dolutegravir based with single drug replacement was successful.

CONCLUSION

This study shows that patients with virological failure on efavirenz therapy experience virologic suppression after switching to dolutegravir-based therapy, even with high NRTI resistance. These findings are relevant to other HIV programs in low-resource settings where switching regimens without viral load testing or resistance testing is used. The results support a global trend of HIV patients switching to dolutegravir-based treatment.

CONFLICT OF INTEREST

None

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHOR CONTRIBUTION

Samia Samad: Conception and frame work, Drafting,
Nazish Misbah: Literature search
Sughand Memon Mir: Data collection
Sadia Ishaque: Review of manuscript
Obaidullah Farooqui: Data analysis, data interpretation
Shehla Baqi: Critical review, final approval of the article, study design

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REVIEW ARTICLE

Surge for narrow-spectrum antibiotics in times of the MDR crisis: Systematic literature review to establish the role of amoxicillin in tonsillopharyngitis

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ABSTRACT

Background: Group A Streptococcus (GAS) is the predominant pathogen accountable for tonsillitis, making it the most prevalent and frequently encountered bacterial cause of upper respiratory infections. Although amoxicillin is a frequently prescribed antibiotic for the treatment of tonsillopharyngitis, alternate agents like macrolides are regularly recommended. The objective of this study was to evaluate and compare the effectiveness of amoxicillin, its side effects, and the associated risk ratio with alternative antibiotic treatments for respiratory tract infections and tonsillopharyngitis in both children and adults.

Material and Methods: An initial search was performed via five basic databases PubMed, Medline, Embase, clinicaltrial.gov and the Cochrane Central Register of Controlled Trials, to identify studies meeting the inclusion criteria spanning from July 2012 to June 2023. The primary search of studies resulted in 6260 from four databases during the study period.

Results: After the initial scan of titles and abstracts, 06 studies were included in the review, which reported clinical cure rates. Four out of six studies reported adverse events.

Conclusion: Our analysis infers that given their narrow spectrum of activity, low incidence of side effects, comparable efficacy and cost-effectiveness, penicillin or amoxicillin can be considered as preferable choice for the management of tonsillopharyngitis and broad-spectrum antibiotics offer no added advantage in disease management.

Keywords: Amoxicillin, Tonsillopharyngitis, Tonsillitis, Acute respiratory infection, Streptococcal infection, GAS, SLR, Systematic literature Review, Antibiotics

BACKGROUND

Tonsillopharyngitis is defined as an acute infection affecting the pharynx, palatine tonsils, or both. Being among the prevalent upper respiratory tract infections, it frequently leads individuals to seek medical care and receive antibiotic prescriptions. While a majority of cases of tonsillopharyngitis result from a viral etiology, which includes rhinovirus, respiratory syncytial virus, adenovirus, and coronavirus, bacterial infections are typically due to group A beta-hemolytic Streptococcus (GABHS), also known as Streptococcus pyogenes. GABHS is recognized as the causative agent for acute pharyngitis in approximately 15-35% of children and 5-15% of adults.[1]

Antibiotics are often indicated for the treatment of GABHS, for alleviating symptoms such as pain and fever), for shortening the duration of the illness, for preventing clinical relapse (i.e., recurrence of symptoms after initial resolution), and for preventing complications (suppurative complications, acute rheumatic fever, post-streptococcal glomerulo-nephritis). [1]

As per the IDSA practice guidelines for the management of group A streptococcal pharyngitis, penicillin or amoxicillin are the antibiotics of choice.[2] Although beta lactams have been in continuous use, there have been no reports of penicillin resistance in GABHS, and the antibiotic remains susceptible and effective. On the other hand, increased rates of resistance are documented for both fluoroquinolones and macrolides, yet, physicians frequently choose these broad-spectrum antibiotics for the treatment of tonsillopharyngitis,

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despite their associated side effects. In fact, there has been a notable surge in the prescription of broad-spectrum antibiotics for the management of upper respiratory tract infections.[3,4]

Narrow-spectrum antibiotics should be preferred over broad-spectrum antibiotics in the treatment of pharyngitis due to several reasons, supported by evidence. Firstly, narrow-spectrum antibiotics target specific bacteria, reducing the risk of disrupting the body's natural microflora and the development of antibiotic resistance.[5] Secondly, they are effective against the most common causative agent of pharyngitis, GABHS, while minimizing the impact on other bacteria.[6]

The excessive use of broad-spectrum antibiotics constitutes another form of inappropriate prescribing and is associated with an increased risk of unnecessary bacterial resistance, drug-related adverse effects, and escalated costs. Antibiotic resistance further increases mortality, cost of care, and length of hospital stays.

Hence, this systematic review aimed to evaluate the available literature comparing the utilization of broad-spectrum antibiotics over amoxicillin in tonsillopharyngitis treatment. The findings from this review will contribute valuable data for evidence-based decision-making and, subsequently, play a pivotal role in policy development, education, and quality improvement initiatives. The assessment will aid in understanding the effectiveness, safety, and impact on patient outcomes, facilitating judicious antibiotic use and reducing the risk of resistance development. Based on this clinical scenario, the following research question was formulated: Do broad-spectrum antibiotics offer any added advantage over amoxicillin in the treatment of tonsillopharyngitis?

MATERIAL AND METHODS

In accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, we conducted a search in the PubMed, Medline, Embase, Clinicaltrial.gov and Cochrane databases for English-language articles published from July 2012 to June 2023.[7] This involved curated Boolean search strings and a thorough review of the bibliography of studies identified through the database searches. These databases were selected due to their accessibility at no cost.

The following keywords were utilized: "Tonsillopharyngitis," "Tonsillitis," "Acute respiratory infection," "Upper respiratory tract infections," "Streptococcal infections," and "Pharyngitis." Experimental studies consisting of randomized control trials; quasi-experimental studies consisting of non-randomized control studies, before-and-after studies, interrupted time series studies; and observational studies consisting of the cohort studies, and case-control studies were included. Review articles, guidelines, and commentaries were excluded from the review. Duplicate studies were also removed. Subsequently, two independent reviewers conducted iterative rounds of blinded title and abstract screening, followed by a thorough review of full-length articles. Any discrepancies were resolved through discussion. All the chosen studies were entered into Excel.

The initial search yielded a total of 6260 studies from five databases over the study period. The review included clinical trials, randomized controlled trials, case-control studies, comparative studies (comparison with other antibiotics) and interventional studies in English. Studies without full-text articles were not included in this review.

Our inclusion criteria involved quantitative studies conducted worldwide that compared oral amoxicillin with other antibiotics for treating tonsillopharyngitis in adults or children across various settings. The inclusion criteria were not restricted by dosage or duration of treatment.

Only studies involving participants meeting the criteria for the diagnosis of tonsillopharyngitis were considered. The primary outcome of interest centered on the resolution of symptoms following the prescription of amoxicillin compared to other antibiotics. Secondary outcomes of tonsillopharyngitis following treatment with amoxicillin, in comparison to other antibiotics, included considerations of dose, duration of treatment, and adverse events.

RESULTS

The preliminary investigation produced 6260 findings from five databases within the designated study period (Figure-I). After eliminating 2647 duplicated studies and excluding 281 with incomplete data, an additional 3326 were dismissed, encompassing conference papers, other intervention measures, systematic reviews, or editorials. Subsequent to the initial scrutiny of titles and abstracts, the review incorporated 6 studies. The qualitative synthesis of the review included 6 studies, whereas the meta-analysis reviewed 5 out of 6 studies.

Two independent authors evaluated the quality of the chosen studies across various domains, including the generation of allocation sequence, concealment of allocation, blinding of participants and personnel, blinding of outcome assessor, incomplete outcome data, and selective reporting. The assessment utilized the 'Risk of bias' tool outlined in the Cochrane Handbook for Systematic Reviews of Interventions (Higgins 2011).[8] The overall risk of bias was categorized as low, high, or unclear. Discrepancies between the authors regarding the quality assessment were resolved through consensus.

The risk ratio (RR) for clinical cure rates in patients treated with amoxicillin compared to other antibiotics was calculated. The results from the included studies were categorized based on the antibiotic treatment strategy. A random-effects model was employed to compute pooled results with a 95% confidence interval (CI). The heterogeneity of the studies was assessed using Cochran's Q test and Higgins I2 statistic. The pooling of overall effect estimates was conducted using Review Manager web (RevMan web, The Cochrane Collaboration, Denmark).

All these studies were released within the past 11 years, with the earliest study dating back to 2000 and the most recent one published in 2022. [9-14] The included studies examined a total of 274194 participants. Three retrospective studies compared penicillin/amoxicillin to broad-spectrum antibiotics including amoxicillin-clavulanate, cephalosporins, and macrolides for the treatment of acute respiratory tract infections along with treatment outcomes and adverse events. (Gerber 2017, Peng Li 2019, Mattan 2022).[12,13,14]

One study compared the clinical efficacy of amoxicillin-clavulanate with amoxicillin for the treatment of pharyngolaryngitis or tonsillitis and the other study compared the performance of amoxicillin and intramuscular benzathine penicillin in relieving manifestations of streptococcal pharyngitis. (Kuroki 2013, Gerber 2017).[10,12]

The last study compared amoxicillin with a placebo in the management of tonsillopharyngitis. (Leelarasamee 2000).[9]

Two studies employed the drug-controlled, randomized, comparative study design to measure the efficiency of amoxicillin against other antibiotics. [11,13] Peng Li *et al*. reported no statistical significance for the clinical and bacteriological eradication efficacy between azithromycin, cefaclor and amoxicillin.[13] Eslami *et al*. reported that once-daily therapy with amoxicillin is as effective as intramuscular benzathine penicillin G for the treatment of GABHS pharyngitis, but penicillin was significantly more effective in reducing exudate and concurrent signs vs. amoxicillin.[11] Mattan *et al*. reported that amoxicillin and penicillin-V treatments were associated with fewer additional primary physician visits compared to other antibiotic treatments. [14] Kuroki *et al*. reported that clinical response rate of treatment with amoxicillin-clavulanate compared to amoxicillin was equivalent.[10] Leelarasamee *et al*. reported that amoxicillin therapy conferred no benefit or harm when compared to a placebo.[9]

Since Leelarasamee *et al*. did not compare penicillin or amoxicillin to another antibiotic agent, it was not included in further analysis, as it did not fulfil our research questions. Out of the remaining 5 studies, only four studies reported adverse events due to the treatments given.

Figure-IV describes the individual assessment of each study included in the meta-analysis. All studies were classified with a low risk of bias.

The pooled analysis with the outcome of treatment success of tonsillopharyngitis was also performed for oral antibiotic therapy with amoxicillin and other antibiotics. Five studies (143954 patients) reported clinical cure rates.

Clinical Efficacy of Amoxicillin versus other Antibiotics: The results indicate no significant difference between amoxicillin and other antibiotics for treating tonsillopharyngitis (Figure-II, Amoxicillin vs. any antibiotics, RR: 1.59, 95% CI: 0.73–3.48, p = 0.25, I2 = 100%). The p-value of 0.25 indicates that there is no statistically significant difference in treatment success between the "any antibiotics" group and the amoxicillin group Figure-II.

Adverse Events of Amoxicillin versus other Antibiotics: Four out of five studies reported adverse events. The results indicate that amoxicillin was associated with a significantly lower risk of adverse events (pooled RR: 0.64, 95% CI: 0.42–0.98, p = 0.04, I2 = 82%).

In this systematic investigation, we examine the documented side effects of amoxicillin in comparison to other antibiotics. The review scrutinized each study, examining its findings on amoxicillin's side effects and comparing them with those of other antibiotics.

Euroki *et al*. provided useful insights on the side effect profile of amoxicillin and its comparison with other antibiotics. The main side effects related to amoxicillin were diarrhea (n=5) and upper airway inflammation (n=1), whereas diarrhea (n=22), urticaria (n=1), and eruptions (n=1) with associated with other antibiotics. Fever was the most often reported side effect related to amoxicillin administration, by Mattan *et al*., with an incidence of n=117 compared to other antibiotics (Fever, n=141). Similarly, Peng Li *et al*. reported diarrhea (n=4), rash (n=2), and nausea (n=3) with amoxicillin as compared to other antibiotics (diarrhea, n=3), (rash, n=5), (nausea, n=3). Lastly, Gerber at al. reported overall 849 adverse events with amoxicillin compared to 189 events reported with other antibiotics, as shown in Table-I.

Individuals who received amoxicillin treatment had a 36% decreased risk of experiencing adverse events compared to those who received other antibiotics, according to the pooled risk ratio (RR) of 0.64. The p-value of 0.04 indicates that the difference in adverse events between the amoxicillin and other antibiotics groups is statistically significant (Figure-III).

Risk of Bias: Using the RoB-2 technique, we assessed the total risk of bias in six studies (Leelarasamee 2000, Haruo 2013, Eslami 2014, Jeffrey 2017, Peng Li 2019, and Mattan 2022).[9-14] While Leelarasamee 2000 and Jeffrey 2017 indicated some concerns in D5 (bias in the selection of reported results), the rest of the research demonstrated a minimal risk of bias in the domains studied. These findings provide assurance about the dependability of the research outcomes in most studies examined in Figure-IV.

Table-I: Displays the characteristics of the studies included.

Author (Year)	Population	Total Participants	Age of Participants, Range (Year)	Study design	Dosages/ Duration of Treatment of Studies	Adverse events of studies
Mattan (2022)	Children and adults suffering from tonsillitis or pharyngitis	242366	3 to >46	Retrospective study	SYR. Amoxicillin 250mg/5ml, TAB. Amoxicillin 500mg	**Amoxicillin:** Fever (n=117) **Comparative drug:** Fever (n=141)
Peng Li (2019)	Children with tonsillitis	256	2 to 12	A drug-controlled, randomized, comparative trial study	Group 1: amoxicillin 30 mg/kg/ day/3 for 10 days Group 2: azithromycin 10 mg/kg/day/1 for 3 days Group 3: cefaclor 20 mg/kg/day/3 for 5 days	**Amoxicillin:** Diarrhea (n=4), Rash (n=2), Nausea (n=3) **Comparative drug:** Diarrhea (n=3), Rash (n=5), Nausea (n=3)
Gerber (2017)	Children With Acute Respiratory Tract Infections	30159	6 months to 12 years	A retrospective cohort study assessing clinical outcomes and a prospective cohort study assessing patient-centered outcomes		**Amoxicillin:** Adverse events (849) **Comparative drug:** Adverse events (189)
Eslami (2014)	Children with pharyngitis	99	6 to 15	Prospective randomized controlled clinical trial	Group 1: Amoxicillin 750 mg orally once daily Group 2: a single shot of BPG 600.000 IU and 1.200.000 IU for children weighed less than 27 kg	
Kuroki (2013)	Children with pharyngolaryngitis or tonsillitis	97	< 15	A drug-controlled, open-label, multicenter study	Group 1: Amoxicillin 30 mg/kg/day/3 for 10 days Group 2: Combination of CVA 6.4 mg/kg/day	**Amoxicillin:** Diarrhea (n=5), Upper airway inflammation (1), **Comparative drug:**

					and AMPC 90 mg/kg/day in two divided doses for 3 days	Diarrhea (n=22), Urticaria (n=1), Eruption (n-1)
Leelarasamee (2000)	Children and adults nonexudative pharyngotonsillitis.	1217	>5	Randomized, double-blinded, placebo controlled trial.	Group 1: Amoxicillin: 1 capsule (250 or 500 mg] 3 or 4 times daily) or syrup was given for 7 days at a dose of 50 mg/kg per day in three or four divided doses. Group 2: placebo	**Amoxicillin:** Nausea (n=4) Vomiting (n=4) Epigastric distress (n=4) Diarrhea (n=1) Rash (n=5) **Placebo:** Nausea (n=3) Vomiting (n=3) Epigastric distress (n=3) Diarrhea (n=1) Rash (n=3)

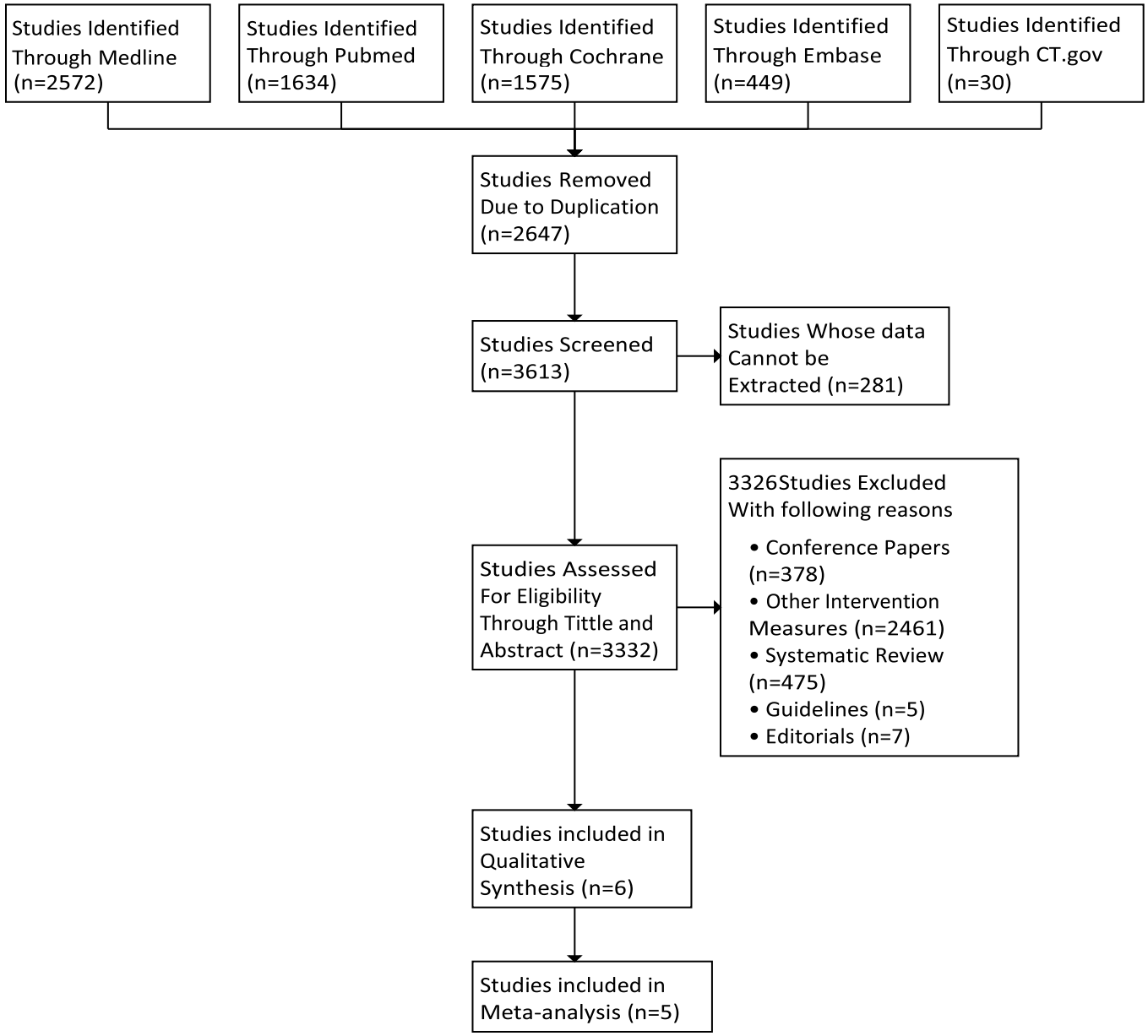

Figure-I: Flow chart illustrating the selection process of studies.

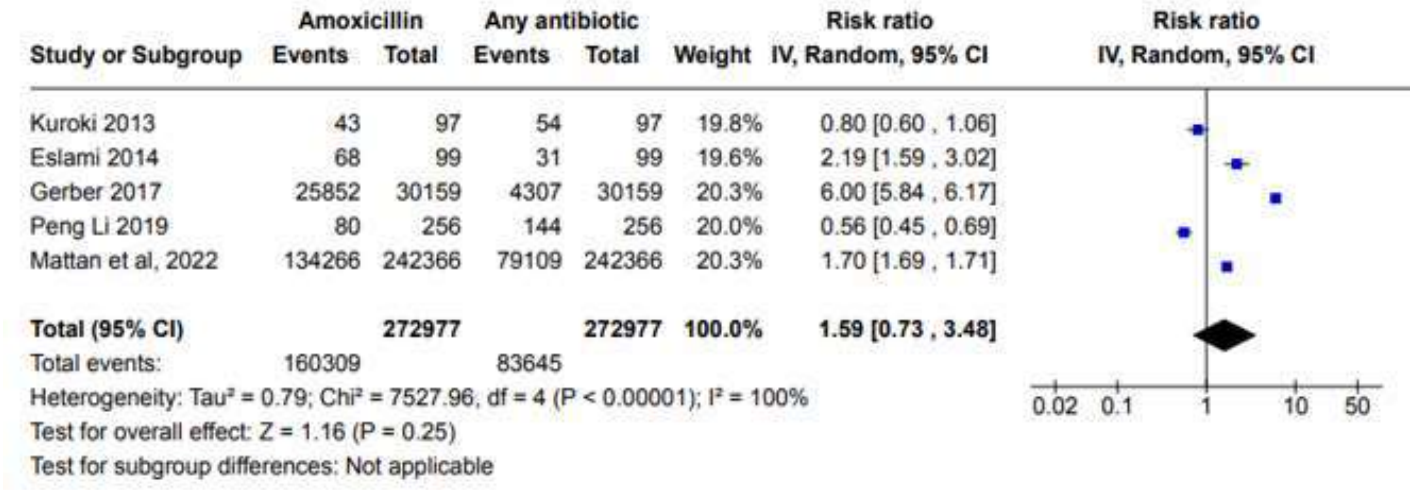

Figure-II: Forest plot of the risk ratio of Amoxicillin vs. another antibiotic regimen

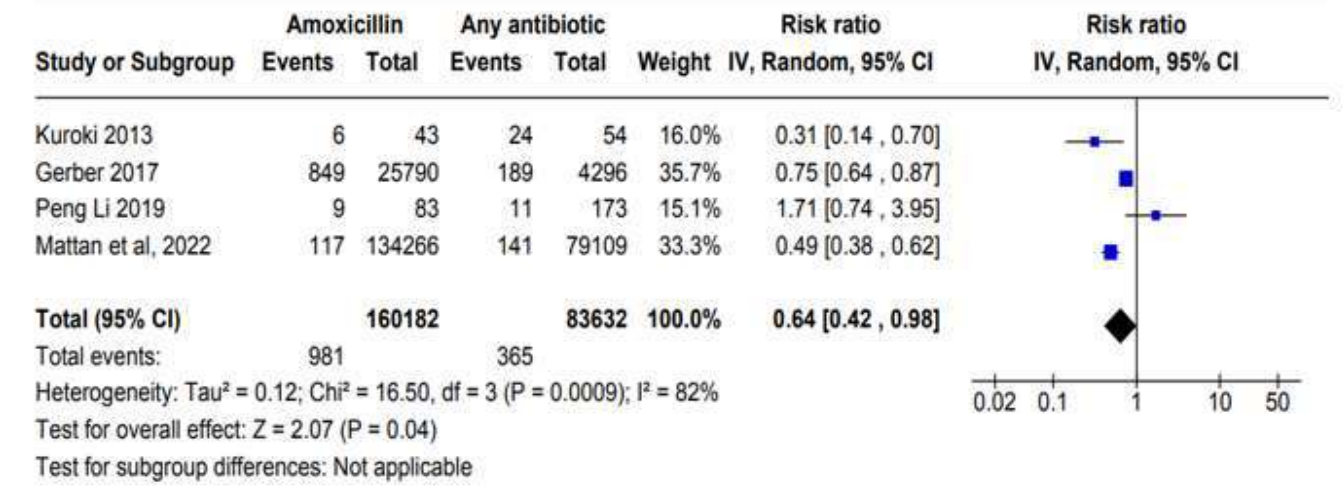

Figure-III: Forest plot of the risk ratio for adverse events due to treatment of URTIs with Amoxicillin vs. another antibiotic regimen

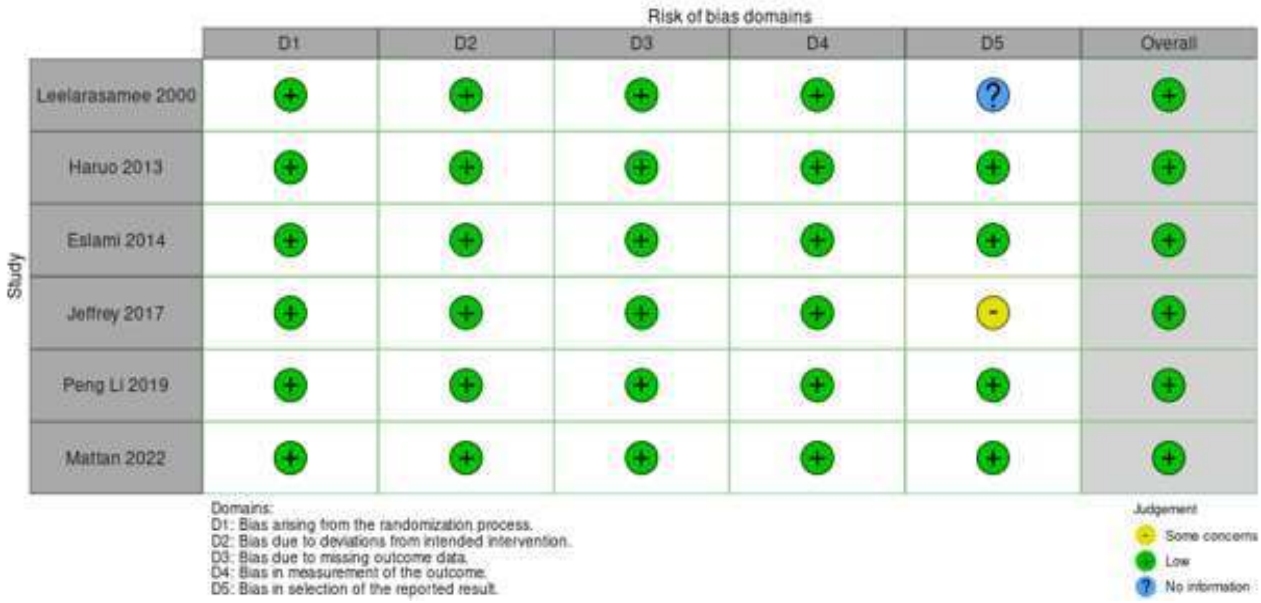

Figure-IV: Critical appraisal according to the RoB-2 tool for assessing Risk of bias in randomized trials and observational studies.

DISCUSSION

In this study, we conducted a systematic review and meta-analysis to analyze if broad-spectrum antibiotics offer any added advantage over narrow spectrum antibiotics like penicillin or amoxicillin in the treatment of tonsillopharyngitis. Our findings suggest broad-spectrum antibiotics are not superior to narrow-spectrum antibiotics and show no significant difference in efficacy or treatment success rate. Amoxicillin was associated with a significantly lower risk of adverse events compared to broad-spectrum antibiotics.

Despite covering an 11-year timeframe in our literature review, we encountered a scarcity of comparative effectiveness studies assessing narrow- and broad-spectrum antibiotic therapy for the prevalent bacterial URTIs, especially tonsillopharyngitis.

We identified three recent Cochrane reviews that aligned with our study objectives. [15-17] Spinks *et al*. assessed the effects of antibiotics for reducing symptoms of sore throat for child and adult patients. They reported, though antibiotics may decrease the incidence of sore throat and lower the chances of certain complications associated with sore throat. However, since the impact on symptoms might be marginal, clinicians need to individually assess whether prescribing antibiotics is clinically justified, taking into account the likely bacterial origin of the sore throat. They stated that it was crucial to recognize the delicate balance between modest symptom relief and the potential risks of antimicrobial resistance. [15]

Another review reported by van Driel ML *et al*. assessed the comparative efficacy of different antibiotics along with the incidence of adverse effects and the risk-benefit of antibiotic treatment for streptococcal pharyngitis. They stated that though antibiotic affects were similar, and all antibiotics caused side effects, but there was no strong evidence to show meaningful differences between antibiotics. Studies did not report on long-term complications; therefore, it was unclear if any class of antibiotics was better in preventing serious but rare complications.[16]

The third review reported by Altamimi *et al*. investigated the evidence regarding the efficacy of two to six days of newer oral antibiotics (short duration) compared to 10 days of oral penicillin (standard duration) in treating children with acute GABHS pharyngitis. They stated that short duration of newer oral antibiotics had comparable efficacy compared to the standard duration 10-day course of oral penicillin. [17]

We did not identify any systematic reviews pertinent to our research objectives. The only other systematic review uncovered in our search focused on antibiotics for recurrent acute pharyngo-tonsillitis and was therefore excluded from our assessment.[18]

We acknowledge that GABHS pharyngitis necessitates the administration of an effective antibiotic, at an appropriate dosage, for an adequate duration to eradicate the pathogen from the pharynx, typically recommended for a 10-day period.[2] Due to their limited spectrum of activity, minimal occurrence of adverse effects, and cost-effectiveness, penicillin or amoxicillin is considered the preferred option for patients, hence, aligning with the outcomes observed in our study.

While numerous treatment guidelines advocate penicillin or amoxicillin as the primary choices for pharyngitis, physicians continue to dispense alternative agents, especially macrolides. This review was conducted to reinforce the existing literature's evidence in favor of narrow-spectrum agents and discourage physicians from prescribing azithromycin, which should be reserved for treating extensively-drug-resistant *Salmonella typhi*. Azithromycin remains the last remaining oral option for this difficult-to-treat infection. The lack of a significant difference in treatment success rates between amoxicillin and other antibiotics raises intriguing questions about the management of URTIs. When choosing an antibiotic, it is crucial to assess factors such as the spectrum of activity, possible side effects, and trends in antibiotic resistance. Our review's results align with previous researches, indicating no statistically significant difference in efficacy between amoxicillin and other antibiotics. Our findings report that broad-spectrum antibiotic treatment provided minimal additional benefit compared to amoxicillin and was more likely to be associated with antibiotic resistance.[19]

Our meta-analysis found a notable and clinically meaningful link between amoxicillin treatment and a lower incidence of side events when compared to other antibiotics. When compared to other antibiotics, amoxicillin had a 36% lower probability of being associated with side effects. When compared with other antibiotics, Peng Li 2019 found that amoxicillin had relatively low incidences of diarrhea (n=4), rash (n=2),

and nausea (n=3), whereas other antibiotics had slightly higher incidences of diarrhea (n=3), rash (n=5), and nausea (n=3). These data imply that amoxicillin may have a comparable safety profile regarding these adverse effects, making it a feasible option for patients needing antibiotic therapy. Finally, the findings of this study state that the most reported side effects of amoxicillin in comparison to other antibiotics, were diarrhea, fever, rash, and nausea. A study conducted by Koga *et al*. indicated a higher frequency of minor side effects in the azithromycin therapy group compared to the amoxicillin treatment group, correlating with our findings.[20]

The safety profile of narrow-spectrum antibiotics versus broad-spectrum antibiotics is already well-known and adequately characterized. The decreased risk of adverse events reported with amoxicillin is significant clinically and has crucial implications for patient safety and treatment outcomes. Healthcare practitioners can potentially reduce the occurrence of adverse events by using amoxicillin, contributing to enhanced patient tolerability and adherence to treatment.

Variations in study designs, patient groups, dosage regimens, and adverse event reporting may have influenced the found heterogeneity among the included studies. Furthermore, the possibility of publication bias should be considered, as research with positive outcomes is more likely to be published, thus impacting the total findings. Considering the limited sample size in our review, attributed to the scarcity of applicable studies, it is recommended that future research endeavors focus on larger, high-quality study samples to improve the reliability and validity of systematic review findings.

Despite these limitations, our meta-analysis provides strong evidence supporting amoxicillin's improved safety profile in treating URTIs. These findings highlight the need for evidence-based prescribing strategies, in which healthcare practitioners should examine the advantages of amoxicillin over other antibiotics to reduce the risk of adverse events while effectively managing tonsillopharyngitis.

CONCLUSION

In conclusion, given its comparable efficacy to other antibiotics and reduced incidence of adverse effects, this research supports the use of amoxicillin as a preferred antibiotic choice for the treatment of tonsillopharyngitis. The findings highlight the significance of evidence-based decision-making in antibiotic selection to enhance patient outcomes and battle the evolution of antibiotic resistance. Future research should focus on filling knowledge gaps, developing personalized treatment regimens, and analyzing the impact of antibiotic resistance on URTI therapy.

CONFLICT OF INTEREST

None

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AUTHOR CONTRIBUTION:

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REVIEW ARTICLE

Giardia lamblia assemblages and their correlation with the severity of diarrhea: A review article

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ABSTRACT

Background: *Giardia lamblia* is responsible for a range of clinical conditions, from asymptomatic to acute or chronic symptomatic forms. The genetic variability of *Giardia lamblia* raises the possibility of a relationship between its assemblages and the clinical severity of giardiasis. We conducted a review of the literature from 2001 to 2023 to explore the association between *G. lamblia* assemblages and the severity of diarrhea.

Material and Methods: A search was performed through databases of PubMed, WHO, Health Protection Agency, the UK and Centre of Disease Control USA websites and Medline. This search yielded 47 studies from 2001 to 2023 as only those articles providing information on the correlation between different assemblages of *G. lamblia* and the severity of the disease were included.

Results: Out of a total of 47 studies that were included, only 26 studies showed a meaningful comparison. 12 studies showed no difference between different *G. lamblia* assemblages and the severity of giardiasis, while 14 studies demonstrated the link between *G. lamblia* assemblage and the severity of the disease.

Conclusion: Numerous studies have attempted to identify the link between its assemblage profile and the symptoms it causes but with little success. However, the findings of these studies are contradictory and inconclusive, making it difficult to establish a clear association. However, the divergence in findings suggests that a definitive correlation cannot yet be established. To fully understand the link between assemblages and severe diarrhea, further molecular studies are needed, especially in large populations residing in endemic regions.

Keywords: *Giardia lamblia, Diarrhea, Assemblages*

BACKGROUND

Giardia duodenalis (syn. *G. intestinalis*, and *G. lamblia*) is an important cause of diarrhea (giardiasis) and a major public health concern worldwide. Around 250 million reported cases of diarrheal illness are caused by giardiasis globally.[1] The estimated prevalence rate of *G. lamblia* in developing and developed countries is 70% and 2% respectively. Several factors such as environmental and socioeconomic factors, and personal hygiene habits account for the prevalence of *G. lamblia* infection.[2,3,4]

G. lamblia is a flagellated protist which belongs to the order Diplomonadida and most commonly detected protozoan parasite in the intestinal tract. *G. lamblia* isolates are categorized into seven assemblages (A-G), based on the characterization of the glutamate dehydrogenase, and small subunit rRNA triosephosphate isomerase genes. Only assemblages A and B infect humans and other hosts such as wild mammals, dogs, cats and other livestock.[5] Type A *G. lamblia* is further divided into subtypes A-I and A-II. Isolation of both assemblages A and B from the individuals have been reported from Europe, Asia, Australia and North America. The true geographical distribution of different assemblages of this parasite with various communities remains less understood. Assemblage A is predominant in Europe[6,7,8] while one study from Australia reported that 70% of children from a day-care center were infected with assemblage B.[9] Water and foodborne transmission remains the main routes of *G. lamblia.* After ingestion of infected cysts from water or food, it is thought that protozoan causes direct damage to the microvilli of the duodenum and upper third of the jejunum which eventually leads to rapid turnover of the mucosal epithelium and changes in absorption and intestinal transit. The other possible route of transmission includes a person-to-person spread

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for instance in day-care settings, in mentally handicapped institutions and during sexual intercourse. Giardiasis is also linked with travel to endemic areas. [10,11]

The clinical manifestations of giardiasis range from asymptomatic infection, acute self-limiting diarrheal illness, and chronic gastrointestinal symptoms with intermittent diarrhea to malabsorption.[12] The majority of infected individuals (60-80%) are asymptomatic. Sub-acute and chronic giardiasis probably leads to weight loss, growth retardation, decreased cognitive functions and poor performance at school in children. In addition, patients with giardiasis may present with cholecystitis and pancreatitis.[13] Several extra-intestinal symptoms such as fever, urticaria, aphthous ulcers, polyarthritis, lymphadenopathy, maculopapular rash, geographic tongue and pulmonary infiltrates have previously been reported.[14] The severity of giardiasis is associated with the virulence and pathogenicity of *G. lamblia* and host factors for example nutritional and developmental status, immune status and age.[1] The possible association between the genotype of *G. lamblia* and the severity of the disease remains debatable. However, several studies have previously attempted to describe the possible association between different genotypes of *G. lamblia* and the severity of the disease. [15,16] This article aims to review the association between various Giardia lamblia genotypes and the degree of severity observed in cases of giardiasis.

MATERIAL AND METHODS

Several sources were used to access data for this review. A search was performed through databases of PubMed, WHO, Health Protection Agency, the UK and Centre of Disease Control USA websites and Medline with keywords *G. lamblia*, a correlation between Assemblages of *G. Lamblia* and severity of diarrhea. This search yielded 47 studies as of April 2023 as only those articles providing information on the correlation between different assemblages of *G. lamblia* and the severity of the disease were included.

RESULTS

Twenty-six studies which provided sufficient and useful information for a reasonable and meaningful comparison were included in this review. Of the 26 studies, 12 studies showed no difference between different *G. lamblia* assemblages and the severity of giardiasis as shown in Table-I, while 14 studies demonstrated the link between *G. lamblia* assemblage and severity of disease as shown in Table-II. The studies that reported the absence of a significant association between *G. lamblia* assemblage and severity of illness were conducted in Albania, Brazil, Mexico, Turkey, Iran, Pakistan, India, Egypt, Cuba, England and Malaysia. In these studies, the authors investigated the possible association of *G. lamblia* genotype with the severity of diseases including the prevalence of genotypes, chronic diarrheal disease, clinical presentation and markers of intestinal inflammation, nutritional status, environmental, socio-economic factors and *G. lamblia* infection. It was observed that children infected with Assemblage B shed more cysts than children with Assemblage A. Furthermore, children with diarrhea due to mixed assemblages shed more cysts than children with assemblages A or B alone.[17]

The studies that reported a significant association between *G. lamblia* assemblage and the severity of the disease were conducted in Spain, Bangladesh, India, Australia, Netherlands, Turkey, Ethiopia, KSA, Egypt, England, Iraq and Iran. A study from Spain showed a significant association of assemblage A with symptomatic giardiasis observed in < 5 years old patients.[32] Two studies from Bangladesh[15,34] showed a significant correlation between assemblage A and the severity of diarrhea. Higher DNA load was noted in assemblage B and was associated with asymptomatic patients.[15] Ajjampur *et al* from India included 452 children with diarrhea in their study that showed assemblage A association with more diarrheal symptoms.[33]A study conducted in Australia that included 353 children under five years of age showed assemblage A's significant association with severe giardiasis.[16] In a study from the Netherlands, the researchers found that assemblage B was associated with persistent watery or profuse diarrhea while assemblage A was linked with intermittent diarrhea. The duration of diarrhea with assemblage B was also prolonged as compared to assemblage A.[6] A study by Aydin *et al* in Turkey revealed Assemblage A identified in 17 symptomatic patients while Assemblage B caused diarrhea in three symptomatic patients.[28] A study from Ethiopia reported that assemblage B was associated with symptomatic infections such as diarrhea, abdominal pain and nausea in 12 of 13 cases.[29] In Saudi Arabia, patients infected with assemblage B were symptomatic, despite a low number of samples (n = 40).[35] Two studies from different parts of Egypt showed variable results. EI

Basha *et al* reported clinical severity with Assemblage A, whereas, Ahmed NS *et al* observed more symptomatic cases with Assemblage B.[38,39] Two studies from Iraq and Iran reported the severity of symptoms associated with Assemblage B. [36,40]

Table-I: Summary of the studies that observed no significant association between Giardia assemblage and the severity of diarrhea.

Author and year of publication	Study Location	Study population	Number of samples/PCR done	Assemblage	Statistically significant association
Cedillo-Rivera *et al*., 2003	Mexico City	Children and adults	26/26	A=21 B =1	None
Berrilli *et al*., 2006	Albania	Children under 9 years	125/22	A=10 B=12	None
Kohli *et al*., 2008	Brazil	Children	108/58	A=9 B=43 Mixed A/B=6	None
Breathnach AS *et al*., 2010	England	Children & adults	267/199	A=48 B=145 Missed A/B=6	None
Balcıoğlu, C *et al*., 2012	Turkey	Children & adults	63/54	A=38 B=16	None
Rafiei A *et al*., 2013	Iran	Children & adults	100/100	A=14 B=27 Mixed A/B=59	None
Tak *et al*., 2014	India	Children & adults	82/82	A=0 B=82	None
Choy HS *et al*., 2014	Malaysia	Children & adults	1252/138	A=69 B=69	None
Fahmy HM *et al*., 2015	Egypt	Children	96/75	A=21 B=54	None
Peubla LJ *et al*., 2017	Cuba	Children	45/36	A=4 B=19 Mixed A/B=13	
Kashinahanji *et al*., 2019	Iran	Children & adults	64/30	A=18 B=12	None
Nawaz *et al*., 2020	Pakistan	Children	76/69	A=20 B=38 Mixed A/B=11	None

Table-II: Summary of the studies that observed a significant association between Giardia assemblage and the severity of diarrhea.

Author and year of publication	Study Location	Study population	Number of samples/ PCR done	Assemblage	Statistically significant association
Homan and Mank, 2001	Netherlands	Children & adults	18/18	A=9 B=9	Yes (B; severe diarrhea)
Read *et al*., 2002	Australia	Children under 5 years	36/23	A=7 B =16	Yes (A; severe diarrhea)
Aydin *et al*., 2004	Turkey	Children & adults	56/56	A=20 B=30 Mixed A/B=6	Yes (A; severe diarrhea)
Haque *et al*., 2005	Bangladesh	Children & adults	322/304	A=36 B=247 Mixed A/B=16	Yes (A: severe diarrhea)
Gelanew *et al*., 2007	Ethiopia	Children & adults	80/59	A=31 B=13 Mixed A/B= 15	Yes (B: severe diarrhea)
Sahagun *et al*., 2008	Spain	Children & adults	108/108	A II = 43 B=61 Mixed AII/B= 4	Yes (A: severe diarrhea)

Ajjampur SS *et al*., 2009	India	Children < 5 years	101/50	A=13 B=22 Mixed A/B=5	Yes (A: severe diarrhea)
Alam *et al*., 2011	Bangladesh	Children & adults	127/117	A=15 B=38 Mixed A/B=11	Yes (A: severe diarrhea)
Al-Mohammed., 2011	Kingdom of Saudi Arabia	Children	40/40	A=23 B=15 Mixed A/B=2	Yes (B: severe diarrhea)
Sarkari B *et al*., 2012	Iran	Children & adults	205/172	A=158 B=6 Mixed A/B=8	Yes (A: severe diarrhea)
Minetti *et al*., 2105	England	Children & adults	247/239	A=82 B=158 Mixed A/B=7	Yes (B: severe diarrhea)
EI Basha *et al*., 2016	Egypt	Children & adults	400/60	A=22 B=38	Yes (A: severe diarrhea)
Ahmed NS *et al*., 2020	Egypt	Children & adults	100/93	A=30 B=37 Mixed A/B=26	Yes (B: severe diarrhea)
Al-Huchaimi *et al*., 2020	Iraq	Children & Adults	75/75	A=22 B=53	Yes (A: severe diarrhea)

DISCUSSION

The review of the data on the association between the *G. lamblia* assemblage and the severity of giardiasis suggests that the correlation between the two is still not well described and is debatable. Data from studies from different parts of the world that investigated the correlation between *G. lamblia* assemblage and the severity of diarrheal illness were contradictory and inconsistent. The apparent discrepancies in the studies reviewed are reflected by the complex interaction between the parasite and the prevalence of genotypes, chronic diarrheal disease, clinical presentation, age and markers of intestinal inflammation, and nutritional, environmental, geographic and socio-economic factors. Sub-optimal sanitary conditions such as poor socioeconomic class, overcrowding conditions, limited access to clean drinking water, big family size and lack of knowledge about hygiene and cleanliness, travelling to the developing world, camping and caravanning are the established risk factors for the acquisition of the *G. lamblia* infection.[40] On the contrary, risk factors such as gender, low birth weight, maternal age at birth, family size, number of siblings and presence of animals or pets were not consistent with the severity of diarrhea. However, poor socioeconomic class and drinking municipality water were found to be associated with severe giardiasis.[32] Furthermore, several other factors including children less than five years of age, the host's nutritional status, the host's immune response, virulence and pathogenicity of *Giardia* isolates, to a large extent, influence both the susceptibility and severity of the diarrheal disease due to *G lamblia*.[7,32] In this review, several studies did not show a significant association between Giardia assemblage and the severity of giardiasis apparently in healthy adults as the main study population. On the contrary, two studies reported a significant association between giardia assemblage A and the severity of illness in children.[15,16] It was not clear if this study population characteristic had a major impact on the presence or absence of a significant association between *Giardia* assemblage and disease. In one study, the researchers did not find any significant association between symptoms and the age, gender or geographical origin of patients.[30] In addition, Hollam-Delgado *et al* found that malnutrition was not a predisposing factor for giardiasis. Malnourished children infected with *G. lamblia* did not develop severe diarrhea in their study.[12]

Studies from different regions of the world have revealed conflicting data on *Giardia* assemblages A and B. The question arises whether assemblage A or B alone or both are more linked with severe giardiasis. In India, Bangladesh, Australia and Turkey, genotypes A and AII were most associated with severe diarrhoea.[15,16, 29,30,32] Whilst assemblage B was more linked with watery and profuse diarrhea in the Netherlands and Ethiopia and assemblage A was linked with intermittent diarrhoea.[6,30] It is interesting to note that assemblage B was also associated with symptomatic patients.[29] It is difficult to ascertain from the above-mentioned studies which

giardia assemblage is most associated with the severity of giardiasis. These studies have several limitations such as a limited study population and a small number of *G. lamblia* isolates. Furthermore, these studies did not investigate the nutritional, immune status and the presence of the co-infections. We suggest that more patients with persistent diarrhea or with specific symptoms of giardiasis should be included in future molecular studies to determine the true association between the *Giardia* assemblage and the severity of the illness. Molecular-based studies from Australia, India, Italy, Ethiopia, and the United Kingdom have reported mixed infections from patients with *G. lamblia*. The mixed infection rate ranged from 2.0 to 25% in these studies and was found to be higher in developing nations.[16 41, 42, 43,44] Mixed infections (both assemblages A& B) are not uncommon and probably due to ingestion of sources such as water contaminated by sewage or slurry with heterogenous Giardia strains.[46] In this review, the mix assemblages rate ranged from 3.7% to 25% from Spain, Bangladesh, India and Ethiopia.[15,30,31, 32] These findings were consistent with the previous studies.[46,47]

In this review article, we have made an effort to determine the correlation between the severity of giardiasis and G. lamblia assemblage by examining various studies conducted worldwide. However, the findings of these studies are contradictory and inconclusive, making it difficult to establish a clear association.

CONCLUSION

Giardia lamblia is a common intestinal parasite. Numerous studies have attempted to identify the link between its assemblage profile and the symptoms it causes but with little success. However, the divergence in findings suggests that a definitive correlation cannot yet be established. This lack of correlation highlights the complexity of the relationship between G. lamblia and human health and underscores the need for further research in this area. To fully understand the link between assemblages and severe diarrhea, further molecular studies are needed, especially in large populations residing in endemic regions.

CONFLICT OF INTEREST

None

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AUTHOR CONTRIBUTION:

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Mohammad Abdul Naeem: Critical revision of the manuscript

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