Journal of Advances in Microbiology Research

E-ISSN: 2709-944X
P-ISSN: 2709-9431
Impact Factor (RJIF): 6.2
JRM 2025; 6(2): 190-194

www.microbiojournal.com
Received: 18-06-2025
Accepted: 22-07-2025

Dr. R Sivaram
Post Graduate Student, Department of Microbiology, Government Stanley Medical College, Chennai, Tamil Nadu, India

Dr. S Hemalatha
Associate Professor, Department of Microbiology, Government Stanley Medical College, Chennai, Tamil Nadu, India

Dr. K Chandrasekaran
Assistant Professor, Department of Microbiology, Government Stanley Medical College, Chennai, Tamil Nadu, India

Dr. V Dillirani
Professor & Head, Department of Microbiology, Government Stanley Medical College, Chennai, Tamil Nadu, India

Dr. M Edwin Fernando
Professor & Head, Department of Nephrology, Government Stanley Medical College, Chennai, Tamil Nadu, India

Correspondence
Dr. R Sivaram
Post Graduate Student, Department of Microbiology, Government Stanley Medical College, Chennai, Tamil Nadu, India

Comparison of modified acid-fast staining and Elisa for the detection of cryptosporidium species antigen to diagnose cryptosporidiosis in renal transplant recipients in a tertiary care centre

R Sivaram, S Hemalatha, K Chandrasekaran, V Dillirani, M Edwin Fernando

DOI: https://www.doi.org/10.22271/micro.2025.v6.i2c.250

Abstract
Background: Cryptosporidiosis is an opportunistic protozoal infection caused by the Cryptosporidium species that infects the epithelial cells of the gastrointestinal tract. The disease manifests as acute, self-limiting watery diarrhoea in immunocompetent individuals. However, in immunocompromised hosts-particularly those with HIV/AIDS, transplant recipients and malnourished children-cryptosporidiosis can lead to severe, persistent, and life-threatening diarrhoea. This study aims to compare the modified acid-fast staining technique with antigen detection using ELISA in the diagnosis of Cryptosporidium species in stool samples of post renal transplant recipients at a tertiary care center.
Materials and Methods: A prospective study was conducted over 6 months (March 2024-August 2024) at Government Stanley Medical College and Hospital, Chennai. A total of 40 stool samples were collected from renal transplant recipients who were more than 18 years of age with complaints of diarrhea. Fresh Stool sample was collected in a wide mouthed screw top container & was subjected to modified acid-fast staining to examine the presence of oocysts of Cryptosporidium and to detect Cryptosporidium antigen by EDITM fecal Cryptosporidium antigen ELISA kit.
Results: 27 post renal transplant recipients with diarrhea were positive for Cryptosporidiosis by detection of oocyst with modified acid-fast technique, whereas 33 post renal transplant recipients with diarrhea were positive for Cryptosporidiosis by detection of antigen with EDI™ fecal Cryptosporidium antigen ELISA kit.
Conclusion: Cryptosporidiosis is a significant opportunistic parasitic infection in renal transplant recipients, mostly due to their immunosuppressed status. Effective care depends on an early and precise diagnosis. Although modified acid-fast staining is inexpensive, they are not very sensitive. On the other hand, antigen detection assays like ELISA have greater sensitivity and specificity, which makes them better suited for early diagnosis, particularly in post renal transplant recipients.

Keywords: ELISA, cryptosporidiosis, modified acid fast stain, renal transplant

Introduction
Cryptosporidium is an obligate intracellular protozoan parasite that infects the epithelial cells of the gastrointestinal tract, causing cryptosporidiosis a diarrheal disease of significant global health concern. The genus includes over 40 species, with *Cryptosporidium hominis* and *Cryptosporidium parvum* being the primary human pathogens [1].
The first human case was reported in 1976 [2]. Cryptosporidium spp. belongs to the family Cryptosporidiidae, which is a member of the phylum Apicomplexa [3].
Oocyst is the infective form to man as well as the diagnostic form excreted in the feces. It is round, small, 4-6 µm in size, surrounded by a cyst wall and bears four sporozoites. The oocysts are acid fast in nature but don't stain by iodine [2].
Cryptosporidiosis is transmitted predominantly via the faecal-oral route, through ingestion of oocysts from contaminated water, food or via direct person-to-person or zoonotic contact [4].
The disease manifests as acute, self-limiting watery diarrhoea in immunocompetent individuals. However, in immunocompromised hosts particularly those with HIV/AIDS, transplant recipients and malnourished children cryptosporidiosis can lead to severe, persistent, and life-threatening diarrhoea [5, 6].

In immunocompetent people, the prevalence in developing countries like India varies from 2.4 to 15%; whereas in the western countries it is 1.4-6% In immunocompromised hosts (HIV positive patients), the prevalence is 12-46% in developing countries (46% in Haiti) and 7-21% in developed countries [5, 6]. Prevalence among renal transplant recipients with diarrhoea in India was found to be 28.3 % [7]. Diagnosis is primarily based on microscopic detection of oocysts using modified acid-fast staining or immunofluorescence techniques. ELISA & Molecular methods, such as PCR, have improved species differentiation and genotyping. Direct fluorescent antibody staining is done to detect Cryptosporidium oocyst by using fluorescent labelled monoclonal antibody directed against cyst wall antigens. This is more sensitive and specific than acid fast staining. Currently, this method is considered as the gold standard test for cryptosporidiosis. The cost of monoclonal antibodies and the need of a fluorescent microscope has limited its use in resource limited countries. PCR is available to detect specific Cryptosporidium genes from both clinical and environmental samples. PCR is more sensitive, takes less time and can differentiate the Cryptosporidium genotypes which plays an important role in outbreak situations. As the cost of the kit is high and needs technical expertise, it is not regularly done [8]. Despite its global impact, effective treatment options are limited. Nitazoxanide is approved for use in immunocompetent individuals, but efficacy in immunocompromised patients remains inadequate [9].

During the first 3 years of post-transplantation, 20% of the recipients develop diarrhea [10], which leads to dehydration and wasting and is associated with increased morbidity [11]. Therefore, rapid and accurate diagnosis of cryptosporidiosis is important in diagnosis & treatment. Diagnosis of Cryptosporidium oocysts in stool samples by conventional microscopy by modified acid fast staining is labor-intensive and requires expertise. Therefore, we aim to compare the Copro enzyme-linked immunosorbent assay (ELISA) method for diagnosing Cryptosporidium antigen with the modified acid-fast staining method for the detection of Cryptosporidium oocyst in stool [10, 12].

Materials and Methods

After obtaining Institutional Ethics Committee clearance, this prospective study was conducted in the Department of Microbiology and Nephrology, Government Stanley medical college and Hospitals in Chennai for a period of 6 months from March 2024 to August 2024. A total of 40 stool samples were collected from Renal transplant recipients who were more than 18 years of age with complaints of diarrhea (>3 bowel movements of liquid consistency for more than three days and less than 14 days) [WHO]. Patients who denied consent, or who had malabsorption syndrome, inflammatory bowel disease, irritable bowel syndrome and patients with known causes of non-infective diarrhea or suffering from persistent or chronic diarrhea with mucus and blood were excluded from the study. Fresh Stool sample collected in a wide mouthed screw top container from post renal transplant recipients with diarrhea was sent to microbiology lab for analysis.

It was subjected to modified acid-fast staining to detect the presence of oocysts of Cryptosporidium. Prepare a thin smear of stool sample on a clean glass slide. Air-dry and fix the smear with methanol for 1 minute. Flood the slide with strong carbol fuchsin (without heating). Let it stand for 5-10 minutes. Rinse gently with water. Decolorize with 1% sulfuric acid for 3 minutes. Rinse thoroughly with water. Stain with methylene blue for 1 minute. Rinse with water and air dry. Examine under oil immersion (1000× magnification). Stool samples were subjected to copro ELISA (EDI™ fecal Cryptosporidium antigen ELISA kit) to detect Cryptosporidium antigen as per manufacturer instructions.

Results

In this study a total of 40 post renal transplant patients with diarrhoea above the age of 18 years were enrolled.

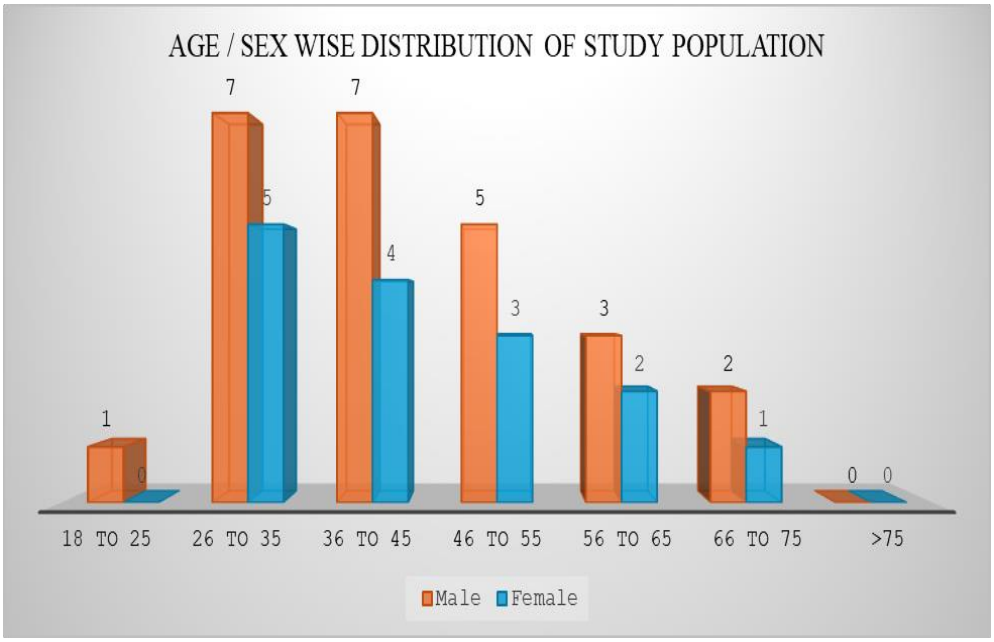

Chart 1: Age / sex wise distribution of study population

Table 1: Age/Sex wise distribution of study population (N=40)

Age in years	Number of post renal transplant recipients with diarrhoea (n=40)		
	Male (25)	Female (15)	Total (40)
18 to 25	1 (4%)	0 (0%)	1 (3%)
26 to 35	7 (28%)	5 (33%)	12 (30%)
36 to 45	7 (28%)	4 (27%)	11 (28%)
46 to 55	5 (20%)	3 (20%)	8 (20%)
56 to 65	3 (12%)	2 (13%)	5 (13%)
66 to 75	2 (8%)	1 (7%)	3 (8%)
>75	0 (0%)	0 (0%)	0 (0%)

In our study the maximum number of post renal transplant recipients with diarrhea was from the age group of 26 to 35 years 12 (30%), followed by 36 to 45 years 11 (28%) & 46 to 55 years 8 (20%) and no cases were in the age group of > 75 years. Majority of the patients 25 (63%) were male, compared to female 15 (37%).

Table 2: comparison of modified acid-fast staining technique and Elisa to diagnose cryptosporidiosis in renal transplant recipients (N=40)

Name of the test	Number positive for Cryptosporidiosis	Number negative for Cryptosporidiosis
Modified acid fast stain technique	27	13
ELISA for Cryptosporidium antigen	33	7

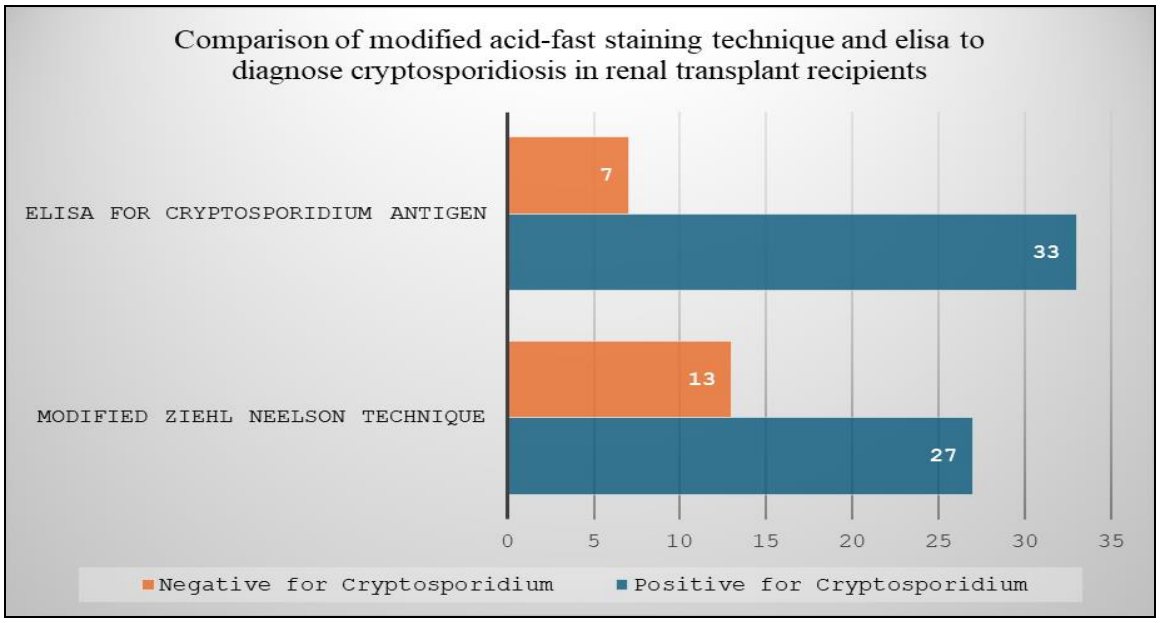

Chart 2: Comparison of modified acid-fast staining technique and elisa to diagnose cryptosporidiosis in renal transplant recipients

In our study, 27 post renal transplant recipients with diarrhea were positive for Cryptosporidiosis by detecting oocyst with modified acid-fast staining technique, whereas 33 post renal transplant recipients with diarrhea were positive for Cryptosporidiosis by detection of Cryptosporidium antigen with EDI™ fecal Cryptosporidium antigen ELISA kit.

Table 3: Age/Sex wise distribution of post renal transplant recipients positive for cryptosporidiosis by detection of oocyst of cryptosporidium with modified acid-fast staining technique (N=40)

Age in years	Number of post renal transplant recipients with diarrhoea positive for Cryptosporidium oocyst (N=27)			Number of post renal transplant recipients with diarrhoea negative for Cryptosporidium oocyst (N=13)		
	Male (19)	Female (8)	Total (27)	Male (6)	Female (7)	Total (13)
18 to 25	1 (5%)	0 (0%)	1 (4%)	0 (0%)	0 (0%)	0 (0%)
26 to 35	6 (32%)	4 (50%)	10 (37%)	1 (17%)	1 (17%)	2 (15%)
36 to 45	5 (26%)	2 (25%)	7 (26%)	2 (33%)	2 (29%)	4 (32%)
46 to 55	4 (21%)	1 (12.5%)	5 (19%)	1 (17%)	2 (29%)	3 (23%)
56 to 65	2 (11%)	1 (12.5%)	3 (11%)	1 (17%)	1 (17%)	2 (15%)
66 to 75	1 (5%)	0 (0%)	1 (4%)	1 (17%)	1 (17%)	2 (15%)
>75	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

From the above table it shows that the maximum number of positive cryptosporidiosis patients by detection of oocysts by modified acid fast staining technique were from the age group of 26 to 35 years 10 (37%), followed by 36 to 45 years 7 (26%) & 46 to 55 years 5 (19%) & most of the cryptosporidiosis positive patients were from male, 19 (70 %).

Table 4: Age/Sex wise distribution of post renal transplant recipients positive for cryptosporidiosis by Edi[tm] Fecal cryptosporidium antigen Elisa Kit) to detect cryptosporidium antigen (n=40)

Age in years	Number of post renal transplant recipients with diarrhoea positive for Cryptosporidium antigen by ELISA (N=33)			Number of post renal transplant recipients with diarrhoea negative for Cryptosporidium antigen by ELISA (N=7)		
	Male (21)	Female (12)	Total (33)	Male (4)	Female (3)	Total (7)
18 to 25	1 (5%)	0 (0%)	1 (3%)	0 (0%)	0 (0%)	0 (0%)
26 to 35	7 (33%)	4 (33%)	11 (33%)	0 (0%)	1 (33%)	1 (14%)
36 to 45	6 (29%)	3 (25%)	9 (28%)	1 (25%)	1 (33%)	2 (29%)
46 to 55	4 (19%)	2 (17%)	6 (18%)	1 (25%)	1 (33%)	2 (29%)
56 to 65	2 (10%)	2 (17%)	4 (12%)	1 (25%)	0 (0%)	1 (14%)
66 to 75	1 (5%)	1 (8%)	2 (6%)	1 (25%)	0 (0%)	1 (14%)
>75	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

As per the table above, the maximum number of positive cryptosporidiosis patients by EDITM fecal cryptosporidium antigen ELISA kit) to detect cryptosporidium antigen were from the age groups 26 to 35 years 11 (33%), followed by 36 to 45 years 9 (28%) & 46 to 55 years 6 (18%) & most of the cryptosporidiosis positive patients by ELISA method were form male patients, 21 (63%).

Discussion

Cryptosporidiosis is one of the most common causes of diarrhea in immunocompromised patients, leading to significant morbidity and mortality worldwide. Most of the laboratories routinely use microscopic methods for the diagnosis of cryptosporidiosis. Unfortunately, this method has many limitations as it requires a minimum threshold of 50,000 oocysts/ml of the stool sample for its detection. Hence it is difficult to detect the parasite by routine microscopy. Moreover, this technique requires expertise for reporting. To overcome all these drawbacks, antigen/antibody-based detection methods such as ICT and ELISA or molecular techniques such as PCR can be used. [12].

This study aimed to compare the detection of cryptosporidiosis by detecting cryptosporidium oocysts using modified acid-fast staining method and by detecting cryptosporidium antigen using EDI[TM] fecal Cryptosporidium antigen ELISA kit. A total of 40 stool samples obtained from renal transplant patients with diarrhea were analyzed.

As per TABLE 1, maximum number of post renal transplant patients with diarrhea were from the age group 26 to 65 years and most of the post renal transplant recipient patients with diarrhea were found to be male 25 (63%). This is in concordance with the study conducted by Raja K *et al* [13] in which the male post renal transplant recipient were 62%. Another study conducted by Lamido TZ *et al* showed that about 50.3% of the cases positive for Cryptosporidiosis from the age group 26 to 35 years, which is a little higher than our study [14].

As per TABLE 2, 27 (68%) post renal transplant recipients with diarrhea were positive for Cryptosporidiosis by detection of oocyst with modified acid-fast staining technique, whereas 33 (83%) post renal transplant recipients with diarrhea were positive for Cryptosporidiosis by detection of Cryptosporidium antigen with EDI[TM] fecal Cryptosporidium antigen ELISA kit which is in concordance with a study conducted by Shalash *et al* [15].

As per table 3 & 4 the most common age groups positive for cryptosporidiosis by both modified acid-fast staining method and ELISA were 26 to 35 years, followed by 36 to 45 years & 46 to 55 years. Also, most of the post renal transplant patients positive for cryptosporidiosis by both methods were male.

In our study the number of patients positive for Cryptosporidiosis by modified acid fast staining technique and EDI[TM] fecal Cryptosporidium antigen ELISA kit is almost similar (27 to 33). The standard procedure for diagnosing cryptosporidiosis is to use microscopy to identify oocyst morphological features following modified acid-fast staining. As it is inexpensive, resource-poor nations continue to use this method. When comparing ELISA and modified acid-fast staining, we can say that the former is a more quick and efficient method that is also very sensitive and specific than staining approach. Despite being somewhat expensive, ELISA should be taken into consideration for diagnosis to identify the low parasite concentration in faecal samples and address the shortage of skilled specialists [15, 16].

Conclusion

When comparing the modified acid-fast staining method with ELISA antigen detection for the diagnosis of Cryptosporidium infection in recipients of renal transplants, it is evident that ELISA is a more accurate and sensitive diagnostic method. Although modified acid-fast staining is still useful since it is easy to use, inexpensive, and widely accessible, its sensitivity is much lower and heavily reliant on the parasite load and operator skill. On the other hand, ELISA offers more sensitivity and consistency, which makes it better suited for screening immunocompromised groups, such as patients underwent kidney transplantation who are more susceptible to cryptosporidiosis. Thus, modified acid-fast staining method may be utilized as a backup technique or in situations when ELISA is not available, but ELISA should be employed whenever resources allow, particularly in high-risk patient populations.

Conflict of Interest

Not available

Financial Support

Not available

References

1. Ryan U, Hijjawi N. New developments in Cryptosporidium research. Int J Parasitol. 2015;45(6):367-73.
2. Apurba Sankar Sastry: Essentials of Medical Parasitology
3. Medical Microbiology 9th Edition by Patrick R. Murray Ph.D. F(AAM) F(IDSA) (Author), Ken S. Rosenthal Ph.D.

4. Efstratiou A, Ongerth JE, Karanis P. Waterborne transmission of protozoan parasites: review of worldwide outbreaks. Int J Hyg Environ Health. 2017;220(4):577-597.
5. Kotloff KL, *et al*. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries: results from the Global Enteric Multicenter Study (GEMS). Lancet. 2013;382(9888):209-22.
6. Checkley W, White AC Jr, Jaganath D, *et al*. A review of the global burden, novel diagnostics, therapeutics, and vaccine targets for Cryptosporidium. Lancet Infect Dis. 2015;15(1):85-94.
7. Chikmath A, Patil K, Vankudre A, Kulkarni S, Seemab S, Sangati M, *et al*. The silent threat: Cryptosporidiosis in renal transplant recipients: Uncovering risk factors, treatment strategies, and the role of food handlers in transmission. Kidney Int Rep. 2025;10(2): S515. Abstract WCN25-2733.
8. Chalmers RM, Katzer F. Looking for Cryptosporidium: the application of advances in detection and diagnosis. Trends Parasitol. 2013;29(5):237-51.
9. Amadi B, *et al*. High dose nitazoxanide in HIV-positive Zambian children with cryptosporidiosis: a randomized controlled trial. Clin Infect Dis. 2009;48(5):540-7.
10. Lanternier F, Amazzough K, Favennec L, Bruneel MMF, Abdoul H, Tourret J, *et al*; ANOFEL Cryptosporidium National Network and Transplant Cryptosporidium Study Group. Cryptosporidium spp. infection in solid organ transplantation: the nationwide "TRANSCRYPTO" study. Transplantation. 2017;101(4):826-30. DOI: 10.1097/TP.0000000000001503.
11. Bhadauria D, Goel A, Kaul A, Sharma RK, Gupta A, Ruhela V, *et al*. Cryptosporidium infection after renal transplantation in an endemic area. Transpl Infect Dis. 2015;17(1):48-55.
12. Elgun G, Koltas IS. Investigation of Cryptosporidium spp. antigen by ELISA method in stool specimens obtained from patients with diarrhea. Parasitol Res. 2011;108(2):395-7. DOI: 10.1007/s00436-010-2079-4.
13. Raja K, Abbas Z, Hassan SM, Luck NH, Aziz T, Mubarak M. Prevalence of cryptosporidiosis in renal transplant recipients presenting with acute diarrhea at a single center in Pakistan. J Nephropathol. 2014;3(4):127-31. DOI: 10.12860/jnp.2014.25. PMID: 25374881; PMCID: PMC4219614.
14. Lamido TZ, Yahaya Y, Yahaya SU, Jummai GF, Adebola O. Comparison of modified Ziehl-Neelsen staining technique with antigen detection using ELISA in the diagnosis of cryptosporidiosis at a tertiary hospital in North-Western Nigeria. Afr J Microbiol Res. 2022;16(9):296-300. DOI: 10.5897/AJMR2022.9646.
15. Shalash IRA, Zalat R, El-Enain G, El-Mohandes M, El-Faramawy M, Aly E. Comparison between modified acid-fast staining and antigen detection assay as diagnostic techniques for Cryptosporidium parvum. World J Med Sci. 2016;13(1):72-78. DOI: 10.5829/idosi.wjms.2016.13.1.102113.
16. Centers for Disease Control and Prevention (CDC). DPDx-Cryptosporidiosis [Internet]. Atlanta (GA): CDC; [cited 2025 Aug 25]. Available from: https://www.cdc.gov/dpdx/cryptosporidiosis

How to Cite This Article
Sivaram R, Hemalatha S, Chandrasekaran K, Dillirani V, Fernando ME. Comparison of modified acid-fast staining and Elisa for the detection of cryptosporidium species antigen to diagnose cryptosporidiosis in renal transplant recipients in a tertiary care centre. Journal of Advances in Microbiology Research. 2025;6(2):190-194.