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Prevalence Of Bacterial and Fungal Pathogens Among End Stage Renal Disease (ESRD) Patients Undergoing Hemodialysis in A Tertiary Care Hospital of Eastern Odisha

Roshni Dandapat[1], Basanti kumari Pathi[2], Nirmala Poddar[3], Kumudini Panigrahi[4], Dr A Raj Kumar Patro[5] and Dipti Pattnaik[6]

[1]P.G resident, Microbiology Department, Kalinga Institute of Medical Sciences (KIMS), Bhubaneswar
[2]Professor, Microbiology Department, Kalinga Institute of Medical Sciences (KIMS), Bhubaneswar
[3]Professor, Microbiology Department, Kalinga Institute of Medical Sciences (KIMS), Bhubaneswar
[4]Professor, Microbiology Department, Kalinga Institute of Medical Sciences (KIMS), Bhubaneswar
[5]Molecular Biology Consultant, Microbiology Department, Kalinga Institute of Medical Sciences (KIMS), Bhubaneswar
[6]Professor & H.O.D, Microbiology Department, Kalinga Institute of Medical Sciences (KIMS), Bhubaneswar

Corresponding Author:
Dr Dipti Pattnaik
Professor & H.O.D , Microbiology Department
Kalinga Institute of Medical Sciences (KIMS), Bhubaneswar
dipti.pattnaik@kims.ac.in
M: 9861045074

Abstract:
After cardiovascular disease, which is the most common cause of morbidity and mortality among patients receiving hemodialysis (H.D.), infections remain the top cause. The aim of this study was to discover fungal and bacterial infections in patients undergoing kidney transplantation with end-stage renal disease (ESRD) and to evaluate the risk factors associated with these infections. **Materials and methods:** An investigation that was prospective and cross-sectional was carried out over the course of two years. Participants in the research were individuals with end-stage renal disease (ESRD) who were over 20 years old, still experiencing hypertension, 48 hours after jugular catheters and arteriovenous fistulas (AVF) were inserted. These patients exhibited symptoms and signs of inflammation at various places. The collection of blood, sputum, urine, and catheter tips was carried out in a sterile environment. Then, they were placed through automated culture and sensitivity testing to identify bacterial and fungal infections. Through the utilization of the SPSS program (version 2.0), statistical analysis was carried out. **Results:** Two hundred and sixty-six percent of the patients who participated in the study were related to microbial illnesses, with forty-three percent of those patients having bacterial infections. The bulk of the clinical samples included Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus (MSSA), and Staphylococcus aureus (MRSA) as the most often isolated bacterial strains. Patients who had dialysis more frequently than twice a week were far more likely to get bacterial infections. Numerous comorbidities affected these individuals. Candida albicans was the most often isolated species, and candidemia was observed in 3% of the patients. **Conclusion:** Patients varied in age from 61 to 70 years, with the majority of them being male. Diabetes mellitus and hypertension were the most common comorbidities among the individuals. Klebsiella pneumoniae, which accounted for nine percent of all MDR gram-negative bacterial isolates, led the pack. Caspofungin, Micafungin, Voriconazole, and Flucytosine were all effective treatments for Candida albicans isolates obtained from the laboratory.

Keywords: *Klebsiella pneumoniae*, *Staphylococcus aureus* (MRSA), *Candida albicans*, Micafungin, Voriconazole, and Flucytosine.

INTRODUCTION

In patients receiving hemodialysis (H.D.), infections are the primary cause of morbidity and mortality; severe infections are usually caused by episodes of bacteremia. The common risk factors predisposing these individuals to infections include diseases that undermine the host's defences, such as ageing, breakdown in the skin and mucosal barriers, co-morbidity, and malnutrition [1].

Most bacteremia instances are thought to be related to vascular access, namely central venous catheters (CVCs). Arteriovenous fistula (AVF) infection risk is thought to be 10 times lower than that of central venous catheter (CVC) infection risk [2]. Due to the fact that the procedure requires vascular access for lengthy periods, there is a significant risk of the transmission of infectious organisms in a setting where several patients are receiving hemodialysis at the same time. It has been demonstrated that infectious germs may be transmitted from one patient to another through the hands of healthcare professionals, as well as through contaminated instruments, supplies, injectable medications, ambient surfaces, or electrical devices. Moreover, patients who are undergoing

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hemodialysis typically endure surgical procedures and hospital stays, which raises the likelihood that they may become infected with a healthcare-associated cause and increases the likelihood that they would be exposed to potential dangers.

Ten percent of patients with end-stage renal disease (ESRD) in the US receive peritoneal dialysis treatment. By comparison, almost 90% of patients receive maintenance dialysis. Antibiotic-resistant organisms (MDR) infections are becoming more common as hemodialysis patients' usage of antibiotics increases and their interactions with the healthcare system become more frequent. [1]. Furthermore, the prevalence of MDR infections has increased. Because uremia impairs cellular immunity, neutrophil function, and complement activation, patients who are getting maintenance hemodialysis are more prone to develop an infection. This is due to the fact that uremia increases the vulnerability of ESRD patients to infectious microorganisms.

The most common types of pathogens found in patients receiving hemodialysis are gram-positive cocci, specifically coagulase-negative staphylococci (CONS), Staphylococcus aureus, and multidrug-resistant organisms, such as vancomycin-resistant enterococci (VRE), methicillin-resistant Staphylococcus aureus (MRSA), and MDR gram-negative rods [3].

Individuals with HIV who are on treatment also exhibit a higher chance of contracting candidemia. The most prevalent species linked to this illness are Candida glabrata, Candida albicans, Candida tropicalis, Candida parapsilosis, and Candida krusei (Pichia kudriavzevii) [4].

Patients who have been receiving hemodialysis for more than three months provided these clinical samples. Isolating and identifying bacterial and fungal infections from these samples was the main goal of this investigation. Additionally, the patterns of antimicrobial sensitivity for both fungi and bacteria could be obtained, and the associated risk factors were investigated.

MATERIALS AND METHODS

Between November 2020 and October 2022, all End all-stage renal disease (ESRD) patients receiving dialysis were included in a prospective cross-sectional study conducted in the Department of Microbiology at Kalinga Institute of Medical Sciences, Bhubaneswar, Odisha, in collaboration with the Department of Nephrology. The study was approved by the Institutional Ethics Committee (KIIT/KIMS/IEC/406/2020) and the Research Committee (KIMS/R&D/PG/2020/28). 150 patients with end-stage renal disease (ESRD) participated in the trial, and several samples were taken for microbial infection screening. Catheter tips, sputum, urine, peripheral venous blood, and swabs were among these samples.

The study included patients with end-stage renal disease (ESRD) who were older than 20 years old, receiving hemodialysis, and exhibiting signs of inflammation 48 hours after the insertion of catheters at different sites, such as the jugular, femoral, subclavian, and acute venous fistula (AVF) catheters. Patients who had fever, chills, headaches, or other inflammatory symptoms within 48 hours of the catheter being inserted or prior to starting dialysis were excluded from the research. Patients who had a positive blood culture before starting dialysis were also excluded.

The tip of the catheter was gathered by clamping it with sterile forceps as near the skin as possible. After that, it was placed in a sterile test tube and delivered to the laboratory that specializes in microbiology. Following that, it was treated in accordance with the usual recommendations [5-7].

Blood samples were taken from two different venipuncture sites, one from the central line and one from the peripheral line, or both from the peripheral lines, in accordance with the recommendations that are considered to be standard [5-7]. Immediately after the collection of swabs from the contaminated site of the catheter, they were placed in a sterile plastic container and delivered to the microbiology laboratory. The collection of urine and sputum samples was carried out in accordance with the established protocols [5-7].

Processing of the catheter tip was accomplished by the utilization of a roll plate and endoluminal flush approach [5, 8]. BacT/ALERT (also manufactured by BioMerieux in the United States) was utilized to cultivate peripheral venous blood. Swabs taken from the contaminated site of the catheter, as well as samples of urine and sputum, were processed in accordance with the standards that are considered standard [5-7]. It was discovered that the antibiotic susceptibilities of the isolated bacteria (aerobic bacteria) were assessed by utilizing the Vitek 2 system (bioMerieux, USA) in accordance with the Clinical and Laboratory Standard Institute (CLSI) 2021 cut-off points. It was determined that fungal infections were present in clinical samples in accordance with the established standards [5-7].

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE 12, 2023

RESULTS

There were a total of twenty-two percent of patients in the research population who fell into the age range of sixty-one to seventy years old. When compared to the female prevalence, which was 36%, the male preponderance was observed to be 64%. There, 47.3 percent of patients had diabetes and hypertension, both of which were common risk factors. The sole risk factor that was detected in 25.3% of the patients was hypertension. In comparison, diabetes was observed in 2.6% of the patients when they were examined. (Table Number One)

Table 1: Demographic and clinical characteristics of hemodialysis patients enrolled for the study (N=150)

Variables	Frequency (%)
Age	
21-30	2%
31-40	7.3%
41-50	22%
51-60	21.3%
61-70	22%
71-80	14%
81-85	4.6%
Sex	
Male	64%
Female	36%
Risk factors	
Diabetes	2.6%
Hypertension	25.3%
Both diabetes and hypertension	47.3%
Systemic lupus erythematosus (SLE)	0.6%
ADPKD (Autosomal polycystic kidney disease)	2.6%

Table 2: Distribution of samples collected from the study group and their culture positivity for bacterial and fungal pathogens

Sample	Aerobic bacteria	Fungi
Catheter tip (n=60)	11 (18%)	03 (5%)
Peripheral venous blood (n=150)	39 (26%)	3 (2%)
Swab (n=60)	6 (10%)	0
Urine (n=90)	35 (39%)	4 (4.4%)
Sputum (n=50)	15 (30%)	2 (4%)
Total	104 (69%)	12 (8%)
p-value: 0.54 (not significant)		

The sample that was collected the most frequently was peripheral venous blood, followed by samples of urine, catheter tips, swabs, and sputum after that. The percentage of bacterial pathogens that were found to be positive in culture was 69% overall. Urine samples had the greatest rate of isolation, which was 39%, followed by sputum samples (30%) and peripheral venous blood tissues (26%). Only 18% of the cultures were positive when they were taken from the catheter tip. The highest percentage of sputum samples (4%), followed by catheter tips (5%), and then urine samples (4.4%), were found to have a positive culture for fungal infections. (The Second Table)

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE 12, 2023

Table 3: Correlation between the number of dialysis and positive aerobic bacterial cultures

No of dialysis	Positive culture	Negative culture	Total cases
1-2 per week	72 (70.5%)	30 (29%)	102 (68%)
3-4 per week	43 (93.4%)	3 (7%)	46 (30%)
>4 per week	2 (100%)	00	2 (1.3%)
X^2	10.204 with 1 degree of freedom		
P value	0.0014 Highly significant		

The proportion of aerobic bacterial culture positive was found to be greatest in patients who were having hemodialysis at a frequency of more than four times per week, which was statistically significant.

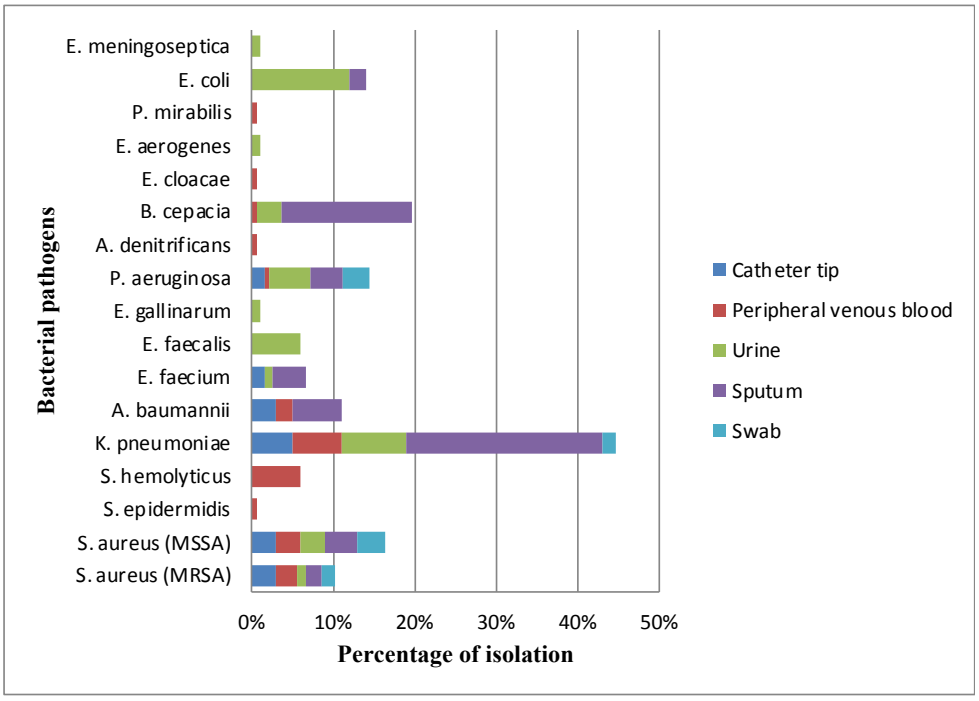

Fig 1: Distribution of aerobic bacterial isolates from various clinical samples

Klebsiella pneumoniae was the most common aerobic bacterial isolate (44.6%) among the clinical samples. ***Klebsiella pneumoniae, Staphylococcus aureus*** (MSSA)***, Staphylococcus aureus*** (MRSA) ***and Pseudomonas aeruginosa*** were the frequently isolated bacterial pathogens. (Figure no 1)

Table 4: Antibiotic susceptibility pattern of Gram-positive isolates

Isolates / Drugs	*S. aureus* (n=22) No (%)	*S. epidermidis* (n=1) No (%)	*S. hemolytic* (n=9) No (%)	*E. faecium* (n=4) No (%)	*E. faecalis* (n=5) No (%)	*E. gallinarum* (n=1) No (%)
Benzylpenicillin	5 (23%)	R	3 (33%)	R	2 (40%)	R
Ciprofloxacin	2 (9%)	1 (100%)	1 (11%)	R	3 (60%)	R
Clindamycin	4 (18%)	R	2 (22%)	R	R	R
Daptomycin	22 (100%)	R	9 (100%)	R	R	R
Erythromycin	6 (27%)	1 (100%)		R	4 (80%)	R
Gentamicin	14 (64%)	1 (100%)	6 (67%)	R	R	R
Levofloxacin	6 (27%)	R	3 (34%)	R	4 (80%)	R

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE 12, 2023

Linezolid	21(95%)	1(100%)	9 (100%)	2 (50%)	5 (100%)	R
Oxacillin	12 (55%)	R	4 (44%)	1 (25%)	R	R
Teicoplanin	20(91%)	1 (100%)	9 (100%)	2 (50%)	5 (100%)	R
Tetracycline	17 (77%)	R	3 (33%)	R	3 (60%)	R
Tigecycline	22 (100%)	1 (100%)	9 (100%)	4 (100%)	5 (100%)	1 (100%)
Cotrimoxazole	14 (64%)	R	R	R	R	R
Vancomycin	18 (82%)	R	R	2(50%)	5 (100%)	1 (100%)
Nitrofurantoin	3 (100%)	-	-	1 (100%)	5 (100%)	1 (100%)

Staphylococcus aureus is the gram-positive isolate that is most commonly seen. The antibiotics daptomycin, tigecycline, and nitrofurantoin were effective against every strain of Staphylococcus aureus I tested. It was shown that the susceptibilities to Vancomycin, Teicoplanin, and Linezolid were, respectively, 95%, 91%, and 82% high.

Klebsiella pneumoniae constituted the Gram-negative bacterial pathogen that was identified the most often. In the order of decreasing frequency, the susceptibility patterns of *Klebsiella pneumoniae* strains were as follows: amoxyclav (41%), nalidixic acid (40%), ceftazidime (34%), cotrimoxazole (31%), ampicillin (31%), and ceftriaxone (31%).

All *the Escherichia coli* There was a one hundred percent susceptibility of the strains to amikacin and meropenem. On the other hand, the susceptibility to Nitrofurantoin, Tigecycline, Ertapenem, Imipenem, Piperacillin + Tazobactam, Cotrimoxazole, and Cefoperazone + Sulbactam was 91%, 92%, 92%, 83%, 67%, 67%, and 67%, respectively.

Burkholderia cepacia had a one hundred percent susceptibility to the drug nitrofurantoin. Gentamicin (89%) and ceftazidime (78%) were effective against Pseudomonas aeruginosa, whereas ticarcillin and clavulanic acid (78%) were also effective against the bacteria.

Table 5: Isolation of fungal pathogens from various clinical samples in patients undergoing H.D.

Fungal pathogens	Catheter tip (n=60)	Peripheral venous blood (n=150)	Urine (n=90)	Sputum (n=50)	Swab (n=60)
C. parapsilosis	-	01 (0.6%)	-	-	-
C. albicans	03 (5%)	02 (1.3%)	03 (3%)	-	-
C. tropicalis	-	-	01 (1%)	02 (4%)	-
Total	03 (5%)	03 (2%)	04(4%)	02(4%)	-

Candida albicans were followed by urine (three percent) and peripheral venous blood (one point three percent) as the most prevalent fungal pathogen that was isolated from the catheter tip (five per cent).

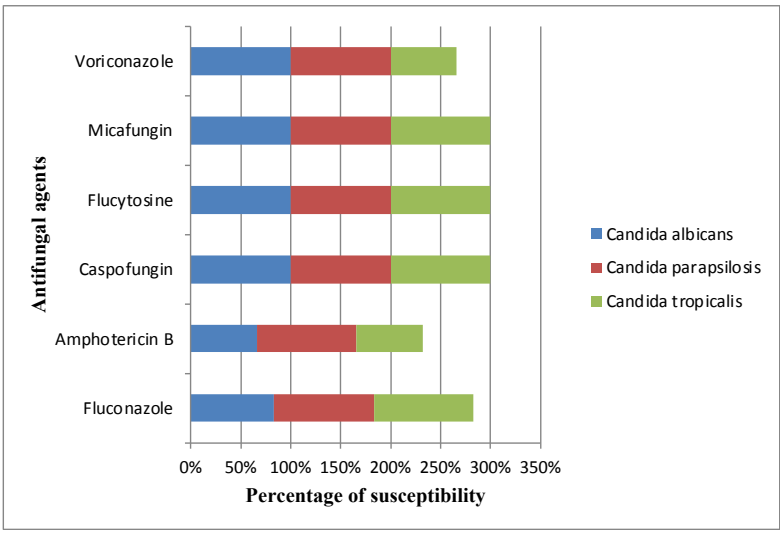

Figure 2: Anti-fungal susceptibility pattern of *Candida isolates*

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE 12, 2023

All ***Candida albicans*** Caspofungin, Micafungin, Voriconazole, and Flucytosine were all effective against the isolates. On the other hand, the sensitivity of the isolates to Fluconazole and Amphotericin B was 83% and 66%, respectively.

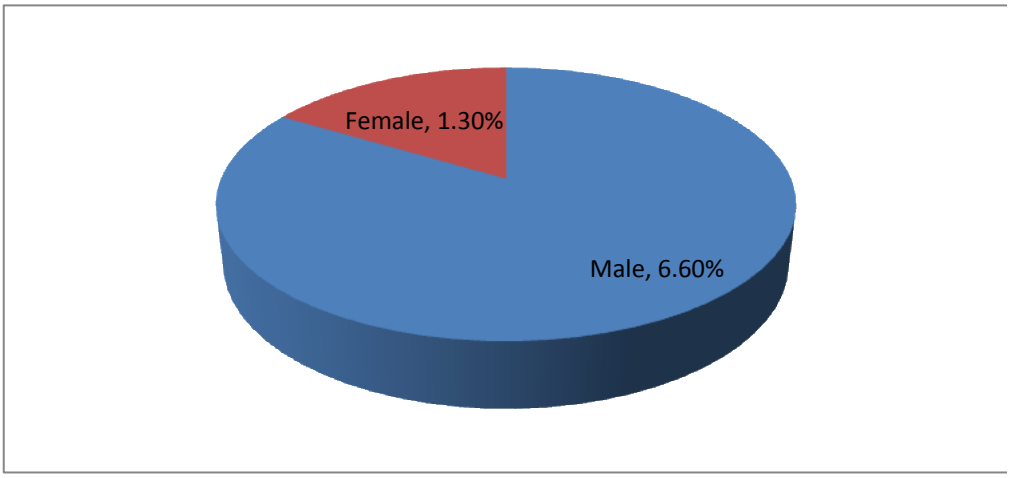

Fig 3: Gender-wise distribution of positive fungal isolates
Fungal pathogens were isolated mostly from males (6.6%)

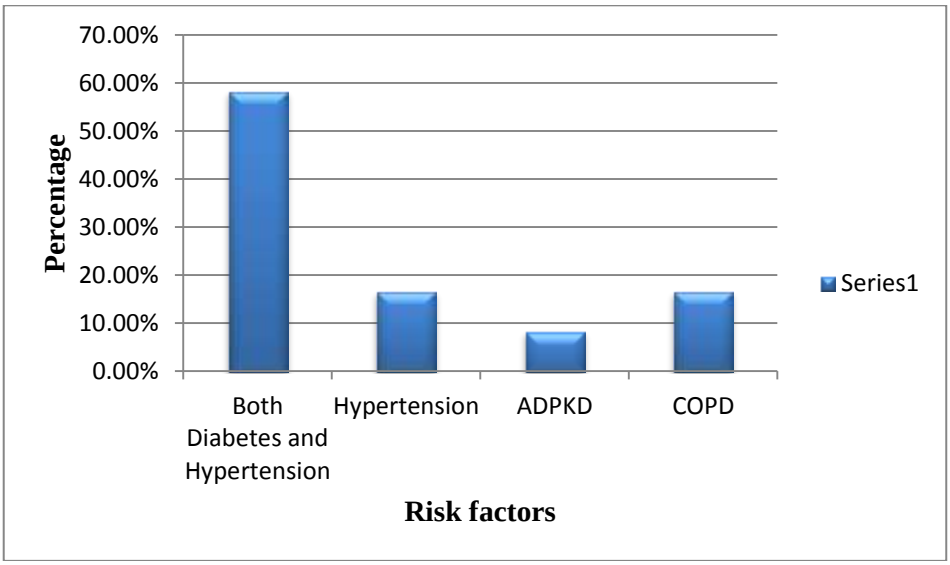

Fig 4. Risk factors associated with fungal infections.

Diabetes mellitus and hypertension 07 (58.4%) were both underlying disorders that were often detected in the group that was participating in the investigation.

DISCUSSION

The bulk of the patients in our study were men, accounting for 96 (64%). The most prevalent age group was between 61 and 70 years old, which is comparable to the findings of Rteil et al. 2020 [9].

According to Elghouat Ghita et al. (2021) and Serefhanoglu et al. (2011), who reported diabetes as a significant risk factor in 29% and 49% of the cases, respectively [10-11], the most prevalent risk factors in our study were diabetes and hypertension, which accounted for 47.3% of the cases. Elghouat Ghita et al. 2021 found that hypertension was the most prevalent risk factor, accounting for 52% of the cases [10].

The percentage of bacterial pathogens that were found to be positive in culture was 68% overall. It was shown that the urine samples had the highest level of isolation (39%), followed by the peripheral venous blood samples (26%), the sputum samples (30%), and then the catheter tips (18%). Culture positivity was found to be at its highest in patients who were getting hemodialysis for more than four days per week (100 percent), followed by patients who were treated for three to four days per week (93.4%). Rteil et al. (2020) discovered that the highest percentage of culture positives came from circulation, 50 percent, followed by sputum, 27.8 percent, and urine, 5.6 percent [9].

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE 12, 2023

Klebsiella pneumonia was the commonest aerobic bacterial isolate (44.6%) in our study, followed by *Staphylococcus aureus* (MSSA) (16.3%), *Escherichia coli* (14%) and *Staphylococcus aureus* (MRSA) (10.2%). Alzhami et al. (2019) also found *Klebsiella pneumonia* (40%), *Staphylococcus aureus* (16.7%), MRSA (9.1%), and *Escherichia coli* (3%) as the predominant bacterial pathogens [12]. Fysaraki M et al. 2013 reported *Staphylococcus aureus* as the commonest bacterial pathogen (36%), followed by *Staphylococcus epidermidis* (17%), *Escherichia coli* (9%) and *Klebsiella spp* (4%) [13].

The antibiotics daptomycin, tigecycline, and nitrofurantoin were effective against every single strain of Staphylococcus aureus that we identified throughout our research. In terms of susceptibility, the results were as follows: 95% for Linezolid, 91% for Teicoplanin, 82% for Vancomycin, 9% for ciprofloxacin, 18% for Clindamycin, and 27% for Erythromycin. These results are comparable to the findings that Masoodi I et al. 2019 [14] reported finding. Both Staphylococcus haemolyticus and Staphylococcus epidermidis strains had the highest sensitivity to the antibiotics Linezolid, Teicoplanin, and Tigecycline. These findings are comparable to those that were discovered by Sahli et al. in 2016 [15].

The most common isolates of Gram-negative bacteria were Acinetobacter baumannii (5%), Burkholderia cepacia (2.8%), Pseudomonas aeruginosa (1.9%), Escherichia coli (4.8%), and Klebsiella pneumonia (9%) among others. Gram-negative bacteria with the highest prevalence was Klebsiella pneumoniae. However, Sahli et al. (2016) discovered that Acinetobacter baumannii was isolated at a rate of 9.1%, whereas Klebsiella pneumoniae was isolated at a rate of 22.7% [15].

Candida albicans (9.3%) was the most often found fungal pathogen, followed by Candida tropicalis (5%), Candida parapsilosis (0.6%), and finally Candida albicans. Five percent of the fungal growth was detected at the catheter tip, while the remaining four percent were detected in sputum, urine, and peripheral venous blood. Conversely, 58.8% of Candida albicans and 41.2% of N. candida albicans species were found to be isolated, according to Serefhanoglu et al. (2011) [11]. Serefhanoglu K et al. (2011) similarly observed a minimal fatality rate after thirty days in the hospital [11]. Men, who made up 6% of the patient population overall, were the ones from whom the bulk of fungal infections were identified. An average hospital stay lasted about eighteen days. Since our hospital is a tertiary care center, our strict infection control approach is most likely to blame for the low isolation of fungal infections.

In our study, the two most common underlying conditions for fungal infection were diabetes mellitus (0.4%) and hypertension. Chronic obstructive pulmonary disease (0.2%) and hypertension (0.2%) were second and third, respectively. The results of Serefhanoglu et al. (2011) and Fysaraki M et al. (2013) were not in agreement with this conclusion. [11], [13]. When compared to central venous catheters (CVC), the use of a fistula allowed for more vascular access for fungal infections. Fysaraki et al. (2013) revealed findings that were comparable to these. However, the research conducted by Serefhanoglu et al. 2011 [13], [11] found that access through CVC was more prevalent. The majority of the persons who participated in our research were literate and came from metropolitan regions. Because urban residents have easier access to medical care, they are more likely to seek hemodialysis treatment on a regular basis.

In the course of our research, we found that all of the Candida albicans isolates were susceptible to the antibiotics caspofungin, micafungin, voriconazole, and Flucytosine. However, the susceptibility to fluconazole and amphotericin B was 83% and 66%, respectively. In addition, similar susceptibility patterns were detected for Candida parapsilosis and Candida tropicalis, which was comparable to the findings that Serefhanoglu et al. 2011 [11] discovered.

Conclusion

It is well-accepted that patients who are receiving hemodialysis are at risk for a number of infections. As a result of an increase in the prevalence of underlying risk factors, such as diabetes mellitus and hypertension, the number of patients who require dialysis is now on the rise. As a result, it is essential to investigate and eradicate the possibility of infection in patients with end-stage renal disease who are receiving continuous hemodialysis.

Because of this, doctors who are giving treatment to patients who have renal illness need to be conversant with local antimicrobial sensitivity patterns in order to pick suitable empirical antimicrobial therapy and to notice the advent of new resistance profiles within the community. It is of the utmost importance to enhance the level of adherence to fundamental infection control techniques, antimicrobial stewardship, and the creation of more efficient ways for prevention and treatment.

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE 12, 2023

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