Sarcouncil Journal of Applied Sciences

ISSN(Online): 2945-3437

Volume- 06| Issue- 03| 2026

Research Article **Received:** 05-02-2026 | **Accepted:** 03-03-2026 | **Published:** 19-03-2026

Clinical Prognosticators of Mixed - Bacterial-Fungal Infections in Immunocompromised Patients

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Abstract: Mixed bacterial-fungal infection is a severe and poorly identified complication in immunocompromised patients, which leads to the delay in diagnosis, improper empirical treatment, prolonged hospital stay, and high mortality. The aim of this retrospective case-control study carried out at a tertiary care hospital was to find out clinical predictors related to mixed infections and assess their effects on the outcomes. Patients with microbiologically confirmed infections, who were immunocompromised, were included; mixed bacterial-fungal infections and cases with only bacterial infections were included respectively. Multi-variable logistic regression was done on 210 patients (70 cases and 140 controls) and neutropenia (OR 3.8, 95% CI 1.9-7.4), prolonged broad-spectrum antibiotic use (more than 7 days) (OR 4.5, 95% CI 2.2-9.1), ICU admission (OR 2.9, 95% CI 1.4-5.8), and As independent predictors of mixed infections, central venous catheterization (OR 3.2, 95% CI 1.6-6.3) was used. There was a great deal of association of these infections with increased mortality (38.5%) as compared to 18.2%, $p < 0.01$) as well as increased length of hospital stay. Timely diagnostic assessment and tailored modalities of treatment can enhance the clinical outcome of high-risk patients when these patients are identified early in life.

Keywords: Bacterial , Fungal , Infection , hospital.

INTRODUCTION

Among the immunocompromised patients infections have continued to be one of the greatest causes of morbidity and mortality. Oncology, transplantation medicine and immunosuppressive therapies have made a big contribution to the survival of patients but the situation has also created a larger number of people who are vulnerable to severe and opportunistic infections [Deinhardt-Emmer, S. *et al*., 2025]. Weakness in both innate and adaptive immune systems especially a failure in neutrophil activity, cellular immunity and mucosal barrier integrity exposes such patients to bacterial and fungal infections [Cumming, E., & Peters, C. 2024].

Although bacterial infections historically have dominated clinical focus, invasive fungal infections have become a significant problem and this is on the patients with hematologic malignancy, solid organ transplantation, excessive corticosteroid exposure, and terribly neutropenic [He, M. *et al*., 2025]. What is more worrying is that there is an incidence of mixed-bacterial-fungal infections which include the isolation of both types of pathogens in the same infectious episode. Such mixed infections are not always adequately diagnosed when using standard clinical care practices, with the initial approaches to diagnosis and treatment being mostly based on bacterial etiologies. The late identification of fungi can result in the wrong empirical treatment, long-term sepsis, long-term hospital stay, and high mortality [Douglas, A. P. *et al*., 2023].

Mixed infections have a complicated pathogenesis. The continued use of wide-range antibiotics alters normal microbiota and increases the growth of fungi. Central venous catheters are invasive devices which offer a space where polymicrobial biofilms form to allow concomitant colonization by bacteria as well as fungi. Also, mechanical ventilation and admission in intensive care unit (ICU) further expose healthcare-associated pathogens. These aspects can be synergistic in the case of severely immunocompromised hosts leading to complicated infections of increased severity and treatment complications [Xu, J. Q. *et al*., 2024].

However, even though they have clinical significance, little information on the particular predictors that contribute to the risk of mixed bacterial-fungal infections in immunocompromised patients is found. The detection of these predictors is necessary to perform risk stratification at an early stage, conduct diagnostic evaluation on time, and choose empirical antimicrobials. Early identification can lead to better outcomes because it will decrease the delays in antifungal treatment and limit complications of infection [Kreitmann, L. *et al*., 2024].

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DOI- https://doi.org/10.5281/zenodo.19117901

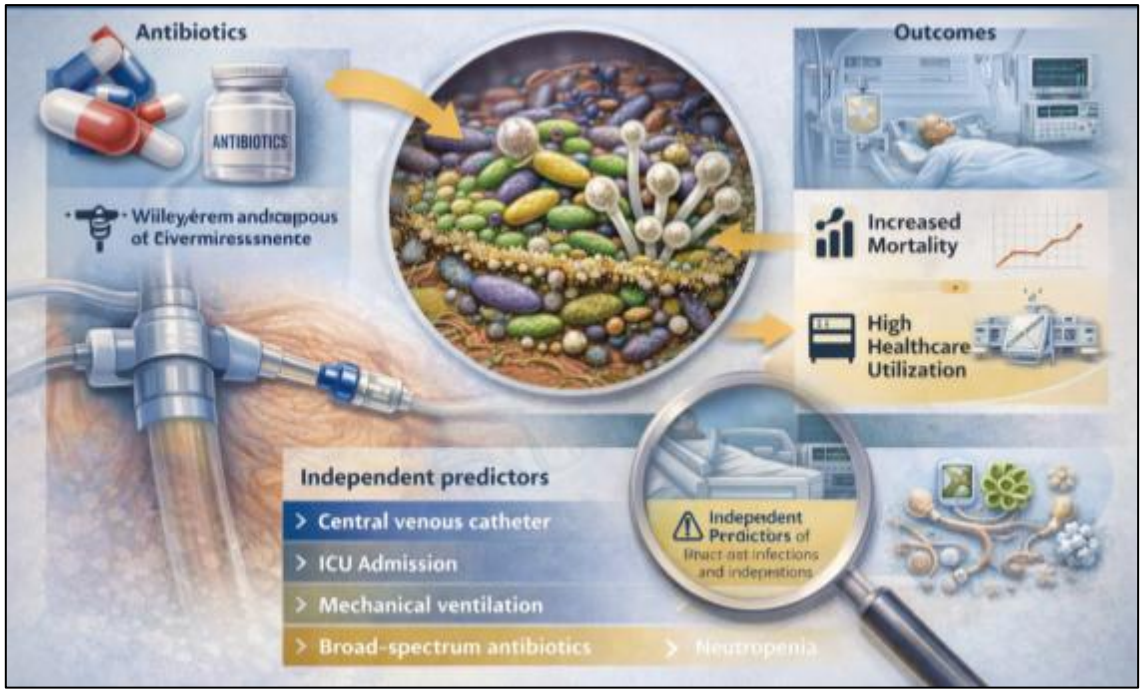

Figure 1 : Mixed Bacterial-Fungal infections in Immunocompromised patients

Thus, the purpose of this investigation was to find independent clinical predictors of mixed bacterial infections with fungi in immunocompromised patients in a tertiary care hospital and to determine the effect of these infections on mortality and healthcare use. The systematic comparison with the patients who had only bacterial infections will help this study to present evidence-based data, which could help guide the clinical decision-making process and optimize the treatment approaches in this population that is at high risk [Naseem, R. *et al*., 2025].

MATERIAL AND METHODS

Methods

Inclusion Criteria

Adult patients (aged 18 years and above) diagnosed with immunocompromised state, patients receiving chemotherapy due to malignancy, patients receiving solid organ transplant, patients receiving immunosuppressive doses of corticosteroids, patients with neutropenia (absolute neutrophil count <500 cells/mm^3), or those taking other immunosuppressive agents were included in the study. Patients with microbiologically confirmed infections were only included during hospitalization to provide accurate diagnosis [Duminuco, A. *et al*., 2024].

Case Group (Mixed Infections)

The case group was a group of patients who were confirmed to have the mixed bacterial-fungal infections during the same hospital stay. Mixed infection was considered the isolation of at least one bacterial pathogen and one fungal pathogen of a sterile site or a clinically significant sample. The two isolates had to be performed in the identical infectious episode within a limit of seven days to ascertain that the two isolates were not different infections [Douglas, A. P. *et al*., 2023].

Control Group

The control group involved patients, whose bacterial infections were confirmed through a microbiological test, and no fungal isolation was observed at the same period of hospitalization. These laboratory diagnostic criteria were the same as those in the case group to make the comparisons relevant. To reduce the confounding and increase consistency of the results, cases and the controls were paired with a 1:2 relationship based on the age and underlying disease. [Maurici, M. *et al*., 2022].

Data Collection

The entire data of the study was extracted out of the electronic medical records at the hospital through a standardized pre-designed data collection form to avoid any errors and to have uniformity. The form measured demographic, clinical, laboratory, and microbiological variables that were needed to be statistically analyzed and identify the factors that are related to mixed infections. The accuracy of data was ensured with the help of independent reviews by the members of the research team [Henry Basil, J. *et al*., 2025].

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Demographic Data

Age, sex, and body mass index (BMI) were the demographic variables. All these variables were gathered to characterize the study population and determine the possible impact of baseline variables on the susceptibility to mixed infections. They also helped to match cases and controls appropriately to minimize the confounding factor with age and sex [Nyberg, S. T. *et al*., 2026].

Clinical Variables

The clinical variables were the underlying disease, the time spent in hospital before the person becomes infected, the nurse intensive care unit (ICU) hospitalization, and necessity of mechanical ventilation. Data on invasive devices including central venous catheters, urinary catheters, total parenteral nutrition and surgery within the last 30 days was recorded as well. Also, a minimum of seven days of exposure to corticosteroids, immunosuppressive therapy and broad-spectrum antibiotics in the 30 days before infection were noted to assess their relationship with the exposure to the risk of mixed infections [Freundlich, R. E. *et al*., 2023].

Laboratory Investigations

Data regarding laboratories were taken within 48 hours of onset of infection to make it relevant with respect to time regarding the infectious episode. These were total white blood cell count, absolute neutrophil count, C-reactive protein (CRP), and procalcitonin concentrations as indices of the inflammatory response and severity of the infection. Renal and liver function data were also noted to determine the general clinical situation and organs involvement, which might be connected with infection or treatment. [Hakki, S. *et al*., 2022].

MICROBIOLOGICAL METHODS

Specimen Collection

To be able to offer quality and minimize contamination, all the clinical specimen was collected according to the general hospital practices. The cultures were the blood cultures, respiratory (sputum and bronchoalveolar lavage) and urine and wound swabs among other sterile body fluids where required. Strict aseptic procedures were followed in the collection of the specimen of the sample in order to ensure reliability and clinical relevance [Harrington, A. *et al*., 2021].

Bacterial Identification

A timely and accuracy diagnosis of bacteria infection was conducted through automated and standardized laboratory techniques. The automated blood culture systems like the BACTEC were used in detecting microbial growth in the blood cultures. The identification of organisms was done through automated identification systems such as VITEK 2. Antimicrobial susceptibility testing was done as per Clinical and Laboratory Standards Institute guidelines to facilitate the selection of the appropriate therapy as well as accurate determination of the patterns of antimicrobial resistance [Alshalhoub, S. *et al*., 2024].

Fungal Identification

To increase the accuracy of the diagnosis, several laboratory tests were used to diagnose fungus. Direct microscopic analysis and culture were routinely done on Sabouraud dextrose agar. The correct identification of Candida species was done using chromogenic media. The identification of the molds was made according to typical morphological presentation in culture. Galactomannan assay was done in instances of suspected invasive aspergillosis. Clinically significant fungal isolates were only included after assessment by an infectious diseases specialist in order to exclude contaminations or colonization [Sedik, S. *et al*., 2024].

Study Variables and Outcomes

Primary Outcome

The main aim was to recognize the independent clinical predictors of mixed bacterial-fungal infections in immunocompromised patients with the view of promoting early detection, enhancing therapeutic interventions and reduction of complications of infections [Reikvam, H. *et al*., 2025].

Secondary Outcomes

To compare the clinical impact of mixed infections and bacterial infections alone, secondary outcomes were to be used in infection-related mortality, length of hospital stay, ICU length of stay, and the requirement of mechanical ventilation [Schmucker, S. 2025].

SAMPLE SIZE CALCULATION

The sample size was determined to produce statistical power of 80 % and a level of significance of 0.05 with the ratio of 1:2 between the cases and the controls. To get sufficient power to do a multivariate analysis, 60 cases and 120 controls were required [Zhou, Y. *et al*., 2025].

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STATISTICAL ANALYSIS

The SPSS software was used to carry out statistical analysis. Categorical and continuous variables were summarized using descriptive statistics. The chi-square and independent t-tests were applied to compare groups, as it was appropriate. The multivariate logistic regression analysis was carried out to find out independent predictors of mixed infections. Outcomes were provided in the form of odds ratio (OR) and 95% confidence interval (CI), and the p-value of less than 0.05 was seen as significant.

RESULTS

The data of 210 immunocompromised patients was taken into consideration, 70 of which (33.3%) were mixed bacterial-fungal infections, and 140 individuals (66.7%) were bacterial infections. The two groups were shown to differ statistically in terms of age, sex or body mass index and the matching of the two groups and minimization of the confounding effects was successful. The demographic features of the two groups are shown in table (1).

Table 1. Demographic Characteristics of the Patients

Variable	Mixed Infection (n=70)	Bacterial Infection Only (n=140)	p-value
Age (years)	53.1 ± 14.8	52.2 ± 15.7	0.71
Male sex	41 (58.6%)	79 (56.4%)	0.77
Body Mass Index	26.4 ± 4.2	25.9 ± 4.5	0.48

Figure (2) illustrates a bar chart comparing age distribution and sex proportion between the two groups.

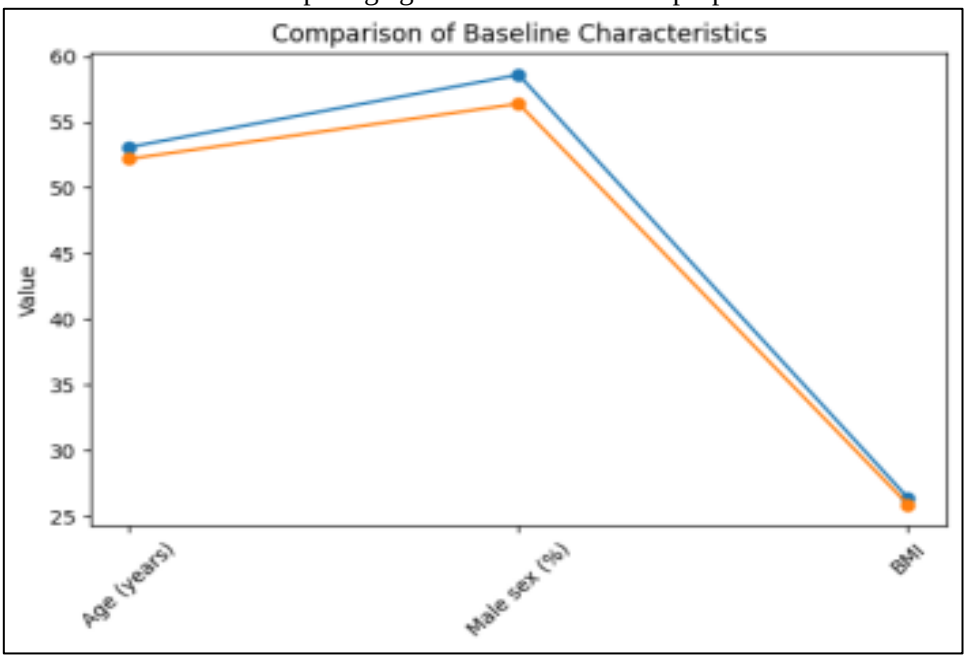

Figure 2. Baseline Characteristics of Study Groups

The univariate analysis showed that there were statistically significant differences of various clinical variables. Neutropenia, taking of broad-spectrum antibiotics (>7 days), hospitalization in the ICU, and central venous catheterization were much more common in the mixed infection group. These comparisons are given in Table (2).

Table 2. Clinical Factors Associated with Mixed Infection (Univariate Analysis)

Variable	Mixed Infection	Bacterial Infection Only	p-value
Neutropenia (<500)	39 (55.7%)	32 (22.8%)	<0.001
Broad-spectrum antibiotics >7 days	46 (65.7%)	41 (29.2%)	<0.001
ICU admission	38 (54.3%)	43 (30.7%)	0.002
Central venous catheter	44 (62.9%)	49 (35.0%)	<0.001
Mechanical ventilation	29 (41.4%)	33 (23.5%)	0.01

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Figure (3) presents a bar chart comparing the proportions of major risk factors between the two groups.

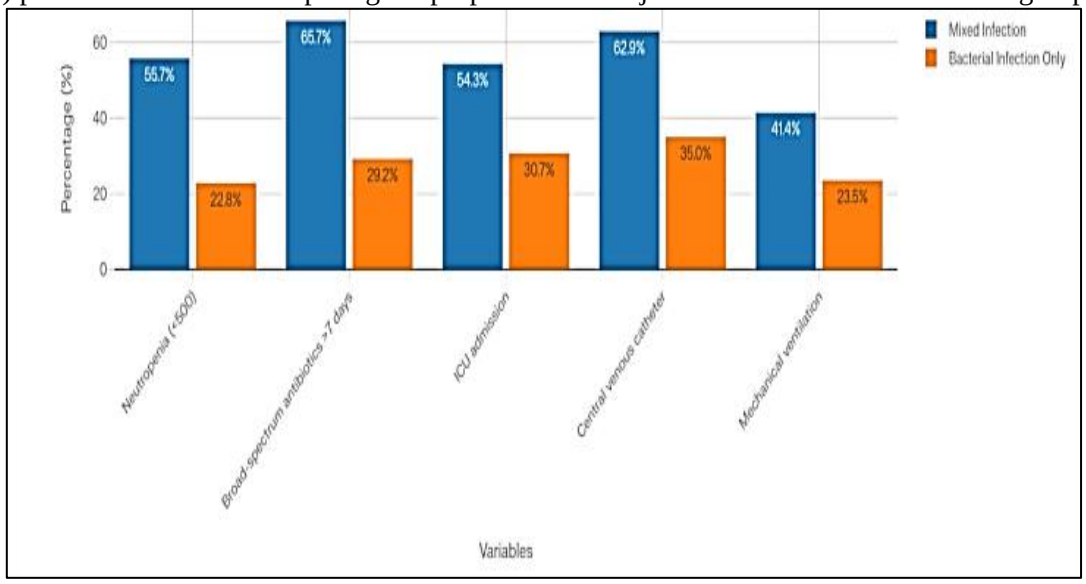

Figure 3: Comparison of Risk factors : mixed vs bacterial infections %

Four independent variables that predicted mixed infection were obtained after adding statistically significant variables into a multivariate logistic regression model. The strongest independent risk factor was the prolonged use of broad spectrum antibiotics. The summary of the multivariate analysis is presented in Table (3).

Table 3. Independent Predictors of Mixed Infection (Multivariate Logistic Regression)

Variable	OR	95% CI	p-value
Neutropenia	3.8	1.9–7.4	<0.001
Broad-spectrum antibiotics >7 days	4.5	2.2–9.1	<0.001
ICU admission	2.9	1.4–5.8	0.003
Central venous catheter	3.2	1.6–6.3	0.001

Figure (4) represents a forest plot displaying the odds ratios (OR) and 95% confidence intervals of the independent predictors.

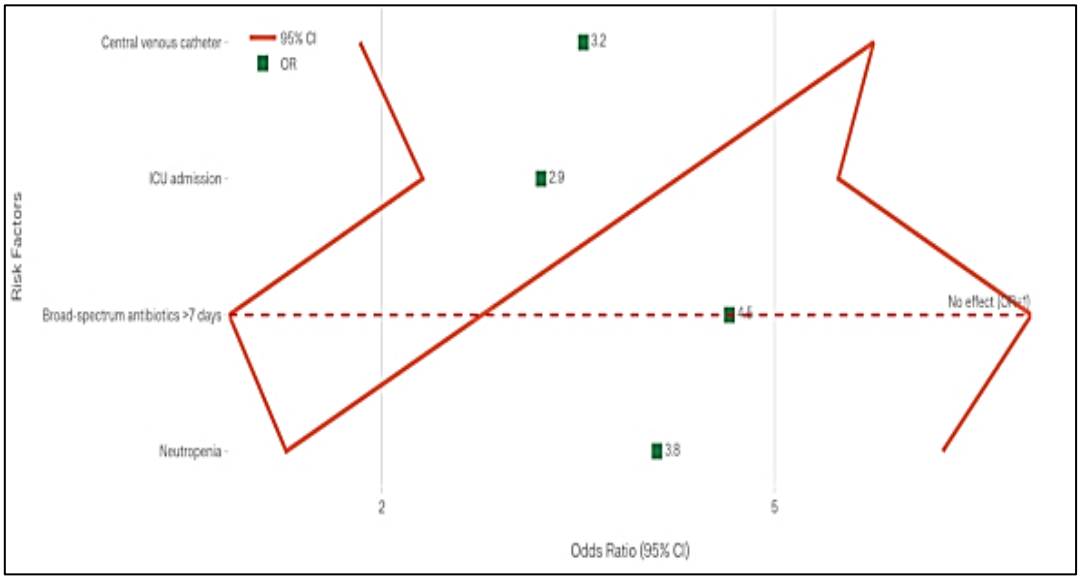

Figure 4: Odds ratios with 95% confidence intervals

Gram-negative bacteria were the most widespread bacterial isolates according to microbiological perspective and Candida species were the predominant fungal isolates. Table (4) gives the microbiological distribution of mixed infection group.

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Table 4. Microbiological Distribution of Isolates in the Mixed Infection Group (n=70)

Microorganism	Number (%)
Klebsiella pneumoniae	20 (28%)
Escherichia coli	15 (22%)
Pseudomonas aeruginosa	13 (18%)
Candida albicans	28 (40%)
Candida glabrata	13 (18%)
Aspergillus spp.	14 (20%)

Figure (5) illustrates a pie chart showing the distribution of fungal isolates in the mixed infection group.

In terms of clinical outcomes, mixed infections were also related to significantly increased mortality and hospital and ICU stays as opposed to the case of bacterial infections. Table (5) shows the comparisons of clinical outcomes of the two groups.

Table 5. Comparison of Clinical Outcomes Between the Two Groups

Variable	Mixed Infection	Bacterial Infection Only	p-value
Mortality rate	27 (38.5%)	25 (18.2%)	0.003
Hospital stay (days)	24.6 ± 8.2	16.3 ± 6.5	<0.001
ICU stay (days)	9.4 ± 4.7	5.8 ± 3.9	<0.001

Figure (5) shows a bar chart comparing mortality rates and mean length of stay between the two groups.

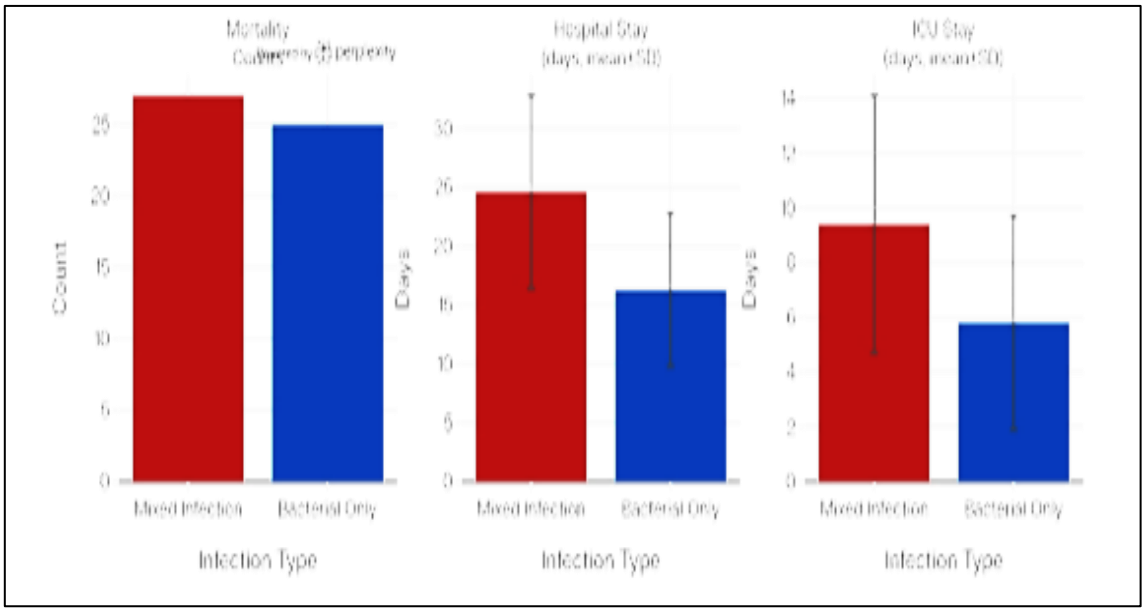

Figure 5: Clinical Outcomes : Mixed vs Bacterial infection only

DISCUSSION

Mixed bacterium-fungal infections are a clinical entity of important clinical concern and potentially life-threatening complication in immunocompromised patients. Mixed nature of one-third of microbiologically confirmed infections in the present study highlights a significant impact of polymicrobial infections to this vulnerable group [Luk, A., & Ostrosky-Zeichner, L. 2025]. Notably enough, the two groups did not differ significantly in terms of baseline demographic characteristics such as age, sex, and body mass index, which proves that the appropriate matching and reduction of confounding took place. This validates the existence of the observed associations and shows that the differences in outcomes were more likely to be caused by clinical and treatment-related factors than inherent demographic differences [Anandani, G. *et al*., 2025].

Neutropenia was identified as a strong independent predictor of mixed infection. This observation biologically is not impossible, as the roles of neutrophils in the innate immune response to bacterial and fungal infections are very active. Extreme neutropenia compromises phagocytosis, intracellular killing and inflammatory signal

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transduction, thereby promoting invasive fungal disease, in particular, candidiasis and aspergillosis [Wattana, M. K., & Estes, M. 2025]. Simultaneously, Gram-negative bacterial infections are very prone to neutropenic patients. The presence of bacterial and fungal pathogens in this case is likely to indicate deep-rooted immune defects but not consecutive independent events. This can be used clinically to add to the argument of early evaluation of antifungals or empirical therapy in persistently febrile neutropenic patients who are not responding well to antibacterial therapy [Bal, W. *et al*., 2025].

Exposure to broad-spectrum antibiotics in the long-term was identified as the greatest independent predictor of mixed infection. The result highlights the significance of antimicrobial pressure in intrusion of host microbiome [Fishbein, S. R. *et al*., 2023]. Broad-spectrum agents inactivate protective commensal flora, that is, especially in the gastrointestinal tract, making fungal proliferation and invasion of the bloodstream easier. Dysbiosis as a result of antibiotics can also result in compromised mucosal immune responses, which further leads to the predisposition to invasive fungal infection. These results support the significant role of antimicrobial stewardship interventions that should reduce unnecessary extended use of antibiotics particularly in immunocompromised patients [Fishbein, S. R. *et al*., 2023].

Mixed infections were also independent of ICU admission and central venous catheterization. The key risk factors among the ICU patients are invasive procedures, mechanical ventilation, exposure to high levels of antimicrobials and severe underlying illness. Central venous catheters, especially, offer a direct access point of microorganisms and are known to be highly susceptible to biofilm formation [Kreitmann, L. *et al*., 2024]. Fungal pathogens particularly Candida species have a high ability to produce biofilms, which increase resistance to host defense and antimicrobial therapy. Co-existence of bacterial and fungal organisms could then be promoted by polymicrobial biofilms on intravascular devices, and is thus causing persistent troublesome infections that are tough to treat [Mahendra, M. *et al*., 2021].

Candida species and Gram-negative bacilli were the commonest fungal pathogens and Bacterial isolates respectively. It has been matched with the transmission of healthcare-related infections and the manifestation of the effects of the antibiotic exposure and invasive devices [Almeida-Bezerra, J. W. *et al*., 2026]. The occurrence of Aspergillus species in a small group of patients is another indication of deep immunosuppression especially among patients with hematologic malignancies or those with long neutropenia. These results indicate that extensive diagnostic methods that encompass fungus-specific tests should be adopted in high-risk individuals instead of the results of bacterial cultures [Bordea, M. A. *et al*., 2025].

Figure 6. Risk Factors and Outcomes of Mixed Bacterial–Fungal Infections

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Mixed infections were characterized by a substantially high rate of mortality and a long hospital and ICU length of stay in contrast with bacterial infections. This higher mortality can be attributed to a number of factors such as a late diagnosis of fungal co-infection, inappropriate first line empirical treatment which is not covered by antifungal agents, synergistic interactions between pathogens which increase virulence and greater underlying immune impairment in the sick patients [Silva, D. L. *et al*., 2025]. The length of stay in the hospital is a probable indication of the severity of infection as well as the complexity of combined antimicrobial treatment. All these results show that mixed infections are not just microbiological phenomena, but they are a serious clinical disorder and have significant prognostic and healthcare implications [O'Toole, R. F. 2021].

Some of the strengths of this study are that microbiologically confirmed diagnoses were used, matching of cases and controls was conducted properly, and multivariate analysis was performed to identify independent predictors. Nevertheless, there are restrictions which should be taken into consideration. Retrospective design can be biased in terms of information, and its single-center nature can be narrow in terms of generalisation. Moreover, the analysis of detailed antimicrobial resistance patterns was not completely carried out, and it is sometimes difficult to distinguish colonization and actual invasive fungal infection in some clinical situations [Marcos-Zambrano, L. J. *et al*., 2021].

CONCLUSION

Immunocompromised patients are prone to mixed bacterial-fungal infections, which are closely linked with neutropenia, extended broad-spectrum antibiotic therapy, ICU hospitalization, and central venous catheterization, and adversely influence clinical outcomes. Efficient diagnosis and treatment protocols should be used to decrease mortality and overall prognosis of this vulnerable group by early detection of high-risk patients and applying specific diagnostic and treatment procedures to them.

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Source of support: Nil; **Conflict of interest:** Nil.

Cite this article as:
Faris, K. B. "Clinical Prognosticators of Mixed - Bacterial-Fungal Infections in Immunocompromised Patients." *Sarcouncil Journal of Applied Sciences* 6.3 (2026): pp 10-19.

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