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E-mail: mmidsp123@yahoo.com


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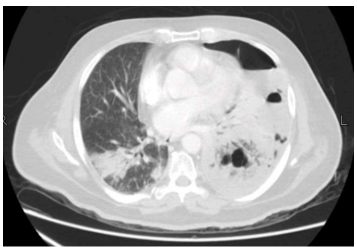

CT Chest with contrast of the patient showing: **Left-sided moderate hydropneumothorax with collapsed upper lobe. Consolidation in bilateral lower lobes with significant cystic changes on left side.** Patient underwent video assisted thoracoscopic surgery (VATS) and pleural tissue culture was sent which grew **Rhizopus species.** Diagnosis**: Pulmonary Mucormycosis**

*Courtesy : Nosheen Nasir, Senior Instructor, Section of Adult Infectious Diseases, Dept. of Medicine, Aga Khan University Hospital, Karachi.*

# *Candida auris:* A Multidrug Resistant Fungus and an Emerging Cause of High Mortality

*Candida auris* was first reported in 2009 from Japan as a cause of otitis media and subsequently reports of blood stream infections emerged from Southeast Asia, India and Middle East. It was initially identified from Pakistan in 2015 when clinical isolates were sent to US Centers for Disease Control and Prevention(CDC) in the setting of an outbreak of invasive infections in what was being identified as *Saccharomyces*at one of the tertiary care centers in Karachi. This led to issuance of an alert to clinicians by CDC in June 2016.[1]

Since its global emergence, *Candida auris* has been associated with invasive infections which are extremely difficult to treat and have high mortality with approximately sixty percent in-hospital mortality reported from centers worldwide.[2] The crude mortality rate from Pakistan was reported at 72 % in a recently published systematic review.[3] The claim to fame of *Candida auris* is the fact that it is multidrug resistant unlike other candida species and is not only resistant to most azoles but displays variable susceptibility even to echinocandins and amphotericin B. Hence, treatment can be very challenging especially in resource limited settings.[4]

*Candida auris* is known to cause invasive infections mostly bloodstream infections (BSIs) and has been isolated from a variety of clinical specimens including bile, urine, pus and tissue culture. Among the risk factors for infection are underlying medical co-morbidities, immunocompromised state, use of invasive devices and exposure to health care facilities especially if patient had stay in intensive care unit. Moreover, the infections are known to occur after prior exposure to broad spectrum antimicrobials particularly antifungals.[5] Alarmingly, *Candida auris* has been associated with ability to cause outbreaks in health care settings. This is being linked to its ability to persist on inanimate objects like mattresses, medical equipment and furniture that comes in contact of patients. It get transferred from these sites to the hands of the health care providers and this is the proposed mechanism of transmission in critical care settings. Hence, CDC has emphasized on good hand hygiene compliance in addition to strict standard and contact precautions as well as terminal cleaning and disinfection of rooms of infected patients with appropriate disinfectants in order to curb patient to patient transmission.[4, 6]

*Candida auris* has been known to harbor resistance to almost all classes of antifungals. Clinical isolates from different parts of the world including Pakistan were greater than 90 percent resistant to fluconazole and approximately 35 percent resistant to amphotericin B.[1] Moreover, the species is known to develop resistance quite rapidly and hence continuous surveillance is required. Although the drug of choice is not clearly defined, empiric treatment with an echinocandin is recommended till susceptibility is available. This can be cost prohibitive in resource limited settings like Pakistan where availability of echinocandin is still a distant possibility for most centers leading to usage of Amphotericin B in such a scenario with variable success. Combination therapy has also been advocated by experts but data on this is scarce. Patients usually require close monitoring for signs of treatment failure and for de novo resistance on therapy. It is also imperative to have an antifungal stewardship program to discourage inappropriate use of antifungals and for early identification of *Candida auris* and implementation of contact precautions and isolation measures to minimize transmission.[6]

In conclusion, *Candida auris* is a deadly pathogen and requires concerted efforts on the part of health care providers, infection preventionists, medical microbiologists and infectious diseases specialists to contain its spread and prevent outbreaks in health care settings.

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**Dr. Nosheen Nasir**
*MRCP (UK), FCPS (Med), FCPS (ID)*
*MSc Epidemiology & Biostatistics*
Senior Instructor
Section of Adult Infectious Diseases, Dept. of Medicine
Aga Khan University Hospital, Karachi.
Email: Nosheen.nasir@aku.edu

ORIGINAL ARTICLE

# Clinical Profile of Patients and Antifungal Susceptibility Pattern of Invasive *Cryptococcus neoformans* Isolates from Pakistan

Faryal Saleem*, Kausar Jabeen*, Joveria Farooqi*, Naima Fasih**, Afia Zafar*

*Department of Pathology and Laboratory Medicine, First Floor, Soparivala building, Aga Khan University Hospital, Karachi, Pakistan.
** Department of Pathology and Laboratory Medicine, Armed Forces Hospitals Southern region, Khamis Mushayt, P.O. Box 101 Zip Code: 61961, Khamis Mushayt, KSA.

## Abstract

**Background**
Cryptococcosis affects immunocompromised as well as immunocompetent individuals. A wide spread improper antifungal use has resulted in antifungal resistance. Data regarding clinical characteristics and antifungal susceptibilities of *Cryptococcus neoformans* from Pakistan is limited.

**Objective**
To determine clinical characteristics of patients and antifungal resistance in invasive *Cryptococcus neoformans*isolates.

**Method**
This study was conducted at Aga Khan University Hospital, Karachi, Pakistan from 2009-2016. Forty-nine *Cryptococcus neoformans* strains were isolated from various clinical specimens. Clinical data for inpatients was collected through medical records and for outpatients, it was collected by interviews via phone calls to patients or attendants. Antifungal susceptibilities were evaluated for amphotericin B, flucytosine and fluconazole by broth microdilution (BMD).

**Results**
Cryptococcosis was seen in immunosuppressed states and in conjunction with chronic infections like hepatitis B (n=2), hepatitis C (n=2) and tuberculosis (n=3). Outcomes were not known for 67% (n=37) of patients. Incidence was higher in adult patients 82% (n=40), and 67% (n=37) cases occurred in males.

For all isolates, MIC50 and MIC90 were within the epidemiological cut off values (ECVs) for 5-flucytosine and fluconazole. One isolate had MIChigher than ECV for amphotericin B.

**Conclusion**
Invasive cryptococcal infections are seen in immunocompromised population as well as in association with certain risk factors such as chronic infections, diabetes and steroid use. Thorough evaluation of all patients must be done for risk factors to ensure better clinical outcomes. Since antifungal resistance is on the rise globally and our findings also show isolated amphotericin B resistance, it is important to perform susceptibility testing of all clinical strains to optimize therapy and for continuous surveillance.

**Key words**
Cryptococcosis, *Cryptococcus neoformans*, antifungal susceptibilities

*Corresponding Author: Faryal Saleem,*
*Department of Pathology and Laboratory Medicine, First Floor, Soparivala building, Aga Khan University Hospital, Karachi, Pakistan.*
*Email: faryal.sk@gmail.com*

## Introduction

Cryptococcosis is an opportunistic infection caused by *Cryptococcus neoformans*, an encapsulated yeast which has a predilection for central nervous system (CNS), but may also cause invasive infections at other sites including lower respiratory tract. It can also present as a disseminated infection particularly in immunocompromised population. More alarmingly, there is a surge of cryptococcal infections in immunocompetent population as well. In 1995, Mitchell *et al* reported 35 out of 118 (30%) cryptococcal infections in immunocompetent individuals in Australia, however, in 2005 Chen *et al* from China reported 91 out of 129 (71%) cases in immunocompetent population.[1,2] Similarly, another study from China by Weng *et al* conducted between 1997-2007 showed that majority of study population,103 out of 154 (70%) patients was apparently healthy.[3]

Invasive cryptococcal infections result in significant morbidity and mortality, with an incidence of nearly one million cases per year globally.[4] The increased incidence of these infections and an overall increase in immunosuppressed population, has led to increased use of antifungal agents for treatment as well as prophylaxis among susceptible populations like HIV/AIDS patients. Subsequently, there are reports of emerging antifungal resistance in *Cryptococcus neoformans*.[5, 6] So far, no study has been conducted regarding clinical characteristics of patients presenting with cryptococcosis in our country. Moreover, antifungal susceptibility profile of clinical isolates of *Cryptococcus neoformans* from Pakistan is also not known.

Hence, we conducted a study to describe the clinical characteristics of patients with proven invasive cryptococcosis and determine the susceptibility profile of *Cryptococcus neoformans* isolated at a major clinical microbiology laboratory of Pakistan from 2009-2016.

**Material & methods**

This retrospective analysis was conducted at the clinical microbiology laboratory of Aga Khan University Hospital, Karachi, Pakistan from 2009-2016. A total of 49 non-duplicate strains of *Cryptococcus neoformans* were isolated from various clinical specimens from all over the country. All isolates were identified by microscopy, colony morphology, and biochemical analysis. This identification was confirmed by commercially available identification panel API 20CAUX (bioMe´rieux, France). Details of clinical information were obtained from inpatients (n=22) through medical records and by telephone for outpatients (n=27) in the course of clinical reporting of cultures. Hence, the study was exempted from ethical approval by the Ethical Research Council of AKU (ERC No: 1373-Path-ERC- 09).

Broth microdilution plates were prepared according to the CLSI M27-A2 method[7]; using antifungal powders for amphotericin B, flucytosine and fluconazole (Sigma-Aldrich, St Louis, MO,USA).Antimicrobial susceptibility profile of these strains was evaluated for resistance against by broth microdilution (BMD) as per methodology recommended by the Clinical Laboratory Standards Institute (CLSI). Since CLSI interpretative break points for susceptibility are not available, the susceptibilities were interpreted using epidemiological cutoff values (ECVs) from previously published literature.[8,9] *Candida parapsilosis* ATCC22019 and *C. krusei* ATCC6258 were used as quality control strains with each batch of antifungal susceptibility testing.

**Data Analysis**

The data was coded and analyzed by using Microsoft® Excel 2010 software. Frequency and percentages of the categorical variables i.e. age, source of specimen and results of antimicrobial susceptibility ($MIC_{50}$ and $MIC_{90}$) for three antifungals, amphotericin B, flucytosine and fluconazole were determined.

**Results**

Among49 clinical isolates of *C. neoformans* 35 (71%) were isolated from cerebral spinal fluid, 10 (20%) from blood and 4 (8%) from miscellaneous samples like tracheal aspirate, bone marrow, urine and ascitic fluid. All isolates were from major urban centers like Karachi (37/49), Lahore (9/49), two from Hyderabad and one from Peshawar. Amongst the patients presenting with invasive cryptococcal disease, 33 (67%) were male. According to the age, patients were categorized into three groups: 2 (4%) cases belonged to pediatric age group (0-18 years), 40 (82%) were adults (19-60 years) and 7 (14%) were elderly (>60 years) patients. The clinical data was collected retrospectively, and details of clinical information could not be obtained for as many as 20 (41%) cases. Of the 29 cases in which history was obtained, invasive cryptococcal disease was seen in immunosuppressed patient population, in conjunction with various chronic infections (Table1). Amongst cases in which history could be obtained, 11 (38%) were HIV reactive, one patient (3%) was found to be HIV non-reactive, whereas HIV status was unknown in 17 (57%) cases. Details of other opportunistic infections could not be obtained in HIV patient population; however, two cases had concomitant pulmonary tuberculosis. Multiple comorbids were seen in HIV non-reactivepatient population as well as patients with HIV status unknown, who presented with invasive cryptococcal infection. In this population, cases of viral hepatitis (n=6), diabetes mellitus (n=6) and those with history of steroid use (n=5) were seen. Hepatitis C was the most common viral hepatitis (3 out of 6 cases). Four cases presented with history of malignancy; hematological malignancies being the most common, with one case each of Hodgkin's lymphoma, Non-Hodgkin's lymphoma and T-cell lymphoma, and one case of hepatocellular carcinoma was also seen. Three patients with history of solid organ transplant were also included; all were renal transplant recipients.

Eighteen out of 29 (62%) patients received an antifungal, however, outcomes were available for 15 cases only. Out of these, 9 patients expired eventually, 4 were treated successfully, and 2 patients were discharged after treatment but were lost to follow up. Outcomes were not known for 33 (67%) patients.

**Table 1: Summary of associated clinical conditions in 29 clinical cases diagnosed with culture proven disseminated cryptococcal infections.**

| HIV status | Comorbidity/ risk factors | Number of cases (%) |
|---|---|---|
| *HIV reactive | | |
| [n=11(38%)] | Without Tuberculosis/OI~ | 9 (31) |
| | With tuberculosis/OI | 2 (6.8) |
| HIV non-reactive | | |
| [n=1 (3%)] | Viral hepatitis, steroid use | 1 (3.4) |
| HIV status unknown | | |
| [n=17(58.6%)] | Steroid use | 5 (17.2) |
| | Viral hepatitis | 5 (17.2) |
| | Diabetes | 6 (20.6) |
| | Malignancy | 4 (13.7) |
| | Hypertension | 3 (10.3) |
| | IVDU# | 1 (3.4) |

*HIV: Human Immunodeficiency Virus
#IVDU: Intravenous drug use
~ Opportunistic Infections

For all 49 *Cryptococcus neoformans* isolates, $MIC_{50}$ and $MIC_{90}$ were found to be within the epidemiological cut off values (ECVs) for 5-flucytosine and fluconazole. However, one isolate had MIC (2 µg/mL) higher than the ECV for amphotericin B (Table 2).

**Table 2: Susceptibilities of *Cryptococcus neoformans* isolates (n=49) against amphotericin, fluconazole and flucytosine**

| Antifungal | Epidemiological cutoff values (ECVs) (µg/ml) | $MIC_{50}$ (µg/ml) | $MIC_{90}$ (µg/ml) | No. of agents susceptible isolates |
|---|---|---|---|---|
| **Amphotericin B** | 1 | 0.5 | 1 | 98 |
| **Fluconazole** | 16 | 8 | 16 | 100 |
| **Flucytosine** | 16 | 4 | 8 | 100 |

## Discussion

This study shows that clinical characteristics in Pakistani patients with invasive cryptococcal infection are consistent with literature published from other part of the world, not only among those suffering from HIV infection, but also with in those having a variety of other immunocompromised states and chronic infections. It is reassuring that almost all isolates are found susceptible to azoles, polyenes and flucytosine, except one which showed higher MIC to amphotericin B.

Invasive cryptococcal infections are seen in immunocompromised as well as immunocompetent population.[10, 11] Targeted therapy with appropriate antifungal agent is required for clearance of infection, failure of which causes considerable morbidity and mortality.[12] As a commonly known fact, mainly affected populations include people suffering from HIV/AIDS, with an estimated 1 million cases of cryptococcal infections per year globally.[4] Studies have shown a decline in incidence of cryptococcal infections in AIDS patients in the US in 1990s, mainly due to early diagnosis, prophylactic antifungal therapy and treatment initiation for HIV.[13] A review regarding serious fungal infections in Pakistan estimates the annual burden of cryptococcal meningitis in HIV infected patients to be about 794 cases.[14] Rates of cryptococcal meningitis have been reported as 2.5% and 9% in two studies in HIV infected population in Pakistan.[15, 16]

This study included local cases of cryptococcal infections, found more in adult patients, with 67.3% of cases occurring in males. The patient distribution is consistent with literature published previously.[17, 18] A study was conducted on 62 cases of cryptococcosis in Brazil in 2009-2010 and similar findings were seen with regards to demographic profile. Majority of the patients from the Brazil study were 20-40 years of age and a higher incidence was seen in male patients (74.2 %), but most patients diagnosed with cryptococcosis had HIV (85%). In this study, other underlying illnesses were candidiasis (30.6%), leprosy (8.1%), toxoplasmosis (12.9%) and tuberculosis (8.1%).[19] Another case series from Karnataka, India, which studied clinical profile of 12 patients with disseminated cryptococcosis, all cases were HIV positive and did not have any other risk factor.[20]

Our data shows that HIV and cancer are not the only underlying comorbids in patients with cryptococcosis in Pakistan. In this study, 4 patients had concomitant malignancies, mainly lymphomas. A study from the largest cancer referral center in Pakistan showed 2 out of 24 (8.3%) culture positive meningitis caused by *C. neoformans* in cancer population.[21] Besides immunosuppressive states, cryptococcosis was seen in association with infections such as hepatitis B, hepatitis C and tuberculosis, which is understandable as their burden is high in the community. In a study done in China by Zhong *et al*, a higher association of hepatitis B was seen in patients with cryptococcal meningitis.[22] Association of tuberculosis and cryptococcosis is seen in certain studies which can be attributed to defective T-cell immunity.[19, 23, 24]

In this study, all clinical isolates were found susceptible to fluconazole and flucytosine, however one isolate was found to have higher MICs against amphotericin B. Literature review from regional countries like India and China and countries like Cambodia and Brazil show that antifungal resistance against *Cryptococcus* species is on the rise.[25-28] Datta *et al* reported higher MICs for fluconazole and itraconazole in 16% and 7% of *C. neoformans* isolates from India.[25] In Brazil, a study by Figueiredo *et al* showed 20% resistance to itraconazole.[28] In general, antifungal resistance is seen mainly with azoles, however, there are reports of resistance against amphotericin B as well.[29, 30] Continuous surveillance of antifungal susceptibility patterns is needed to monitor the changes in sensitivity profile of *Cryptococcus* species.

This study is the first of its kind from Pakistan. Major strength of the study is that a considerable number of cases of invasive cryptococcal disease were included. The collection of isolates was from different parts of the country and susceptibility testing was performed on by CLSI recommended BMD method. One of the limitations of this study is that the strains of *Cryptococcus* isolates were not identified by molecular methods. Another limitation was lack of availability of clinical information for 41% of cases including HIV status which was known only in 12 patients.

Due to lack of access to healthcare facilities and availability of diagnostic modalities, the actual burden of cryptococcal infections in our population is likely to be underestimated. At times diagnosis is not considered as HIV status is not known or other risk factors are not taken into consideration. Further studies with larger number of cases and details of clinical presentations may be able to better highlight the risk factors involved in invasive cryptococcal infections.

## Conclusion

In summary, our finding suggests that in Pakistan, empirical

use of antifungal drugs can be used as per available guidelines in invasive cryptococcosis as clinical isolates are susceptible to azoles, polyenes and flucytosine. Since, isolated resistance to amphotericin B is seen in one of the study isolates, routine susceptibility testing for all invasive cryptococcal isolates is recommended. Routine surveillance of antifungal resistance is imperative to ensure optimum therapy and eventual clearance of infection as well as to monitor trends of antifungal resistance in invasive cryptococcal strains. To ensure early diagnosis and therapy, there is a need to keep a high index of suspicion and exploration of risk factors, not only in HIV/AIDS patients but also those with impaired T-cell immunity or apparent immunocompetent status.Studies with a larger sample size and details of clinical information will be helpful to further ascertain predisposing risk factors of invasive cryptococcal infections in our population.

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ORIGINAL ARTICLE

# Outcome of Intermediate Risk Pediatric Febrile Neutropenia – a Single Center Prospective Study from Pakistan

Muhammad Rahil Khan*, Muhammad Shamvil Ashraf*, Sundus Iftikhar**

*Department of Pediatric hematology/oncology, The Indus Hospital, Korangi Campus, Karachi, 75190, Pakistan
**Head of Department of Statistics and training unit, The Indus Hospital, Korangi Campus, Karachi, 75190, Pakistan

## Abstract

### Purpose
Febrile Neutropenia (FN)is an oncological emergency, which requires early recognition and prompt antibiotics administration for better outcome.We aimed to determine the outcome and association of demographic, clinical and laboratory data of intermediate risk pediatric febrile neutropenia treated with piperacillin / tazobactam and amikacin in a tertiary care hospital of Pakistan.

### Methods
All intermediate risk pediatric febrile neutropenia less than 16 years of ageadmitted in the pediatric oncology ward of The Indus Hospital from May to July 2016 were enrolled prospectively. Relapsed patients, low and high-risk FN were excluded. Outcome defined as success if discharged after completion of antibiotics or as failure if antibiotics changed, antifungal added or need of intensive care due to hemodynamic instability and death.

### Results
Total 141 episodes occurred in 136 children. Success rate was 83 %(n=117) and failure 17%(n=24). Major reason of failure was change in antibiotics (79.2%) due to persistent fever > 72 hrs.Three deaths occurred in the entire cohort due to probable fungal infection. On multivariable analysis, four independent risk factors were found to be significant for failure- ANC< 100 at presentation (aOR (95% CI):11.45 (1.70-77.19), p= 0.012), CRP=20mg/dl (aOR (95% CI):3.94 (1.03-15.12), p=0.045), respiratory rate (aOR (95% CI):1.48 (1.17-1.88), p=0.001) and heart rate (aOR (95% CI):0.96 (0.93-1.00),p=0.048).

### Conclusion
Our approach to treat intermediate risk febrile neutropenia was optimal. Strategy of low and high risk needs investigation. Based on our results, scoring model can be formulated for further refinement of our current management.

**Key Words**
febrile neutropenia, intermediate risk

*Corresponding Author: Muhammad Rahil Khan, Department of Pediatric hematology/oncology, The Indus Hospital, Korangi Campus, Karachi, 75190, Pakistan. Email: rahil.khan@tih.org.pk*

## Introduction
Febrile Neutropenia (FN) in children on chemotherapy is asignificant cause of mortality and morbidity. Itrequires prompt evaluation and early treatment withantibiotics.[1] This becomes more important in low income countries like Pakistan where infections such as diarrhea, pneumonia and malaria are high constituting major causes of mortality in children under five years.[2] Such FN patients presenting in the emergency department (ED) are routinely started on early empirical broad-spectrum antibiotics without waiting for laboratory results. This approach of early empirical therapy has resulted in decreasein infection related mortality in developed countries to 1-3%.[3]

To avoid antibiotic resistance, FN patients are stratified into low and high risk and treated accordingly with risk-adapted antibiotics. This risk stratification is based on the patient's primary diagnosis, treatment and episode related factors such as height of fever and blood counts.[4]

The choice of empirical antibiotics depends on regional and institutional local pathogens and antibiotic sensitivity and resistance pattern.[5] This ranges from multidrug treatment with an additional gram negative regimen to a more recent approach of monotherapy with antipseudomonal beta lactam or carbapenem in uncomplicated FN patients.[1, 4]

In Pakistan, there is scarcity of data on FN, its risk stratification and outcome in pediatric population. To our knowledge there are only two studies on pediatric FN in Pakistan[2, 6] but with limitations. We at The Indus Hospital (TIH), stratify our FN patients into three categories – high, intermediate and low risk based on multiple factors as demonstrated in table 1 and treat accordingly with different regimen. Such stratification has also been used by other groups.[7, 8] Based on antibiotic sensitivity pattern, our intermediate risk FNs (IRFN) are treated with a combination of piperacillin/tazobactam and amikacin. This study intended to determine the outcome of IRFN and explore factors associated with the outcome.

## Material and Methods
### Study design and setting
This was a prospective, observational and non-intervention studyconducted from May – July 2016 in pediatric oncology

unit of TIH after approval from Institutional Review Board (IRB). TIH treats more than 800 new cancer patients peryear with 60 febrile neutropenia admitted in pediatric oncology unit (POU) in a month. POU is 50 bedded including ward with isolation rooms, emergency department (ED), daycare, procedure room, pediatric intensive care unit (PICU) and high dependency unit.

The details of demography (age and gender of the patient, primary cancer diagnosis, chemotherapy phase and last date of chemotherapy administration, use of colony stimulating factor), clinical (presenting complains, time to reach ED after fever at home, height of temperature on presentation, examination details including vital signs, soft tissue infection or degree of mucositis if present, inpatient course, change of antibiotics, reason of change, last date of antibiotics) and laboratory investigations in ED and in ward (CBC including absolute neutrophil count (ANC), blood cultures, C-reactive protein (CRP), Chest X-Ray (CXR), Urine and Stool detailed report (D/R), cultures, repeat cultures, Computed Tomography (CT) scan of chest and ANC at discharge were documented in the predesigned case report form.

**Patients' Selection and FN Management**

All IRFN patients less than 16 years of age admitted from the ED or daycare unit in the ward and received piperacillin / tazobactam and amikacin were included. Informed consent was taken from guardian or patient, if eligible. Relapsed patients, all low and high risk febrile neutropenia**,** febrile patients admitted on antibiotics other than piperacillin / tazobactam and amikacin were not enrolled in the study.

On presentation, detailed history and complete examination was done and relevant hematological(CBC,CRP) radiological (CXR–if respiratory symptoms present)and microbiological investigations(blood cultures and if any focus- stool, urine or skin cultures) were carried out in ED. The first dose of piperacillin / tazobactam and amikacin were given within an hour of arrival to ED after taking cultures. During admission, same antibiotics were continued if remained stable. Amikacin was stopped after 72 hours if cultures were negative. Patients were discharged if remained afebrile for 36-48hrs with no complications or signs of clinicalillness,resolution of initial focus and rising ANC. In case of persistent fever > 72hrs, culture positivity or hemodynamic instability at any point time during admission, cultures were repeated and antibiotics were switched to carbapenem with/ without vancomycin and colomycin or other antibiotic according to sensitivity and shifted to PICU if indicated. If patients remained febrile > 96 hours, CT chest (HRCT) was done for invasive fungal infection (IFI)and empirical antifungal (amphotericin- B) was added.

**Definitions**

**Fever:**Êaxillary temperature >38.5$^0$C or two consecutive readings of >38.0$^0$C for 2 hÊ

**Neutropenia:** ANC <0.5 × 10$^9$/l, or expected to fall below 0.5 × 10$^9$/l.

**Success outcome**– IRFN completed five days course of piperacillin/ tazobactam, amikacin and discharged safely without complication.

**Failure outcome**- change in antibiotics due to either clinical deterioration, prolonged fever for more than 72 hrs or cultured organism resistant to piperacillin-tazobactem, need for antifungal treatment or fluid resuscitation requiring PICU admission and death.

**Risk Stratification** – defined in table.1

**Table 1- Risk Stratification Definition[7,8]**

| Low Risk | Intermediate Risk | High Risk |
|---|---|---|
| Patients of<br>• Acute lymphoblastic leukemia (ALL) on maintenance therapy,<br>• Solid tumor with Post chemo day > 10 ( except B-NHL ) with<br>o possible daily follow up,<br>o contactable on phone,<br>o leaving at one hour distance from hospital,<br>o no appearance of illness (hemodynamically stable),<br>o no obvious focus of infection like pneumonia, diarrhea sinusitis,, abscess and no other significant co-morbidity | Febrile Neutropenia with all ALLs (except low risk), acute myeloid leukemia, B-Non-Hodgkin Lymphoma (BNHL) and solid tumors with post chemo day < 10 and no high risk features | Febrile neutropenia with signs of hemodynamic instability which include<br>• difficulty in breathing,<br>• oxygen saturation < 90 %,<br>• tachycardia,<br>• hypotension,<br>• weak peripheral pulses,<br>• poor capillary refill,<br>• oliguria,<br>• altered consciousness,<br>• convulsions,<br>• fluid resuscitation<br>• need of inotrope |

**Statistical Analysis**

Data were entered and analyzed using SPSS version 21. Mean (SD) were computed as appropriate for all the quantitative variables-age, vital signs, laboratory investigations like Hemoglobin (Hb), White Cell Count (WCC). Frequency and percentage were computed for all the qualitative variables i.e., gender, primary diagnosis, chemotherapy phase. Independent sample t-test were applied as appropriate to assess significant difference in all the aforementioned quantitative variables between the outcomes. Chi-square test/Fisher exact test were applied as appropriate to assess significant association of all the aforementioned quantitative variables with outcome. Univariate and multivariable logistic regression was applied to assess risk factors associated with regimen failure. All the variables with P-value <0.25 were included in multivariable analysis. P-value <0.05 was considered significant.

**Results**

A total 141 admissions of IRFN occurred in 136 children during the study period. Table 2 enumerates the description of demographical, clinical and laboratory features of our cohort. Hundred and four episodes were leukemic patients. Granulocyte

**Table 2- Descriptive analysis of intermediate risk febrile neutropenia**

| Variables | Leukemia n=104 n (%) | Lymphoma and solid organ cancers n=37 n (%) | Overall n=141 n (%) |
|---|---|---|---|
| Age inyears (mean ± SD) | 7.8 ± 4.0 | 9.3 ± 3.9 | 8.2 ± 4.0 |
| Gender | | | |
| *Female* | 38 (36.5) | 19 (51.4) | 57 (40.4) |
| *Male* | 66 (63.5) | 18 (48.6) | 84 (59.6) |
| Phases of chemotherapy | | | |
| *Induction* | 39 (37.5) | 13 (35.1) | 52 (36.9) |
| *Consolidation* | 29 (27.9) | 21 (56.8) | 50 (35.5) |
| *Interim maintenance* | 13 (12.5) | 2 (5.4) | 15 (10.6) |
| *Delayed intensification* | 17 (16.3) | 1 (2.7) | 18 (12.8) |
| *Maintenance* | 6 (5.8) | - | 6 (4.3) |
| Chief compaints at presentation | | | |
| *Fever only* | 46 (44.2) | 13 (35.1) | 59 (41.8) |
| *Fever with other symptoms* | 58 (55.8) | 24 (64.9) | 82 (58.2) |
| Temperature at presentation | | | |
| *37 $^{o}$c* | 45 (43.3) | 14 (37.8) | 59 (41.8) |
| *>37 $^{o}$c and <=38 $^{o}$c* | 41 (39.4) | 18 (48.6) | 59 (41.8) |
| *>38 $^{o}$c* | 18 (17.3) | 5 (13.5) | 23 (16.3) |
| Duration of fever | | | |
| *<24 hours* | 100 (96.2) | 34 (91.9) | 134 (95) |
| *24-48 hours* | 2 (1.9) | 1 (2.7) | 3 (2.1) |
| *>48 hours* | 2 (1.9) | 2 (5.4) | 4 (2.8) |
| Patients with soft tissue infection | 6 (5.8) | 3 (8.1) | 9 (6.4) |
| Heart rate per min (mean ± SD) | 121 ± 21 | 125 ± 16 | 122 ± 20 |
| Respiratory rate per min (mean ± SD) | 25 ± 4 | 25 ± 4 | 25 ± 4 |
| Systolic blood pressure mmHg (mean ± SD) | 101 ± 11 | 100 ± 10 | 101 ± 11 |
| Diastolic blood pressure mmhg (mean ± SD) | 59 ± 11 | 60 ± 10 | 59 ± 11 |
| Absolute Neutrophil Counts (ANC) on admission | | | |
| <100 | 63 (60.6) | 24 (64.9) | 87 (61.7) |
| >=100 | 41 (39.4) | 13 (35.1) | 54 (38.3) |
| C-Reactive Protein mg/dl | | | |
| Below median (<20 mg/dl) | 54 (51.9) | 17 (45.9) | 71 (50.3) |
| Above median (>=20mg/dl) | 50 (48.1) | 20 (54.1) | 70 (49.6) |
| Hemoglobin g/dl (mean ± SD) | 8.2 ± 1.7 | 8.7 ± 1.3 | 8.3 ± 1.6 |
| White Blood Cells 10^9/L | | | |
| <1000 | 62 (59.6) | 24 (64.9) | 86 (61) |
| >=1000 | 42 (40.4) | 13 (35.1) | 55 (39) |
| Platelet 10^9/L (mean ± SD) | 115.5 ± 133.6 | 124.3 ± 99.6 | 117.8 ± 125.3 |
| Chest X-ray (n=94) | | | |
| *Normal* | 64 (61.5) | 21 (56.8) | 85 (90.4) |
| *Infiltrates* | 8 (7.7) | 1 (2.7) | 9 (9.6) |
| Culture Repeated | 19 (18.3) | 6 (16.2) | 25 (17.7) |
| *Type of culture (n=25)* | | | |
| *Blood* | 19 (100) | 5 (83.3) | 24 (96) |
| Reason for repeating culture (n=25) | | | |
| *Persistence of fever* | 16 (84.2) | 6 (100) | 22 (88) |
| *New symptom/ focus of infection* | 1 (5.3) | - | 1 (4) |
| *Other* | 2 (10.5) | - | 2 (8) |
| Absolute Neutrophil Counts at discharge | | | |
| *<100* | 27 (26) | 4 (10.8) | 31 (22) |
| *>=100* | 77 (74) | 33 (89.2) | 110 (78) |
| Outcome | | | |
| *Success* | 85 (81.7) | 32 (86.5) | 117 (83.0) |
| *Failure* | 19 (18.3) | 5 (13.5) | 24 (17.0) |
| Reason for failure (n=24) | | | |
| *Change in Antibiotics* | 15 (78.9) | 4 (80) | 19 (79.2) |
| *Need for fungal Treatment* | 1 (5.3) | - | 1 (4.2) |
| *Need for PICU* | 1 (5.3) | - | 1 (4.2) |
| *Combination* | 2 (10.5) | 1 (20) | 3 (12.5) |

colony stimulating factor (G-CSF) was used as part of the chemotherapy protocol in 17.7% episodes (n=25). Nearly 73 % events occurred in induction and consolidation. Blood cultures were positivein only 3.5% of patients. Overall 117 (83%) events were treated successfully with our regimen and 24 (17%) didn't respond. Major reason of failure was change in antibiotics (n=19, 79.2%) mainly due to prolonged fever of more than 72 hours (n=12, 63.2%) followed by bacterial growth (n=7, 36.8%). Three (2% of events) died due to probable invasive fungal infection (IFI) on CT chest. Four (2.8%) episodes needed PICU either due to hemodynamic instability or IFI requiring mechanical ventilation. Around61.7% had ANC <100/mm3 on admission and 78% had ANC >100/mm3 on discharge.

Table.3 demonstrates univariate and multivariable analysis of factors associated with failuresin leukemia patients. On univariate analysis, variables found to be significant for failure were consolidation phase (OR:7.56( 95% CI 1.56-36.7) p=0.012) , RR (OR:1.22 (95% CI 1.07-1.38)p= 0.002), soft tissue infection (OR:5.12 (95% CI 0.95-27.71) p=0.058), platelet count(OR:0.98 (95% CI 0.97-0.99) p=0.006), WCC< 1000(OR:3.032 (95% CI 0.93-9.9), p=0.066), CRP=20mgl/dl (OR:2.33(95% CI 0.93-

**Table. 3 Univariate and multivariable analysis of leukemia patients**

| Variables | Univariate Analysis | | Multivariable Analysis | |
|---|---|---|---|---|
| | **Odds Ratio (95% CI)** | **P-value** | **Adjusted Odds Ratio (95% CI)** | **P-value** |
| Age in years | 0.98 (0.87-1.11) | 0.793 | 1.10 (0.92-1.31) | 0.313 |
| Gender | | | | |
| *Male* | 0.98 (0.35-2.76) | 0.976 | 0.75 (0.21-2.72) | 0.658 |
| *Female* | Ref | | Ref | |
| Phases of Chemotherapy | | | | |
| *Induction* | 3.54 (0.63-19.8) | 0.15 | | |
| *Consolidation* | 7.56 (1.56-36.7) | 0.012 | | |
| *Interim Maintenance, Delayed intensification and maintenance* | Ref | | | |
| Chief Complaints | | | | |
| *Fever only* | Ref | | | |
| *Fever with other symptoms* | 1.93 (0.67-5.54) | 0.224 | | |
| Temperature at Presentation | | | | |
| *37 °c* | Ref | | | |
| *>37 °cand<=38 °c* | 0.49 (0.15-1.57) | 0.227 | | |
| *>38 °c* | 1 (0.27-3.72) | 1.00 | | |
| Heart rate per min | 0.997 (0.97-1.02) | 0.813 | 0.96 (0.93-1.00) | 0.048 |
| Respiratory rate per min | 1.22 (1.07-1.38) | 0.002 | 1.48 (1.17-1.88) | 0.001 |
| Systolic blood pressure mmhg | 1.01 (0.97-1.06) | 0.635 | | |
| Diastolic blood pressure mmhg | 0.99 (0.95-1.04) | 0.697 | | |
| Hemoglobin g/dl | 0.84 (0.62-1.12) | 0.24 | | |
| Platelet 10^9/L | 0.98 (0.97-0.99) | 0.006 | | |
| Soft tissue infection | | | | |
| *Yes* | 5.12 (0.95-27.71) | 0.058 | | |
| *No* | Ref | | | |
| White blood cell count 10^9/L | 0.54 (0.21-1.42) | 0.213 | | |
| *<1000* | 3.032 (0.93-9.9) | 0.066 | | |
| *>=1000* | Ref | | | |
| C-Reactive Protein mg/dl | | | | |
| *Below Median (<20 mg/dl)* | Ref | | Ref | |
| *Above Median (>=20mg/dl)* | 2.33 (0.93-5.87) | 0.072 | 3.94 (1.03-15.12) | 0.045 |
| Absolute Neutrophil Counts at Admission | | | | |
| *<100* | 7.21 (1.58-33.15) | 0.011 | 11.45 (1.70-77.19) | 0.012 |
| *>=100* | Ref | | Ref | |

5.87), p=0.072) and ANC<100(OR:7.21 (95% CI 1.58-33.15) p=0.011). When these factors were selected for multivariable analysis, ANC< 100 at presentation (aOR:11.45(95% CI 1.70-77.19) p= 0.012), CRP=20mg/dl (aOR:3.94 (95% CI 1.03-15.12) p=0.045), RR (aOR:1.48 (95% CI 1.17-1.88), p=0.001) and HR (aOR:0.96 (95% CI 0.93-1.00)p=0.048) were found to be the risk factors associated with failure.

**Discussion**

Pediatric oncology is one of the success stories in medicine. The survival in developed countries has reached approximately 80%.[9] Unfortunately in many low middle-income countries is still dismal between 5-10%.[10] Multiple factors are related to low survival. The lack of National standard of care policies and protocols is one major reason of poor outcome.[11]

FN is one of the leading cause of mortality in cancer children especially in Pakistan where FN burden is high.[6, 12] Regrettably, in Pakistan, national guidelines for febrile neutropenia doesn't exist. Institutional FN guidelines are being followed but results are not much published regarding the outcome of their strategies. In this study we validated our institutional approach of risk stratification. We have shown that our stratification of FN into low, intermediate and high risk has good results in the intermediate risk category. This success is comparable to other's FN strategy as documented by Timothy *et al* (91.5%).[13] The rate of change of antibiotics (17%) were much lower (61%) than seen by Chamberlain *et al*.[14]

Infection related mortality in our cohort were much lower (n=3, 2.1%) incontrast to 22-27% deaths seen in other regional studies from Pakistan and India.[2, 15] This might be due to inclusion of all low and high risk FNs in their study. The other factor could be poor supportive care and high percentage of bacteremia-25.8% by Mahmud *et al*[2] and 36% by Dubey *et al*.[15] Low bacteremia in our study (3.5%) seems to be true representative rather than being false negative results as patients with positive blood cultures had prolonged fever and needed a change in antibiotics. So it's less likely to be an issue of laboratory yield. Contrary to above published reports with high bacterial culture rate, studies with low rate (8.5- 16%) have documented low mortality in FNs.[13] This confirms that decreased rate of bacterial infection in this study is an important factor for our low mortality.

Immediate antibiotic administration, ideally within an hour, after arriving in emergency department (ED) is now considered an important factor for better outcome.[16] Although exact time was not calculated, but one cause to success could be early institution of antibiotics in our ED. We have a dedicated pediatric oncology ED so there are less chances for patients being missed or delayed in general pediatric ED. Our ED team arewell-trained to prescribe and administer first dose of antibiotics in all neutropenic fevers without waiting for investigation results.

The other reason for our better achievement could be early arrival of patients to hospital once their symptoms appeared at home. Majority (95%) of the patients reached within 24 hours of symptoms. This is in contrast to only 27% FN patients reaching hospital earlier than 24 hours seen by Alia *et al*.[6]

The ANC<100/mm$^3$ at the time of admission was the leading independent failure risk factor in our study. Patients with ANC<100/mm$^3$ had 11.45- fold failure risk on multivariable analysis. Similar relationship of ANC <100/mm$^3$ with FN complications was also identified by others.[17,18] The other important variable for predicting unfavorable outcome in this and various studies was CRP. CRP is an acute phase reactant and a marker for invasive bacterial infection.[3] This study showed that baseline CRP>20mg/dl had 3.45 fold higher chances of failure. Similar significance of CRP was also elaborated by others[17, 19, 20] but their CRP cut off levels were different.

Some factors found significant for high riskin other studies were statistically not substantial in our study. These include peak temperature of more than 38.5°C or 39°C at presentation, <7 days of chemotherapy, female gender and age less than 5 years.[8, 18, 20] Although hemoglobin and platelet level had significance on univariate analysis in our study as documented by Amman *et al* and Rondinelli *et al* but were not statistically significant in the multivariable analysis.[8, 19]

The strength of our study are its prospective nature and comparable sample size to other prospective FN studies done in children.[21, 22] Based on our results, we can propose a scoring model and further document the validation of risk factors identified. Multiple factors based scoring system had been tested earlier by different investigators.[8,18,19,23] This helps in identifying high-risk patients needing aggressive approach on presentation and on the other hand minimizing over treatment of low risk FNs.

Our study was a single center study, one of the major limitation. Absolute monocyte count< 100/mm3[8, 21] and undernutrition[17] have been associated with adverse outcomes in other studies. Our study failed to assess and document significance of these factors.

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ORIGINAL ARTICLE

# Spectrum of Uropathogens and their Antibiotic Susceptibility Pattern – Four Years Data from a Reference Laboratory of Karachi, Sindh.

Saima Naseem*, Samina Baig**, Fatima Fasih*, Shaheen Sharafat*

*Department of Pathology, Dow International Medical College, Dow University of Health Sciences
**Department of Microbiology, DDRRL, Dow University of Health Sciences, Karachi

## Abstract

**Background**
Urinary tract infections are amongst the most common bacterial infections. It is therefore important to investigate spectrum of uropathogens as well as their antibiotic susceptibility in order to implement appropriate use of antibiotics. The aim of the present study is to investigate common pathogens isolated from urine samples and to reveal antibiotic susceptibility patterns of these pathogens against routinely used antibiotics.

**Method**
This retrospective study was conducted at the Dow Diagnostic Research and Reference Laboratory during 2009 – 2012. All urine samples collected during the study period were investigated for culture growth and sensitivity patterns. Isolated uropathogens were then tested against antibiotics by Kirbu bur disc diffusion method.

**Results**
Out of 14,787 urine samples investigated, 4,479 (30%) showed significant bacterial growth. Among those which showed bacterial growth, 2647 (59%) were from females patients while 1832(40.9%) were from male patients. Overall, frequency of gram negative bacteria was higher 3919(87%) compared to gram positive bacteria 560 (12%). Of the gram negative organisms, 2268 (58%) samples were positive for *Escherichia coli* while in the gram positive; *Enterococci species* was most frequent 283 (50.5%). The antibiotic sensitivity profile of the isolated microorganisms showed that Imipenem, Piperacillin/Tazobactam, Amikacin and Fosfomycin were most effective antibiotics for gram negative bacteria and Vancomycin and Chloramphenicol were most effective against gram positive bacteria.

**Conclusion**
Our data demonstrated that prevalence of urinary tract infection is 30 %. *E.coli* is the most common gram negative bacteria and *Enterocccus* is the most common gram positive bacteria isolated from the investigated urine samples. Antibiotic susceptibility pattern showed that gram negative bacateria are highly sensitive to Imipenem, Piperacillin/ Tazobactam, Amikacin and Fosfomycin and gram positive bacteria are highly susceptible to Vancomycin and Chloramphenical. These antibiotics can be used for empirial treatment of urinary tract infection. It is necessary to have proper and effective use of antibiotics for treatment of UTI to avoid recurrence and emergence of resistant strains.

*Correspondence Author: Saima Naseem,*
*Assistant Professor, Department of Pathology, Dow International Medical College, Dow University of Health Sciences, Karachi, Pakistan.*
*Email: drsaima107@hotmail.com*

**Keywords**
Urinary tract infection, Gram positive bacteria, Gram negative bacteria, multidrug resistant.

## Introduction

Urinary tract infections (UTIs) are considered as one of the frequently reported infections in both outpatients & hospitalized patients.[1] UTIs are identified as persistent presence of actively dividing microorganisms in the urinary tract as well as microbial colonization of the urine.[2,3] Clinical manifestations of UTIs can vary from mild cystitis to pyelonephritis and even septicemia if not appropriately treated.[4] Each year approximately 150 million people suffer from UTIs globally, rendering the disease as one of the major reasons of economic burden, particularly in developing countries.[5] UTIs contribute to major utilization of antimicrobial drugs.[6] Patients suffering from UTIs are usually given antibiotics empirically before the outcome of urine culture and sensitivity report. Therefore there is high chance of development of antibiotic resistance in urinary pathogens due to indiscriminate use of antibiotics.[1] Various research studies have reported about changing pattern of uropathogens and their sensitivity to commonly available antibiotics in the last 20 year due to antibiotic resistance which has now become a major health problem all around the world and varies in different countries.[1] For the effective treatment of the UTIs, it is necessary to have recent data on spectrum of uropathogens causing UTIs and their antibiotic sensitivity pattern in a particular geographical areas.[6] It will help physicians to prescribe most appropriate empirical treatment in critically-ill patients. Taking into account these objectives a retrospective study was conducted to investigate the spectrum of uropathogens in urine samples and their antibiotic susceptibility pattern.

## Material & Methods

The study was performed at the Dow Diagnostic Research and

Reference Laboratory (DDRRL), Dow University of Health Sciences (DUHS) Karachi. All urine samples collected during the study period from 2009-2012 were investigated for culture and sensitivity tests followed by investigation of antibiotic resistance pattern. Urine samples were received from both outpatient and inpatient departments. Urine samples were collected in disposable, wide mouth sterile containers. Urine samples were processed on immediate basis after collection in bacteriology section. In case of any delay, the samples were refrigerated at 4-6°C. The samples were examined using direct microscopy for white blood cell count. The samples were inoculated on Cysteinelactose electrolyte deficient media by semi-quantitative plating method using a calibrated loop carrying 0.001ml of urine. The plates were incubated at 37°C for 24 hrs. Cultures without any colony after 24hrs incubation were further incubated for 48hrs before reporting no bacterial growth. Samples with colony count equal or more than $10^5$ Cfu/ml were considered positive. Identification and interpretation of cultures of bacterial isolates was done by using standard microbiological method.[1]

Susceptibility testing to antibiotics was performed by disc diffusion method on Mueller Hinton agar (MHA) as recommended by clinical laboratory standard institute (CLSI).[6] Required antibiotics were placed on the inoculated MHA plates according to whether the testing bacteria are gram positive or gram negative. The antibiotic discs used for susceptibility testing included Amoxicillin / Clavulanic acid, Ampicillin, Amikacin, Aztreonam, Imipenem, Piperacillin / Tazobactam, Gentamycin, Ceftriaxone, Cefuroxime, Ofloxacin, Ciprofloxacin, Cotrimaxazole, Cefixime, Ceftazidime, Nitrofurantoin, Pipemidic acid, Fosfomycin, Erythromycin, Tetracycline, Vancomycin, Chloramphenicol, Penicillin, Clindamycin, Fusidic acid & Cloxacillin. These agar plates were then incubated for 24 hrs at 37°C. Inhibition zones were measured in mm. Interpretation of inhibition zone was done according to CLSI guidelines. Statistical analysis of the results was done by SPSS version 16. Descriptive statistic was computed in percentages.

## Results

A total 14,787 urine samples were submitted for culture & sensitivity from 2009-2012. Of these, 4,479 urine samples were positive for bacterial growth. Among these, 1832 (4%) samples were of male patients and 2647 (59%) samples were of female patients. The patients were between the age ranges of 1-90 years with mean age 45 years. Frequency of infections was much higher in the patients between 21-30 years of age followed by 51-60 years of age as compared to other age groups (Figure1). The isolated microorganisms from urine samples included both Gram negative as well as Gram positive bacteria with the former being more frequent (87%) (Table1). Of the gram negative bacteria, most frequent isolate was *Escherichia coli (E.coli)* (57.8%) followed by *Klebsiella species (sp.)* (23%), *Enterobacter sp.* (5.6%), *Citrobacter frundii* (4.7%), *Pseudomonas sp*. (3.3%), *Pseudomonas aeruginosa* (3.0%), *Proteus mirabilis* (1.3%), *Proteus vulgaris* (0.4%), *Acinetobacter sp*. (0.4%) and *Citrobacter diversus* (0.2%). In gram positive group most frequent isolate was *Enterococcussp*.(50.5%) followed by

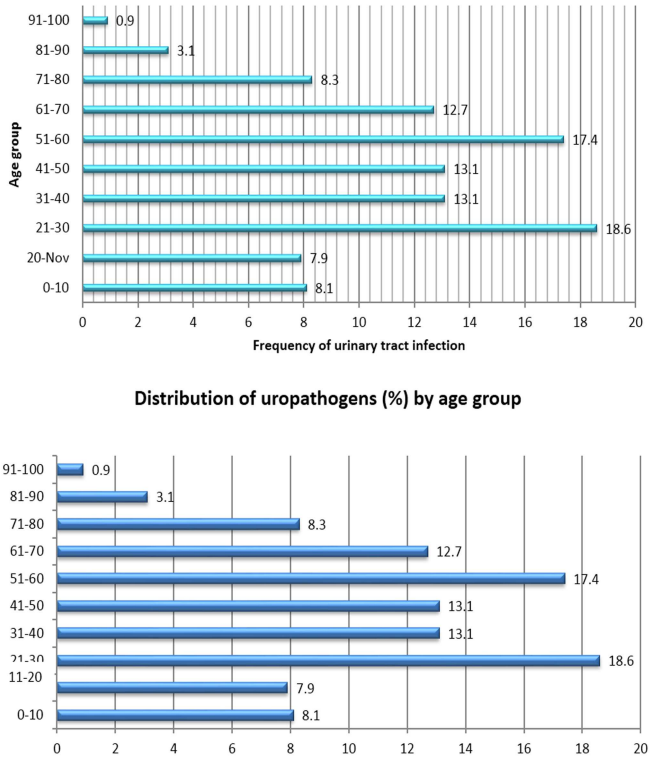


**Fig** 1. **Distribution of Urinary tract infections (%) by age group in urine samples tested during 2009-2012 at a reference laboratory at Karachi ( N=4479)**

**Table 1: Bacteria isolated from urine samples during 2009-2012 at a reference laboratory at Karachi ( N=4479)**

| Gram Negative Bacteria(n=3919) | | Gram Positive Bacteria(n=560) | |
|---|---|---|---|
| **Name of bacteria** | **No. of isolate (87%)** | **Name of bacteria** | **No. of isolate (12%)** |
| *Escherichia coli* | 2268 (57.8%) | *Enterococcus species* | 283 (50.5%) |
| *Klebsiella species* | 890 (22.7%) | | |
| *Enterobacter species* | 223 (5.6%) | | |
| *Citrobacter frundii* | 188 (4.7%) | *Staphylococcus aureus* | 177 (31.6%) |
| *Pseudomonas species* | 132 (3.3%) | *MSSA* | 112(63.3%) |
| *Pseudomonas aeruginosa* | 121 (3.0%) | *MRSA* | 65 (36.7%) |
| *Proteus mirabilis* | 54 (1.3%) | *Streptococcus species* | 100 (17.8%) |
| *Proteus vulgaris* | 17 (0.4%) | | |
| *Acinetobacter species* | 16 (0.4%) | | |
| *Citrobacter diversus* | 10 (0.2%) | | |

*Staphylococcus aureus* (32%) & *Streptococcus sp.* (18%). Imipenem and Tazobactam were the most susceptible antibiotics for *Enterobacteriaceae* (Table 2). Imipenem and Amikacin were found to be most sensitive antibiotics for non-fermenters (Table 3). Antimicrobial susceptibility pattern of gram positive bacteria, revealed Vancomycin and Chloramphenicol displaying maximum susceptibility (Table 4).

**Discussion**

Antimicrobial drug resistance is a major health problem faced by the patients due to irrational use of antimicrobial drugs. The epidemiology and susceptibility profile of uropathogens varies in different regions as well as within the same region over a period of time. Regular surveillance of antimicrobial resistance pattern is necessary both globally and at local level.[7] In our study frequency of urinary tract infections (UTIs) was found to be 30.2% which is in accordance with the results of study done by Chongtham U. *et al*[8] (30%) but in contrast to Kulsoom B. *et al*[2] (74.10%) & Prakash D. *et al* (53.8%)[9]. There could be several reasons of disparity found in the frequency of uropathogens isolated in different studies such as duration of study, urine culture methodology, varying population size, diverse population, variable level of personal hygiene and environmental factors.[10, 11] Our data demonstrated that UTIs were more common in women compared to men. These results are in line with the previous studies conducted by Shilpi T. *et*

**Table 3: Antimicrobial susceptibility pattern of non-fermenters isolated from urine samples during 2009-2012 at a reference laboratory in Karachi( N=4479)**

| Antibiotics | Acinetobacter species (n=16) | Pseudomonas species (n=132) | Pseudomonas aeruginosae (n=121) |
|---|---|---|---|
| Amoxicillin | **64.5*** | - | - |
| Ampicillin | 9.5 | - | - |
| Amikacin | **83.0*** | **71.2*** | **74.4*** |
| Aztreonam | 53.5 | 36.4 | 49.6 |
| Imipenem | **92.5*** | **78.8*** | **87.6*** |
| Pipericillin/ Tazobactem | 21.5 | **76.5*** | **81.0*** |
| Gentamycin | 21.5 | 49.2 | **62.0*** |
| Ofloxacin | 9.0 | 53.0 | 53.7 |
| Ciprofloxacin | 7.0 | - | - |
| Cotrimaxazole | 18.0 | - | - |
| Cefexime | 16.0 | - | - |
| Ceftazidime | 46.0 | 52.3 | 51.2 |
| Nitrofurantoin | 22.5 | - | - |
| Pipemidic acid | 27.5 | - | - |
| Ceftriaxone | 11.0 | - | - |
| Cefuroxime | 14.0 | - | - |
| Fosfomycin | 28.0 | - | - |

*Microorganisms showing significant sensitivity to antibiotics.

**Table 2: Antimicrobial susceptibility pattern of *Enterobacteriaceae* isolated from urine samples during 2009-2012 at a reference laboratory in Karachi (N=4479)**

| Antibiotics | Escherichia coli (n=2268) | Klebsiella species (n=890) | Enterobacter species (n=223) | Proteus Mirabilis (n=54) | Proteus vulgaris (n=17) | Citrobacter diversus (n=10) | Citrobacter frundi (n=188) |
|---|---|---|---|---|---|---|---|
| Amoxicillin | 43.6 | 38.4 | 45.7 | 53.7 | 11.8 | 40.0 | 29.3 |
| Ampicillin | 11.9 | 3.3 | 23.8 | 18.5 | 11.8 | 20.0 | 9.6 |
| Amikacin | **84.0*** | **74.6*** | **77.1*** | **68.5*** | 53.0 | **100*** | **72.3*** |
| Aztreonam | 42.6 | 39.1 | 37.7 | 50.0 | 53.0 | 40.0 | 40.4 |
| Imipenem | **98.3*** | **94.7*** | **93.3*** | **94.4*** | **70.6*** | **100*** | **88.3*** |
| Pipericillin/ Tazobactem | **79.0*** | **72.5*** | **80.7*** | **96.3*** | **70.6*** | **90.0*** | **72.3*** |
| Gentamycin | 54.7 | 55.7 | **59.9*** | 51.9 | 35.3 | **70.0*** | 53.2 |
| Ofloxacin | 32.9 | 54.4 | 48.9 | 44.4 | 47.1 | 50.0 | 44.7 |
| Ciprofloxacin | 32.8 | 52.4 | 30.9 | 27.8 | 41.2 | 50.0 | 37.2 |
| Cotrimaxazole | 24.6 | 35.4 | 33.6 | 16.7 | 11.8 | 30.0 | 34.0 |
| Cefexime | 32.9 | 34.0 | 33.6 | 31.5 | 17.6 | 20.0 | 23.4 |
| Ceftazidime | 39.5 | 45.4 | 53.8 | 50.0 | 29.4 | **60.0*** | 53.2 |
| Nitrofurantoin | **81.4*** | 37.4 | 55.2 | 16.7 | 11.8 | **80.0*** | 34.0 |
| Pipemidic acid | 19.6 | 37.8 | 18.8 | 24.1 | 17.6 | 30.0 | 23.4 |
| Ceftriaxone | 38.3 | 40.9 | 50.7 | 50.0 | 41.2 | **60.0*** | 50.0 |
| Cefuroxime | 33.6 | 36.6 | 39.9 | 42.6 | 11.8 | 40.0 | 34.6 |
| Fosfomycin | **63.5*** | **64.6*** | **67.3*** | 48.1 | 41.2 | **70.0*** | **59.0*** |

*Microorganisms showing significant sensitivity to antibiotics.

**Table 4: Antibiotic sensitivity pattern of gram positive bacteria isolated from urine samples during 2009-2012 at a reference laboratory in Karachi**

| | Gram Positive Bacteria | | |
|---|---|---|---|
| **Antibiotics (N=4479)** | **Enterococci species (n=283)** | **Stapylococci aureus (n=177)** | **Streptococci species (n=100)** |
| Erythromycin | 23.3 | 35.6 | 44.0 |
| Amoxicillin | **78.8*** | - | - |
| Ampicillin | **71.7*** | - | - |
| Amikacin | - | **84.7*** | - |
| Clindamycin | - | **61.0*** | 37.0 |
| Fusidic acid | - | 57.6 | - |
| Cloxacillin | - | 36.7 | - |
| Gentamycin | 37.5 | **62.7*** | - |
| Ofloxacin | 41.7 | 44.6 | - |
| Ciprofloxacin | 43.8 | - | - |
| Cotrimaxazole | 9.9 | 45.2 | - |
| Tetracyclin | 19.8 | 42.9 | - |
| Vancomycin | **84.8*** | **88.1*** | **87.0*** |
| Chloramph-enicol | **70.7*** | **66.1*** | **74.0*** |
| Penicillin | 58.0 | 23.7 | **66.0*** |
| Nitrofurantoin | **69.6*** | - | - |
| Pipemidic acid | 5.7 | - | - |
| Fosfomycin | **73.9*** | - | - |
| Ceftriaxone | - | - | **62.0*** |

*Microorganisms showing significant sensitivity to antibiotics.

*al*[12] & Somashekara SC *et al.*[13] The reason behind high prevalence of UTI in females is due to shorter urethra, sexual activity, urinary incontinence & close proximity of the urethral meatus to anus.9,14 In this study, gram negative bacilli constituted 87% of the total isolates while gram positive constituted 12%. *E.coli* (57.8%) was found to be most predominant isolated microorganism among the gram negative bacilli followed by *Klebsiella sp.* (22.7%) which has also been reported in the studies conducted by Paryani J. P. *et al*[15], & Chongtham U. *et al.*[8] & Lakshminarayana SA *et al.*[16] *Enterococcus sp.*(50.5%) was found to be the most common etiological agent amongst the gram positive bacteria. This is in contrast to study done by Manikandan S. *et al*[17] according to which *Staphylococcus aureus* was the most common isolated gram positive bacteria. In the current study UTIs were significantly higher in patients between 21-30 years of age which is in accordance with the study conducted by Kulsoom B. *et al*[2] and Bitew A. *et al.*[11] However the second commonly affected age group was found to be patients having age between 51- 60 years which has also been reported in a study by Nwadioha S. I. *et al.*[18] Literature search showed that elderly males have a higher incidence of UTIs compared to elderly females.9 According to the results of antibiotic susceptibility, the most sensitive antibiotic against *Enterobacteriacea* was Imipenem and Tazobactam followed by Amikacin and Fosfomycin. It is consistent with the results of the study by Yadav M. *et al*[19] and Niranjan V*et al.*20 Among the non-fermenters, Imipenem and Amikacin were found to be most sensitive antibiotics. These findings were also reported by Yadav M. *et al,*[19] Shilpi T. *et a,*[12] Prakash D. *et al*[9] and Paryani J.P. *et al.*[15] Among the gram positive bacteria, Vancomycin and Chloramphenicol were the most sensitive antibiotics. Same results are reported by Yadav M. *et al*[19] and Bitew A. *et al*[11] and Kashef N. *et al.*[12]

Low level of resistance was noticed against Aminoglycoside drugs in both gram negative and gram positive organisms and hence may be useful in empirical treatment of UTI. Patients have limited access to Aminoglycosides as it is available in injectable form and therefore displayed low resistance rates.[19] However high resistance was seen against third generation Cephalosporin, Ampicillin, Amoxicillin, Ciprofloxacin and Cotrimoxazole. This high proportion of resistance is due to easy availability, indiscriminate use of antibiotics in both community and hospital sectors. Moreover non-evidence based unchecked doctor's prescription practices and non-compliance of patients are also prime reasons of emergence of antibiotic resistance.[22] Limitations of study are that patient data (symptomatic vs asymptomatic or critically ill vs stable patients) is not available as the study is retrospective. Source of the patients is not known whether hospitalized or community based. Moreover source of the urine sample is also not present in the data if it is catherized or midstream urine. Further how many samples are from the same patients is not noted for the indication of colonization .

## Conclusion

The current study displayed that Imipenem, Piperacillin/Tazobactam and Amikacin were the most effective drugs against Gram-negative bacteria while Chloramphenicol and Vancomycin were the most sensitive drugs against Gram-positive bacteria.

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# Bacterial Isolate of Neonatal Sepsis and their Susceptibility Pattern in POF Hospital Wah Cantt.

Tahira Tehseen, Ayaz Hussain Qureshi, Lubna Ghazal

Wah Medical College, Wah Cantt. Pakistan

## Abstract

**Background**
Neonatal sepsis is the occurrence of microorganisms in a normally sterile site (blood) of the neonates which accompanied by signs and symptoms of infection and systemic inflammatory response in the first month of life. Over world while 1.6 million deaths were recorded due to neonatal sepsis, caused by bacterial infection. The objective of the study is to find the frequency of baccteremia causing neonatal sepsis and their susceptibility pattern at Neonatal Intensive Care Unit (NICU) of Pakistan Ordinance Factory (POF) Hospital Wah Cantt.

**Material & Methods**
**Study design:** Descriptive Cross sectional study.
**Study setting:** Microbiology section of POF Hospital laboratory Wah Cantt.
**Study sample:** A blood sample of (n=168) patients (one each), clinically diagnosed as neonatal sepsis (both early and late onset), admitted to NICU were taken. Inclusion Criteria: Before administration of antibiotics, in six months' period from December 2014 to June 2015, exclusion Criteria: Patients who were already on antibiotics.
**Data analysis:** All blood samples were analyzed for bacterial pathogens and their antibiotic susceptibility was assessed by standard microbiological methods. By using SPSS, frequency chart and tables were developed to display the results.

**Results**
Out of N=168 blood samples, fifty one (30.4%) isolates were gram- positive and one hundred and seventeen (69.6%) were gram-negative bacteria. Among gram- positive isolates, *Staph. aureus (31.4%) and* Methicillin resistant coagulase negative staphylococcus (MRCoNS) (31.4%) were most frequently found while *Klebsiella* species (65.8%) was most frequent isolate in gram-negative bacteria. Gram-negative pathogens exhibited sensitivity mostly to amikacin (76.9%)and gram-positive isolates were sensitive to vancomycin (95.2%).

**Conclusion**
The present study concludes that gram negative bacteria predominantly as the causative agent of neonatal sepsis in our setting. Isolated bacteria showed high resistance to commonly prescribed antibiotics. Establishment and implantation of infection control practices are required to overcome this grave situation.

**Keywords**
Neonatal sepsis, Microorganisms, Antibiotic sensitivity.

*Corresponding Author: Tahira Tehseen,*
*Wah Medical College, Wah Cantt. Pakistan*
*Email: drtahira_ tehseen@hotmail.com*

**Background**
Neonatal sepsis is the occurrence of microorganisms in a normally sterile site (blood) of the neonates and accompanied by signs and symptoms of fever, lethargy, poor cry, difficulty to arouse, refusal to suckle, abdominal distension and unstable body temperature associated with bacteremia or meningitis.[1] Neonatal septicemia is of two types, early onset sepsis (EOS) and late onset sepsis (LOS). During the first 5-7 days of life, the fulminant multisystem illness encountered is EOS whereas LOS is most commonly recognized after the first week of life.[2] Onset of infection within the first six days of life reflects vertical transmission from mother to infant, while at seventh day of life or later is likely to be acquired through horizontal transmission.[3] Over world while 1.6 million deaths were recorded due to neonatal sepsis, caused by bacterial infection.[4] This emergent medical condition required prompt diagnosis and relevant treatment, to prevent complications and death due to septicemia[5,6] The frequency of bacteria causing neonatal sepsis and their susceptibility pattern vary at different countries, even at different hospitals in same country.[7] The most common pathogens for neonatal sepsis in Europe and America were gram-negative organisms, in 1960. Which were replaced by group B *Streptococcus* in 1970`s, and coagulase negative staphylococci during late 1980`s and 1990`s. Gram negative organisms still remain the main cause of neonatal sepsis in most of the developing countries.[8]

In Asia, according to studies common organisms isolated from blood culture in neonatal sepsis were gram positive cocci including coagulase negative staphylococci (CoNS), *Staphylococcus aureus and enterococcus* while gram negative rods revealed, *E.coli*, *Pseudomonas* spp., *Enterobacteriaeceae* and *klebsiella* spp.[2,7]

Another study from Southern India, revealed that coagulase negative staphylococcus (CoNS) found to be the largest group 262 (37.6%) of isolates, followed by *Klebsiella* species 129(18.5%), *Pseudomonas* spp. *98*(14.1%), *Acinetobacter* spp.

49 (7%) and *Enterobacter* spp. 34(4.9%). Gram-negative organisms like *Enterobacter* spp., *Pseudomonas* spp. and *Klebsiella* spp., were found to be sensitive to amikacin and ciprofloxacin. In gram positive organism, coagulase negative staphylococcus (CoNS) was sensitive to ciprofloxacin.[9] In Pakistan isolated organisms in neonatal unit were, *E.coli* 22 (33.37%), *Klebsiella* spp. 22 (34.37%) and *Pseudomonas* spp. 8(12.5%), which were sensitive to ceftazidime, amikacin and imipenem.[10,11]

Neonates with sepsis are at high risk of losing life if there is delay in initiating treatment. The empirical antibiotic therapy should be started immediately to achieve the aim. So it is mandatory to identify the etiological agents and their antibiotic susceptibility pattern. Diversity of bacteria causing neonatal sepsis and their susceptibility pattern varies in time and region due to irrational use of antibiotics and change in life style.[5] The indecision regarding the choice of antibiotics can be reduced by regular survey of etiological agents and their antibiotic susceptibility patterns.

Awareness of predictors of positive blood culture and antimicrobial susceptibility pattern of common pathogens in a given area is crucial in guiding local empirical choice of antibiotics.[6]

The study was planned to identify the bacteria causing neonatal sepsis and their susceptibility pattern in POF Hospital Wah Cantt, so as to start specific, cost effective treatment at the earliest.

**Materials & Methods**

The descriptive Cross sectional study was designed at Microbiology section of POF Hospital laboratory Wah Cantt. A blood sample of (n=168) patients (one each), clinically diagnosed as neonatal sepsis (from birth till 28 days both early and late onset neonatal sepsis) admitted to NICU were taken.

Inclusion Criteria: Before administration of antibiotics, in six months' period from December 2014 to June 2015, without discrimination of gender & prematurity or birth weight.

Exclusion Criteria: Patients who were already on antibiotics. All blood samples were analyzed for bacterial pathogens and their antibiotic susceptibility was assessed by standard microbiological methods. By using SPSS, frequency chart and tables were developed to display the results.

Blood samples were collected in Brian heart infusion (BHI) broth for culture during period of study. The blood samples were incubated for 24 hrs at 37ºC under aerobic conditions. Subcultures were done by collecting the inoculums from BHI broth and inoculating on blood agar and MacConkey agar on three alternative days. The subcultures were incubated for 18-24 hrs at 37ºC under aerobic conditions. The agar plates were examined for growth of bacteria and their colonial morphology. The bacterial growth was subjected to gram stain and biochemical tests. Gram-negative bacilli were identified by using Oxoid Microbact 24E test strips. Gram positive cocci were identified by catalse and coagulase test. Antimicrobial susceptibility testing for gram-positive organism, was carried out on Muller Hinton agar using discs of penicillin (10 units), cefoxitin (30µg) (Oxoid, Basingstoke,UK), amoxicillin/clavulanic acid (20/10 µg), erythromycin (15 µg), linezolid (30 µg), ciprofloxacin (5 µg), clindamycin (2µg) and doxycycline (30 µg) and E strip of vancomycin (MIC). Gram-negative isolates were subjected to antimicrobial susceptibility test using discs of ampicillin (10µg), amoxicillin/clavulanic acid (20/10g), cefuroxime (30µg), ciprofloxacin (5µg), meropenem (10µg), cefepime (30µg), amikacin (30µg), gentamicin (10µg) and piperacillin/tazobactam (100/10µg), by Modified Kirby-Bauer disc diffusion method, according to CLSI recommendations.[12] ATCC 25923 *Staphylococcus aureus*, and ATCC 25922 *E.coli* were used as control strains.

**Results**

In a total of N= 168 positive blood culture isolates of neonatal sepsis, fifty one (30.4%) isolates were gram- positive and one hundred and seventeen (69.6%) were gram-negative bacteria. In the patients revealing isolation of gram-negative bacteria, the mean age was 4.8 + 5.4 days, and in gram-positive bacteria the mean age was 6.24 + 8.07 days. Out of 117 Gram-negative isolates, 63 (53.8%) were from male patients and 54 (46.2%) were from female patients. Gender distribution for 51 Gram-positive isolates were, as 36 (70.65%) and 15 (29.35%) for male and female patients respectively, (Table1).

Among gram- positive isolates, *Staph. aureus (31.4%)*, Methicillin resistant coagulase negative staphylococcus (MRCoNS) (31.4%) and methicillin resistant *Staphylococcus aureus* (MRSA) (23.5%), were frequently found (Figure1), while in gram-negative bacteria, *Klebsiella* species (65.8%) and *E.coli (24.8%)* were frequently isolated bacteria.

The major contribution of *Klebsiella* species found *Klebsiella oxytoca (34.2%) and Klebsiella pneumoniae (31.6%) (*Figure2*).*

Gram-negative organisms were found to be sensitive to amikacin (76.9%), piperacillin/tazobactam (75.2%), ciprofloxacin (63.2%) and cefepime (61.5%), and resistant to ampicillin (93.2%), gentamicin (60.7%), amoxicillin/clavulanate (75.2%), cefuroxime (74.4%) and meropenem (62.4%). (Table 2). Gram-positive isolates were sensitive to vancomycin (95.2%), linezolid (82.4%), amikacin (74.5%), and amoxicillin/clavulanate (60.8%), and resistant to penicillin (88.2%), doxycyclin (75.2%) and erythromycin (60.8 %). (Table 3). Twelve MRSA were isolated, among them 91% to vancomycin and linezolid, 50% to ciprofloxacin and amikacin, 41% were sensitive to clindamycin and erythromycin and 25% to doxycycline. (Table4)

**Table 1: Demographic characteristics of study groups**

| Characteristics | Gram-negative isolates | Gram-positive isolates |
|---|---|---|
| Number of patients | 117 | 51 |
| Age of the patients(in days) | 4.8 + 5.4 | 6.24+ 8.07 |
| Gender (M/F) | 53.8% /46.2% | 70.65%/29.35% |

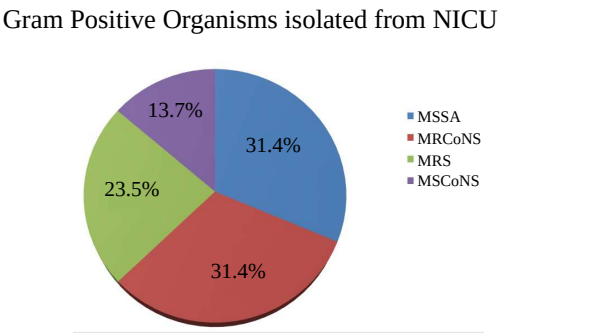


**Fig 1. Percentage of Gram-Positive isolates from NICU**

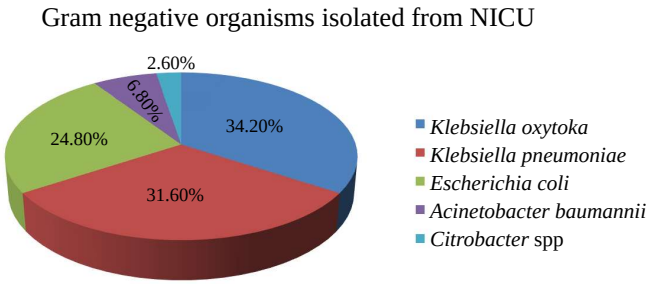


**Fig 2. Percentage of Gram-negative isolates from NICU**

**Table 2: Antimicrobial sensitivity and resistance pattern of Gram-negative isolates**

| Antimicrobials | Sensitive | Resistance |
|---|---|---|
| Amikacin | 76.9% | 23.1% |
| Piperacillin/tazobactam | 75.2% | 24.8% |
| Ciprofloxacin | 63.2% | 36.8% |
| Cefepime | 61.5% | 38.5% |
| Gentamicin | 39.3% | 60.7% |
| Meropenem | 37.6% | 62.4% |
| Cefuroxime | 25.6% | 74.4% |
| Amoxicillin/clavulanate | 24.8% | 75.2% |
| Ampicillin | 6.8% | 93.2% |

**Table 3: Antimicrobial sensitivity and resistance pattern of Gram-positive isolates**

| Antimicrobials | Sensitive | Resistance |
|---|---|---|
| vancomycin | 95.2% | 4.8% |
| Linezolid | 85.4% | 14.6% |
| Amikacin | 74.5% | 25.5% |
| Amoxicillin/clavulanate | 60.8% | 39.2% |
| Erythromycin | 39.2% | 60.8% |
| Doxycycline | 24.8% | 75.2% |
| Penicillin | 11.8% | 88.2% |

## Discussion

Timely diagnosis and therapy are important for the avoidance of morbidity and mortality due to neonatal sepsis in NICU.[13] The precise prediction of likely pathogens and antimicrobial resistance patterns is a fundamental requirement for successful therapy. The distribution of the causative pathogens for sepsis in our study showed that these infections were caused mainly by gram-negative bacteria, mostly *Klebsiella oxytoca, Klebsiella pneumoniae*, and *E.coli*. The gram-positive bacteria included methicillin sensitive *Staph. auerus* (MSSA), methicillin resistant *Staphylococcus auerus* (MRSA), methicillin resistant coagulase negative staphylococci (MRCoNS), and less commonly with methicillin sensitive coagulase negative staphylococci (MSCoNS) while a study in Africa and Asia have shown *Klebsiella, pseudomonas, Enterobacteriaceae and E.coli as predominant gram negative organism and Staph. aureus as* common gram positive organism. [4, 14]

Coagulase negative staphylococci (CoNS ) are the common colonizer of skin and mucous membranes of neonates, they are the common cause of blood culture contamination.[15] It is still hard to decide which blood isolates of CoNS represent true infection and which are contaminants.[16] Standard precautions were observed.[17] Historically the predominant organisms associated with neonatal sepsis have changed in years. In the past gram-positive bacteria dominated over gram-negative bacteria, but now the frequency of gram-negative organisms has increased in the recent years.[18]

Our study revealed the resistance pattern of gram negative isolates as ampicillin (93.2%), amoxicillin/clavulanic acid (75.2%) and cefuroxime (74.4%) and that of gram positive isolates as penicillin (88.2%), doxycyclin (75.2%) and erythromycin (60.8%). Effective antibiotics were amikacin (76.9%), Piperacillin/tazobactam 75.2%, ciprofloxacin (63.2%), and cefepime (61.5%) for gram negative bacteria, while vancomycin (95.2%), linezolid (82.4%), and amikacin (74.5%) for gram positive bacteria. MRSA showed highly sensitive pattern for vancomycin and linezolid i.e. eleven out of twelve were sensitive to vancomycin and linezolid. According to the

**Table 4: Sensitivity/resistance pattern of antimicrobial against MRSA (n=12)**

| | Clindamycin | Erythromycin | Doxycycline | Ciprofloxacin | Amikacin | vancomycin | Linezolid |
|---|---|---|---|---|---|---|---|
| **Sensitive** | 5 (41%) | 5 (41%) | 3 (25%) | 6 (50%) | 6 (50%) | 11(91%) | 11(91%) |
| Resistant | 7 (59%) | 7 (59%) | 9 (75%) | 6 (50%) | 6 (50%) | 1 (9%) | 1 (9%) |

study conducted in Tanzania, about 82% *Klebsiella pneumoniae* and 76% *E.coli were* resistant to gentamicin and ampicillin while methicillin resistant *Staphylococcus aureus* among gram-positive organisms was the second common isolate.[6] Another study from Uganda reported that most of the gram negative bacteria isolated in their study were resistant to ampicillin.[19] Where as a study in Pakistan conducted at NICU of Multan Hospital, gram negative isolates were sensitive to piperacillin/ tazobactam, cefoperazone/sulbactam and imepenem,[20] moreover imipenem was the most effective drug against gram-negative bacteria.[21]

Swift treatment with antibiotics is necessary for encouraging outcome of neonatal sepsis. In our study, *Staphylococcus* spp. and gram negative bacilli were frequently found to be resistant to ampicillin, thus indicating that the use of ampicillin to manage neonatal sepsis in our set up may be ineffective. Since *Staphylococci* are mostly resistant to penicillin therefore vancomycin will be a better choice for the coverage of the gram-positive pathogens. Gram-negative pathogens, *Klebsiella oxytoca*, *Klebsiella pneumoniae* and *E.coli* are resistant to commonly used antibiotics in our setup.

## Conclusions

The present study concludes that gram negative bacteria predominantly as the causative agent of neonatal sepsis in our setting. Isolated bacteria showed high resistance to commonly prescribed antibiotics. Study suggested that amikacin and piperacillin/tazobactam should be given as empiric regimen.

Establishment and implantation of infection control practices are required to overcome this grave situation.

## Recommendations

The knowledge of prevailing bacterial pathogens and their antibiotic sensitivity patterns in the region are essential to overcome the problem of neonatal sepsis. Continued surveillance is mandatory to assess the resistance pattern at a certain center and empirical antimicrobial therapy must be tailored according to the local as well as regional data.

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ORIGINAL ARTICLE

# *Mycobacterium Tuberculosis* Detection in Pulmonary Specimens from Prospective Immigrants; Laboratory Data from Pakistan

Yusra Shafquat*, Kausar Jabeen*, Muhammad Irfan**, Khalid Wahab*, Rumina Hasan*

*Department of Pathology and Laboratory Medicine, Aga Khan University
**Section of Pulmonary and Critical Care, Department of Medicine, Aga Khan University

## Abstract

**Introduction**
Pakistan is a high tuberculosis (TB) burden country. As such, pre-immigration TB screening for Pakistani travelers is required by several countries. Positivity rate amongst this group has not been reported previously.

**Objectives**
To assess the culture positivity rate of *Mycobacterium tuberculosis* (MTB) in pulmonary specimens received from prospective immigrants in Pakistan.

**Method**
Data for MTB culture requested during 2008-2016 from prospective immigrants were retrieved from laboratory database. TB culture request was based on physician's discretion. Identification and cultivation of MTB was performed at AKU laboratory using standard methods.

**Results**
A total of 172 cases, for which TB smear and culture was requested were included. MTB culture was positive in 13/172 (7.5%) cases of which 5 were smear positive and 8 smear negative. One case was smear positive and culture negative. Three additional cases were positive for non tuberculous mycobacteria (NTM).

**Conclusion**
Our data shows a high culture positivity rate in prospective immigrants screened for TB. Use of rapid diagnostic tests such as Xpert MTB/Rif in immigrants' pre and post migration from high TB incidence areas may lead to an early diagnosis and treatment.

**Introduction**
Tuberculosis (TB) remains one of the world's "deadliest" communicable diseases.[1] In 2015, globally, an estimated 10.4 million people developed TB, 60% of which belonged to India, Indonesia, China, Pakistan, Nigeria and South Africa.[1] Although TB exists mainly in high-burden developing countries (HBCs), an increase in cases in low incidence countries has also been observed.[2] Factors responsible for this increase are HIV co-infection, immigration from TB HBCs, multidrug resistant TB (MDR-TB) and overcrowding within poor communities of large cities.[3] Of these, immigrants from TB HBCs, especially in the first few years have a significant contribution.[4] In the United States and Canada, as well as in various European countries, over 50% of notified TB patients have been reported to be foreign-born,[5] with reactivation of latent TB.[6] Similar reports from European countries such as Denmark, Holland, Sweden, the United Kingdom and Switzerland suggest that foreign-born cases tend to have higher TB incidence; 100/ 100,000 as compared to native population; 15/100,000.[3] These higher rates of imported TB are making TB control more difficult in developed settings.[7]

The findings of a recent meta analysis by Aldridge RW *et al* regarding the pre-entry screening programmes showed that the prevalence ranged from 19.7 cases to 335.9 cases per 100000 cases.[8] However the prevalence was high in immigrants from HBCs. Another meta-analysis by Chanon HY *et al* on the high risk TB population after immigration showed that the cumulative incidence of TB was 2794 per 100000 persons.[9] The rate was higher in the population found to be at higher risk of TB at pre-entrant screening. These meta-analyses conclude that the prevalence and incidence of TB is high in both pre and post immigration population from HBCs and emphasizes on the importance of screening at both levels. In addition to the increasing incidence and prevalence of imported TB, the number of MDR and XDR cases is also increasing notably from Asia and Africa.[10] Immigrant screening for TB is a major component of immigration policies of many countries. The World Health Organization (WHO) recommends symptoms questionnaire or sputum smear for screening and chest X-ray for diagnosis in smear negative cases.[11] Centers of Disease Control and Prevention (CDC), USA recommends PPD skin test or Gamma interferon release assay (IGRA) followed by chest X-ray and TB culture and susceptibility testing.[12]

TB is a major health problem in Pakistan with an estimated

*Corresponding Author: Kausar Jabeen,*
*Associate Professor, Department of Pathology and Laboratory Medicine, Aga Khan University Hospital, Stadium road, Karachi, Pakistan*
*Email:kausar.jabeen@aku.edu*

incidence of 510,000 and a prevalence of 270 per 100,000 population with a 4.2% new of MDR-TB cases.[1,13,14,15] It is estimated that 51% of cases are concentrated in the province of Punjab, followed by 23% in the province of Sindh, 15% in the Khyber Pakhtoonkhwa, and 3.5% in Baluchistan, with the remainder being distributed within the tribal and northern areas and in Azad Kashmir. Therefore, every immigrant travelling from Pakistan has to go through multiple TB screening tests at the local immigration clinics. The proportion of immigrants in which TB is diagnosed during screening is not known.

In this study, we determined the frequency of isolation of *Mycobacterium tuberculosis* (MTB) in pulmonary specimens submitted to our laboratory from immigrants undergoing TB screening.

## Material & Methods

### Setting

Our study was conducted at a Clinical Microbiology Laboratory, Karachi, Pakistan, that receives approximately 20 specimens/year from immigrants for MTB culture. These requests are generated by the prescribing physicians working for or referred from immigration screening programs on the basis of positive TST or significant findings on chest radiographs.

### Study design

Cross-sectional study

### Specimen selection

All the pulmonary samples (sputum or bronchial washings) from immigrants undergoing TB screening identified from laboratory database were included. Data was retrieved from 2008-2016 and included age, gender, year of isolation, specimen type, positivity and susceptibility pattern against isoniazid, rifampin, streptomycin, ethambutol, ethionamide, ofloxacin, capreomycin, amikacin and kanamycin. Duplicate specimen from the same patient was excluded.

### Microbiological methods

**Isolation of Mycobacterium tuberculosis (MTB)**

Acid Fast Bacilli (AFB) smear: AFB smears were performed using Kinyoun's and Auramine O stains. The following quantitation was used: 1-19/40 fields AFB – Rare, 20-199/40 fields AFB seen – (1+), 5-50/field AFB – (2+), >50/field AFB (3+).

### AFB culture

TB culture was performed using Lowenstein-Jensen (LJ) (Remel™, Thermo fisher scientific™, Kansas, USA), Mycobacterium Growth Indicator Tube (MGIT), (BD, Thermofisher scientific, Dublin, Ireland) and Middlebrook 7H10 agar (BD, Thermofisher scientific) for all of the specimens. MTB was isolated from clinical specimens using standard microbiological procedures, and it was identified by typical colony morphology and PNB (para-nitrobenzoic acid) sensitivity. In addition to these standard identification procedures pigmentation and rate of growth were also observed.

### Antimicrobial susceptibility test

Agar proportion method was used to determine susceptibility against isoniazid (1 µg), rifampin (5 µg), streptomycin (1 µg), ethambutol (25 µg), ethionamide (25 µg) (BD, Thermofisher scientific™, Dublin, Ireland), ofloxacin (10 µg) (Oxoid™, USA), capreomycin (4 µg/ml), amikacin (5 µg/ml), kanamycin (4 µg/ml) (Sigma Aldrich®). Middlebrook 7H10 agar supplemented with 10% oleic acid albumin dextrose catalase (OADC) was used for susceptibility testing.[16] MTB H37Rv was used as a control.

### Ethical approval

This study has been exempted from ethical approval by the institutional ethical review committee (ERC number: 4067-Pat-ERC-16). Due to social and financial impact of a positive TB result on prospective immigrants' future, patients were not contacted and no clinical information was collected. This study is based on laboratory data only. The results were reported by the laboratory to the prescribing physicians.

## Results

A total of 172 immigrants were recruited during the study period. These included 87 male (50.8%). Mean age of study participants was 53 years (SD±20.5). Pulmonary specimens included were mostly sputum 168 (97.6%) and bronchoalveolar lavage 4 (2.3%). In all those patients, who had a positive TST or radiological findings consistent with TB and could not produce sputum, bronchalveolar lavage was done. Of these 172 immigrants, 13 were positive for MTB. These included 5 smear positive culture positive cases and 8 smear negative culture cases. Additionally, one sputum specimen was smear positive and culture negative however, the patient had a prior history of TB treatment. A total of 13 cultures were positive for *Mycobacterium tuberculosis* (Table 1). Three cases grew NTM. On susceptibility testing 10 (77%) cases were both isoniazid and rifampicin susceptible, 1 (7.6%) case was isoniazid monoresistant, 1 (7.6%) MDR and 1 (7.6%) XDR. Xpert MTB/Rif data was available for four culture positive cases and of these three were Xpert MTB/Rif positive and one was negative.

## Discussion

A rising trend of imported TB is being observed worldwide. In US alone it has been observed that the rate is 13.4 times higher in foreign born as compared to the general population.[17] TB culture plays an important part in immigrant screening policies, especially in high risk and vulnerable population.

Our study reports 7.5%culture positivity rate in pulmonary specimens from asymptomatic individuals who were screened for immigration purposes. As observed in the population based National TB prevalence survey conducted in Pakistan from

**Table 1: shows the frequency of smear and culture positivity of TB in study population and their susceptibility pattern**

| Cases | Smear results | Culture results | Susceptibility pattern |
|---|---|---|---|
| Case 1 | Positive | Positive | Both rifampicin and isoniazid susceptible |
| Case 2 | Positive | Positive | Both rifampicin and isoniazid susceptible |
| Case 3 | Positive | Positive | Both rifampicin and isoniazid susceptible |
| Case 4 | Positive | Positive | Both rifampicin and isoniazid susceptible |
| Case 5 | Positive | Positive | XDR TB † |
| Case 6 | Negative | Positive | Both rifampicin and isoniazid susceptible |
| Case 7 | Negative | Positive | Both rifampicin and isoniazid susceptible |
| Case 8 | Negative | Positive | Both rifampicin and isoniazid susceptible |
| Case 9 | Negative | Positive | Both rifampicin and isoniazid susceptible |
| Case 10 | Negative | Positive | Both rifampicin and isoniazid susceptible |
| Case 11 | Negative | Positive | Both rifampicin and isoniazid susceptible |
| Case 12 | Negative | Positive | Isoniazid monoresistant* |
| Case 13 | Negative | Positive | MDR TB** |

*Mono-resistance: resistance to any one first-line anti-TB drug
**Multidrug resistance: resistance to both isoniazid and rifampicin
†Extensive drug resistance: Multidrug resistance alongwith resistance to any fluoroquinolone and to at least one of three second-line injectable drugs (capreomycin, kanamycin and amikacin).[25]

2010 to 2011, total number of smear positive cases were 233/8521(2.7%) and 278/8521 (3.2%) culture positive cases. The survey also showed that 39% of the 207 definite AFB smear positive cases had no cough but abnormal radiological findings. The findings of the prevalence survey are consistent with findings of our study, both indicating high prevalence of TB in an otherwise asymptomatic population of Pakistan.[13]

In the health and demographic survey of Pakistan 2012-2013, 33 percent of males and 10 percent of females migrated to other countries, 29 percent of which was urban population. The most common reason for this immigration were mostly work and educational opportunities. In case of females, marriage was the commonest factor identified.[18] Our study lacks clinical and the demographic data of the immigrants included.

Over the past three decades, the TB notification data from industrialized countries has been variable but the outcome is same: decreased incidence in native population and an increasing incidence in foreign population. However the data from various studies suggest that the diagnosis of active TB was lower at the time of immigration.[19] It was also seen in Taiwan that the average annual TB notification rate in the foreign-born population was greater than that in the Taiwan-born population (94.0/100,000 vs. 72.0/100,000). 73% of these foreign born belonged to mainland China and Vietnam.[20]

In a study conducted in Turin, Italy from 1991 through 2010, a total of 27,358 socially marginalized immigrants attended the screening program out of which a total of 557 (2%) were definite cases of active TB. 75% of this population belonged to high TB burden countries. Similarly, prevalence of TB in 2.7% in immigrants was observed in a study conducted in an otherwise low TB incidence area in Western Europe.[4]

Culture still remains the gold standard for diagnosis of active tuberculosis. The main drawback of culture is that it takes 2-8 weeks that causes delay in diagnosis and immigration process. AFB smear is also rapid and inexpensive but it has low sensitivity of 64% and specificity of 98%.[21] Rapid molecular methods should be evaluated for inclusion in the screening policies.

Conventional PCR assay is known to have 77.3% and 99.9% sensitivity and specificity.[22] Comparatively, sensitivity of the Xpert MTB/Rif in smear and culture-positive pulmonary specimens was found to be 100% and in case of smear-negative pulmonary specimens, the sensitivity and specificity of the test is 74.2% and 98.3% respectively.[23] Xpert MTB/Rif will not only aid in rapid diagnosis but also in early start of therapy preventing transmission and emergence of MDR TB. As suggested by Samaraweera S *et al* addition of Xpert MTB/Rif in the algorithm of immigration screening policy may help in identifying cases early.[24]

Our study does not represent the cumulative TB positivity rate from all the immigrants from Pakistan. We have only included the samples of individuals referred by the physicians to our laboratory after initial screening. The proportion of cases that are referred to other laboratories is not known to us. The rate of positivity reported therefore might be an overestimate.

The high positivity rate highlights an urgent need to estimate the actual burden of active TB in this population. Further multicenter studies are required to determine the cumulative positivity rate in this population. This will help to make a policy to screen these individuals effectively.

In summary, our study finds that there is a high culture positivity rate (7.5%) in asymptomatic individuals who underwent screening for immigration purposes. This was a single center study and we suggest further multicenter population based studies to determine the actual culture positivity rate in this population. There is also a need to evaluate the role of rapid diagnostic methods such as Xpert MTB/Rif as they will lead to early diagnosis and management of these individuals.

**Declarations**

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## Disclaimer

Preliminary data of this study has been presented in:

i. 45th Union World Conference on Lung Health, Barcelona, Spain, 2014. The abstract has been published in the abstract book.
ii. 38th Annual PAP Conference/ 3rd Joint Conference of the Societies of Pathology in Collaboration with the Royal College of Pathologists & British Association of Pakistani Pathologists, Lahore, Pakistan, 2015

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## Instructions to Authors

**Scope**
The Infectious Diseases Society of Pakistan sponsors the Infectious Disease Journal of Pakistan (IDJ). The Journal accepts Original Articles, Review Articles, Brief Reports, Case Reports, Short Communications, Letter to the Editor and Notes and News in the fields of microbiology, infectious diseases, public health; with laboratory, clinical, or epidemiological aspects.

**Criteria for publication**
All articles are peer reviewed by the IDSP panel of reviewers. After that the article is submitted to the Editorial Board. Authors may submit names and contact information of 2 persons who potentially could serve as unbiased and expert reviewers for their manuscript, but IDSP reserves the right of final selection.

**Submission of the Manuscript**
Manuscripts must be formatted according to submission guidelines given below, which are in accordance with the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" (originally published in *N Engl J Med* 1997;336:309-15). The complete document appears at www.icmje.org. Please submit one complete copy of the manuscript and all enclosures to **The Managing Editors, Infectious Diseases Journal of Pakistan, Department of Pediatrics & Child Health, The Aga Khan University, Stadium Road, P.O. Box 3500, Karachi 74800, Pakistan.** An electronic copy of the manuscript must also be sent to pak_idj@yahoo.com**.** All manuscripts submitted to IDJP must be accompanied by an Authorship Declaration stating that *'The authors confirm that the manuscript, the title of which is given, is original and has not been submitted elsewhere. Each author acknowledges that he/she has contributed in a substantial way to the work described in the manuscript and its preparation'*. Upon submission a manuscript number will be assigned which should be used for all correspondence.

**Manuscript Categories**

**I. Original Articles**
Articles should report original work in the fields of microbiology, infectious disease or public health.The word limit for original articles is 2000.

**Title page**
This should list the (i) title of the article, (ii) the full names of each author with highest academic degree(s), institutional addresses and email addresses of all authors. (iii) The corresponding author should also be indicated with his/her name, address, telephone, fax number and e-mail address. (iv) A short running title of not more than 40 characters (count letters and spaces) placed at the foot end of the title page.(v) a conflict of interest statement should also be included in this section.

**Abstract**
Abstract should not exceed 250 words and must be structured in to separate sections headed *Background, Methods, Results and Conclusions*.

Please do not use abbreviations or cite references in the abstract. A short list of four to five key words should be provided to facilitate.

**Background**
The section must clearly state the background to the research and its aims. Controversies in the field should be mentioned. The key aspects of the literature should be reviewed focusing on why the study was necessary and what additional contribution will it make to the already existing knowledge in that field of study. The section should end with a very brief statement of the aims of the article.

**Materials and Methods**
Please provide details of subject selection (patients or experimental animals). Details must be sufficient to allow other workers to reproduce the results. The design of study and details of interventions used must be clearly described. Identify precisely all drugs and chemicals used, including generic name(s) and route(s) of administration All research carried out on humans must be in compliance with the *Helsinki Declaration*, and animal studies must follow internationally recognized guidelines. The authors are expected to include a statement to this effect in the Methods section of the manuscript. A description of the sample size calculation and statistical analysis used should be provided.

**Results**
Present results in logical sequences in the text, tables and illustrations. Articles can have a maximum of 5 illustrations (in a combination of figures and tables) per article. The results should be in past tense and repetition of results presented in the tables should be avoided. Exact *P*-values should be reported along with reporting of OR and RR with their Confidence Intervals where applicable.

**Discussion**
Emphasize the new and important aspects of the study and conclusions that follow from them. Do not repeat the details from the results section. Discuss the implications of the findings and the strengths and limitations of the study. Link the conclusions with the goals of the study but avoid unqualified statements and conclusion not completely supported by your data.

**Acknowledgments**
Acknowledge any sources of support, in the form of grants, equipment or technical assistance. The source of funding (if any) for the study should be stated in this section. Please see below for format of **References, Figures and Tables**.

**II. Review Articles**
Authoritative and state of the art review articles on topical issues are also published, with a word limit of 2000. It should consist of critical overview of existing literature along with reference to new developments in that field. These should be comprehensive and fully referenced. Articles should contain an Abstract; Main Text divided into sections, Conclusions and References.

**III. Brief Reports**
Short clinical and laboratory observations are included as Brief Reports. The text should contain no more than 1000 words, two illustrations or tables and up to 10 references.

**IV. Case Reports**
Instructive cases with a message are published as case reports. Routine syndromes or rare entities without unusual or new features are invariably rejected. The text should contain no more than 1000 words, two illustrations or tables and up to 10 references. The authorship should not exceed 3-4 persons.

**V. Letter to the Editor**
These may relate to material published in the IDJP, topic of interest pertaining to infectious diseases, and/or unusual clinical observations. A letter should not be more than 300 words, one figure and 3-5 references.

**VI. News and Views**
Informative, breaking news updates in infectious diseases from around the world (approx. 200 words).

**VII. Notices**
Announcements of conferences, symposia or meetings may be sent for publication at least 12 weeks in advance of the meeting date. Details of programs should not be included.

**References**
Number references consecutively in the order in which they are first mentioned in the text. Identify references in text, tables and legends by Arabic numerals (in superscript). References cited only in tables or in legends to figures should be numbered in accordance with a sequence established by the first identification of the particular table or illustration. Bibliography should be given in order. Authors, complete title, journal name (Abbr), year, vol, issue, page numbers. According to "Uniform Requirements of Manuscripts submitted to Biomedical Journals", as cited in N Engl J Med 1997; 336:309-15.

**Tables and Figures**
Data reported either in a table or in a figure should be illustrative of information reported in the text, but should not be redundant with the text. Each table must be presented on a separate sheet of paper and numbered in order of appearance in the text. Table should be numbered consecutively in Arabic numerals. Tables and Figures legends should be self-explanatory with adequate headings and footnotes.Results which can be described as short statements within the text should not be presented as figures or tables.

**Illustrations**
Illustrations should be numbered, given suitable legends and marked lightly on the back with the author's name and the top edge indicated. Original drawings may be submitted although high quality glossy photographs are preferable. They should be kept separate from the text. If possible, figures should be submitted in electronic format as either a TIFF (tagged image file format) or JPEG format. Minimum resolution for scanned artwork is:

- Black& white line illustration (e.g. graphs): 600 dpi
- Black & white halftone illustrations (e.g. photographs): 300 dpi
- Color illustrations: 400 dpi (note that color images should be split CMYK not RGB)

**Plagiarism**
Authors should refrain from plagiarism and should double check their work before submitting it for publication. Adequate references should be provided for text from other sources.

**Authorship criteria**
Those who have contributed sufficiently to the conceptualization, design, collection and analysis of data and writing of the manuscript should be granted authorship. Ideally all authors should be from the same department except for studies that are multi center or multispecialty.

**Instructions updated - April 2012.**
**Editor IDJ**

## MEMBERSHIP APPLICATION FORM

# MEDICAL MICROBIOLOGY & INFECTIOUS DISEASES SOCIETY OF PAKISTAN

No. _____

Name

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Department & Division

Field of Interest

Designation | PMDC No.

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Signature Date

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Comments: _____

Signature General Secretary: _____

**FULL MEMBERSHIP:**

Should be at least medical graduates registered with PMDC and having postgraduate qualification in any field.

**ASSOCIATE MEMBERSHIP:**

Ph. D, Master degree & M. Phill in biological sciences, BSc in Nursing & allied medical science.

**PRIVILEGES OF MEMBERSHIP:**

**FULL MEMBER:**

All the members shall have the right to:
1. Participate in all activities of the society.
2. Vote according to constitution of the society.

**ASSOCIATE MEMBERS:**

All the members shall have the right to:
1. Participate in programs of the society.

Please send your Application form by hand or by mail only.
Membership fee will only be received in cash/ on-line deposit / pay order or bank draft made out to Infectious Disease Society of Pakistan.

**Mailing Address and Contact Nos:**

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