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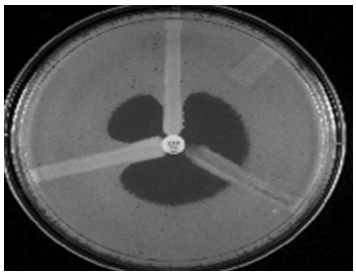

Modified Hodge test; ESBL detection.

Courtesy: AFIP, Rawalpindi.

Polio Eradication from Pakistan – Will dream come true ?

When WHO's global polio eradication initiative was started in 1988, it was expected that by year 2000, this infectious disease would be eradicated for the second time in planet. This dead line was extended several times and latest date of 31st December 2012 set by WHO was again missed. The Global polio eradication initiative in its year ending report writes "2012 ends with fewest wild polio cases ever" with only 218 cases reported worldwide an encouraging contrast to 650 cases in 2011.[1]

During the last twenty four years, Pakistan has remained one of the few countries attracting maximum focus of WHO in controlling this disease. Pakistan's polio eradication initiative was started in 1994 with implementation of National immunization days. From an estimated 2500–3000 cases per year, this number was reduced to 156 in only four years by 1998. With few peaks of cases observed in 1999 and 2003, there has been gradual and consistent downward trend until 2007 when the figure touched all time low of 31 cases.[2] All this was achieved through a coordinated and concerted effort in which communication interventions had played a consistently central role. It was quite a daunting and challenging task to target the poorest, most marginalized, hardest to reach and those without access to health services. The underserved population in Pakistan where immunity is too low to stop the circulation of wild poliovirus, has environment conducive to its spread. The involvement of local influentials, doctors, imams and teachers in remote areas remained a turning point in the initiative's communication strategy in Pakistan. Women folk comprising of lady teachers, young students, lady health workers and volunteers were the mainstay of polio eradication teams as only females are allowed to enter the premises of majority of our rural houses.[2,3]

The euphoria experienced in 2007, with only 32 cases reported, however was short lived. The overall declining security environment of the country were directly proportioned to the lukewarm and interrupted polio eradication efforts. Pakistan along with Afghanistan and Nigeria has remained among the top three countries with maximum number of wild polio cases in the years 2011 and 2012. There were 198 wild polio cases reported from Pakistan in 2011, the highest in the world for the year. In 2012, however, Pakistan witnessed 58 cases, second highest after Nigeria.[1]

As the year 2012 came close to the end and WHO was almost to laud global polio eradication initiative's efforts to combat the disease, Pakistan witnessed one of the worst carnage in its drive against polio. In mid December 2012, nine grass root level health workers were brutally assassinated while traveling from house to house to administer polio vaccine. As if this was not enough another six female health workers and male doctor were shot dead on new year's day near a community centre in Swabi district in the north west of Pakistan.[4,5] These brutal attacks took place primarily in two locations, Khyber Pakhtunkhwa province which in 2012 accounted for more than 40% of all Pakistan's polio cases, and Karachi the largest city of Pakistan with a population of over 20 millions. The sad incidences have been widely condemned by the UN Secretary General, Ban Kimoon saying this as "senseless and inexcusable". Due to safety concerns, the UN was forced to halt its participation in the vaccination campaign. Unfortunately, these tragedies have come at a time when the independent monitoring board of the global polio eradication initiative in its November 2012 report had appreciated Pakistan's efforts to curb polio.

As health care professionals, we are aware of the huge repercussions which are to follow in the aftermath of these unfortunate incidents. These incidents will surely pose challenges on many fronts, beyond public health. The international community which has already held Pakistan responsible for virus causing outbreak in Western China in 2011 would point fingers at Pakistan when the polio figures would be released in 2013. Heidi Larsan, an anthropologist currently leading a team studying issues around public trust in vaccines and the implications for immunization programmes and policies at the London School of Hygiene and Tropical Medicine, termed the killings of health care workers as "game changer" in the drive against polio.

The recent wave of killings has infact added fuel to the fire in our country's efforts to put vaccination drive on the track. It has added a formidable challenge to the multidimensional problems for vaccination including hard and inaccessible terrain in the parts with armed insurgency, hostile attitude of parents to refuse vaccination, the deteriorating hygiene and sanitation conditions and population movement across border with Afghanistan. The heads must now roll on, the health experts join hands and National Polio Control Programme must now gear up in devising workable strategies to refocus on the vaccination strategies. The 65th World Health Assembly in May 2012 passed a resolution labelling polio as an emergency. Now that WHO's global polio emergency action plan 2012–13 and the endgame strategic plan 2013–18 have been framed, it is high time authorities concerned in Pakistan also come up with emergency action and strategic plans to bolster the efforts of WHO.[6]

It is time to prioritize our actions. The health experts, bureaucracy, political forces alongwith government, NGO's & WHO need to work in collaboration. We must immediately address systemic polio eradication impediments, get vaccinaton back on track providing necessary security cover for around 100,000 vaccinators & use all possible communication means to reach out masses with positive information to diffuse deadlock and mistrust. Whole of the world would monitor Pakistan's efforts closely in the year 2013. As long as single child in Pakistan remains infected, children all over Pakistan are at risk and our desire of polio eradication would remain a dream.

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ORIGINAL ARTICLE

Clinical and Demographic Pattern of HIV Positive Patients in Two Tertiary Care Hospitals in Pakistan: 20 Years Experience

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Abstract

Objective
To describe the demographic pattern, modes of transmission, clinical presentations and management of adult HIV positive patients presenting at two HIV care centers in Pakistan over the course of 20 years.

Method
This was a retrospective study of all HIV positive patients presenting to the two HIV clinics. Medical records of all the HIV positive patients were reviewed, and information about age, gender, country of residence, mode of transmission, marital status, travel history, WHO clinical stage, opportunistic infections, treatment given and outcome were recorded on the data extraction sheet shared between the two centers.

Results
647 patients were seen between January 1991 and March 2011. The mean age of the patients was 35.6 years ± 12.8 SD; nearly two thirds were male. The commonest mode of transmission was heterosexual (67.1%). Over 50% presented in WHO class 3 and 4, while nearly a quarter were co-infected with tuberculosis. 348 patients were started on anti-retroviral therapy and 51.4% are currently receiving treatment at these centers. Mortality was highest among those who presented in pre morbid clinical states.

Conclusions
The demographic pattern of HIV in Pakistan is evolving. Heterosexual acquisition appears to be the predominant mode of transmission, followed by intravenous drug usage.

Keywords
Anti Retroviral Therapy, AIDS, HIV.

Introduction
The first case of HIV in Pakistan was reported in 1987 but over the years (1987-2010), the number of HIV positive cases has steadily increased.[1] According to UNICEF 2009 report, the estimated number of HIV positive patients in Pakistan is 98,000.[2] Although initially portrayed as an HIV free country, Pakistan is now identified as a country with concentrated epidemics in high risk groups.[3] In the early 1990s, majority of infected persons were deported workers from the Gulf States and their spouses but now the infection has been increasingly diagnosed among other demographic groups within the country.[4] The reported prevalence of HIV among intravenous drug users (IDU) in Pakistan was 9% in 2004, which rose to 15.8% in 2006-2007, and was estimated to have exceeded 20% by 2007-2008.[5] According to the latest report, the prevalence in 2010 was 36.7%, suggesting that HIV may spread in Pakistan along similar patterns as other South Asian countries; beginning from high risk population subgroups, crossing the barrier and spreading in the general population.[3,6] High risk sexual behaviors, poverty, economic instability, social discrimination, low HIV awareness among healthcare givers and the general population, illiteracy and extensive internal and external labor migration have all contributed to spread of the disease among various groups.[5,7,8] Pakistan is now following the "Asian Epidemic Model" which identifies clusters of HIV explained on the basis of intermix of commercial sex workers, injection drug use and poor infection control practices.[5,8]

The clinical presentation of HIV/AIDS is complex, progressing over years from no symptoms to disease characteristics of severe immunodeficiency. The initial clinical presentation may mimic symptoms of common endemic diseases in that particular region.[9] Although few studies have previously been conducted in Pakistan on the clinical presentation of HIV and AIDS, the number of the participants in these studies was small and the results could not be extrapolated to the larger population.[10,11] Demographic data relating to individual groups such as intravenous drug abusers or sex workers are known, but overall comprehensive HIV data among the general population is not known.[12] Generally, many clinicians in Pakistan are unaware of the clinical spectrum of HIV /AIDS. In a study conducted in Lahore regarding knowledge, attitudes and practices of health care providers for managing sexually transmitted infections including HIV, only 45% of the doctors had correct knowledge about transmission and prevention of HIV, and only 21% had seen a patient with advanced HIV infection.[13]

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With the global introduction of antiretroviral treatment (ART) in the mid 1990's and prophylaxis for opportunistic infections, the course of HIV / AIDS has changed radically, and it has become a chronic disease now rather than a fatal disease. In May 2006, Pakistan's National AIDS Control Program (NACP) approved ten public and three private clinics within the country for HIV patient management. Initially NACP provided first line ART but since 2008, second line ART through the Global Fund are provided free of cost to all registered AIDS patients at these centers.

The aim of this study was to describe the clinical and demographic profile of HIV positive patients seen over a period of 20 years. To our knowledge this is the largest number of case series of HIV positive patients reported from Pakistan. This study should serve to educate healthcare providers about risk factors and clinical features of HIV /AIDS in Pakistan so that timely screening, recognition and proper management can be undertaken. It will also raise positive awareness about treatment availability and encouraging outcomes.

Materials & Methods

This was a retrospective study conducted at the 2 of the three large NACP registered private centers for HIV treatment in Pakistan: The Indus Hospital (TIH), Karachi, and Shaukat Khanum Memorial Cancer Hospital and Research Centre (SKMCHRC), Lahore. Ethical approval was obtained from the ethics review boards in each participating institution.

Medical records of all adult HIV positive patients who presented from January 1991 to March 2011 were reviewed by the principal investigators and co-investigators and information was recorded on a data extraction sheet which was shared by the two centers so that the information extracted was uniform and consistent. Records for 196 cases registered prior to November 2007 were transferred to TIH from another private hospital where the principal investigator used to be a consultant.

Information about patients' age, gender, residence, mode of transmission, marital status, travel history, WHO clinical stage, opportunistic infections, treatment given and outcome was collected and recorded on the data extraction sheet.

Testing method

Prior to 2006, HIV testing was done using a single ELISA based test followed by a confirmatory western blot. CD4 and viral loads were not done routinely because of its cost. HIV serology, CD4 and viral load testing became free with the establishment of NACP in 2006. Cases are now confirmed using one rapid test and two ELISA based tests as recommended by WHO. HIV RNA is assayed with Amplicor HIV-1 Monitor Test (Roche Diagnostics) that detects above 500cps/ml and CD4 testing is performed by flow cytometry.

Tuberculosis (TB), a co-morbidity, was diagnosed on the basis of a positive acid fast bacilli smear on Ziehl-Neelsen staining or isolation of mycobacteria in tissue or sputum culture, or clinical, radiological or histopathological features consistent with TB. Symptom resolution with antituberculous therapy was also considered to be a case of TB.

Statistical analysis

Demographic and clinical variables were analyzed using SPSS software package (version 16.0; Chicago, IL, USA). Differences between groups for categorical variables were analyzed using Chi-Square, or Fisher's exact test, as appropriate; Student's t-test were used to compare normally distributed continuous variables and Wilcoxon rank sum tests for not normally distributed variables. A p-value of <0.05 was considered statistically significant.

Results

From January 1991 to March 2011, a total of 647 patients were recorded. The mean age of the patients was 35.6 yrs± 12.8 SD. Almost two-thirds of the patients were male (62.1%). Other baseline characteristics and demographics such as marital status, occupation, diagnosis year, risk factors for HIV exposure are shown in tables 1 and 2. Prior to 2000, majority of the patients gave history of travel outside Pakistan where they presumably acquired the infection (Fig 1).

The commonest mode of transmission was heterosexual contact (67.1%), while 9% patients gave history of intravenous drug

Table 1: Baseline characteristics at registration of HIV infected patients at 2 tertiary hospitals (n = 647)

	No. Patients (%)		
Baseline Characteristics	**TIH n=308**	**SKMCH&RC n=339**	**Total n=647**
Gender			
Male	227 (73.7%)	175 (51.6%)	402 (62.1%)
Female	78 (25.3%)	164 (48.4%)	242 (37.4%)
Transgender	3 (1.0%)	--	3 (0.5%)
Marital status			
Married	216 (70.1%)	218 (64.3%)	434 (67.1%)
Single	63 (20.5%)	43 (12.7%)	106 (16.4%)
Divorced	12 (3.9%)	42 (12.4%)	54 (8.3%)
Minor	8 (2.6%)	35 (10.3%)	43 (6.6%)
Unknown	9 (2.9%)	1 (0.3%)	10 (1.5%)
Occupation			
Professional	28 (9.1%)	31 (9.1%)	59 (9.1%)
Business	50 (16.2%)	11 (3.2%)	61 (9.4%)
Unskilled/other	122 (40.3%)	150(44.2%)	112 (17.3%)
Housewives	64 (20.8%)	103 (30.4%)	167 (25.8%)
Minor	10 (3.2%)	21 (6.2%)	31 (4.8%)
Student	7 (2.3%)	9 (2.7%)	16 (2.5%)
Unemployed	15 (4.9%)	10 (2.9%)	25 (3.9%)
Unknown	10 (3.2%)	4 (1.2%)	14 (2.2%)

abuse. In a third of the patients, the mode of transmission could not be determined because either they did not disclose exposure to any known risk factor, or the history could not be ascertained because of patient's moribund state at presentation.

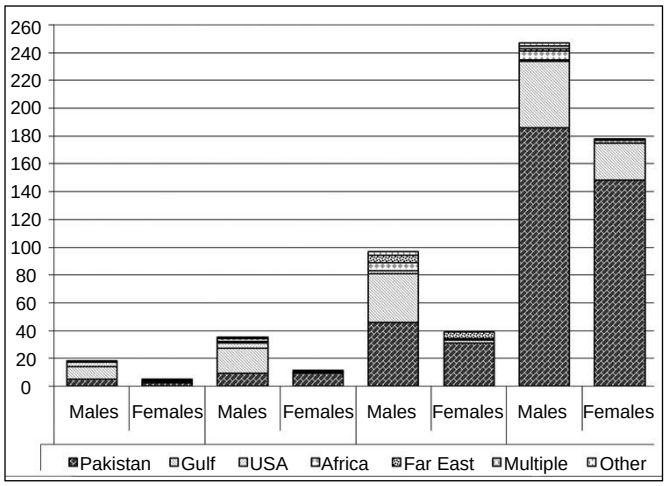

Fig 1. Travel history of patients

The most common manifestations of HIV/AIDS in our study were weight loss (67%), fever (59%) and diarrhea (57%) followed by oropharyngeal candidiasis (32%). Nearly one-fourth of the patients were co-infected with TB (Table 3).

The median CD4 before initiation of antiretroviral therapy was 149 cells/uL. 348 patients were started on ART and 67% of the patients self reported good adherence to the treatment and kept scheduled appointments. Mortality was highest in patients whose initial visit was in WHO category IV. Clinical outcome with and without ART as well as management of HIV is shown in table 4.

Discussion

Our study is one of the largest profiles of HIV adult patients and their presentation in the past 20 years. As observed globally, in our study also, men outnumbered women. Similar gender profile has been observed in another Pakistani study, except for a small cluster of patients from a small town near Lahore,

Table 2: Demographics

Characteristics	No. Patients (%) Male n=402	Female n=242	Transgender n=3	Total n=647
Median age (min, max), y	38 (1, 70)	34 (2, 65)	37 (30, 42)	
Year of Diagnosis				
1991-1995	18 (4.5%)	5 (2.1%)	0 (.0%)	23 (3.6%)
1996-2000	35 (8.7%)	11 (4.5%)	0 (.0%)	46 (7.1%)
2001-2005	101 (25.1%)	45(18.6%)	1 (33.3%)	147(22.7%)
2006-Mar 2011	248 (61.7%)	181(74.8%)	2 (66.7%)	431(66.6%)
Referred by				
Self	139 (34.6%)	71 (29.3%)	--	210 (32.5%)
Deported	34 (8.5%)	9 (3.7%)	--	43 (6.6%)
Other physician	69 (17.2%)	26 (10.7%)	3 (100.0%)	98 (15.1%)
Relative	11 (2.7%)	9 (3.7%)	--	20 (3.1%)
NGO	149 (37.1%)	127 (52.5%)	--	276 (42.7%)
Risk factors for HIV exposure				
Heterosexual contact	169 (42.0%)	105 (43.4%)	--	274(42.3%)
Homosexual/bisexual	47 (11.7%)	0 (.0%)	3 (100.0%)	50 (7.7%)
Injecting drug use	45 (11.2%)	13 (5.4%)	--	58 (9.0%)
Maternal fetal	21 (5.2%)	11 (4.5%)	--	32 (4.9%)
Blood products	16 (4.0%)	11 (4.5%)	--	27 (4.2%)
Unknown	104 (25.9%)	102 (42.1%)	--	206(31.8%)
Spouse HIV statuse*	n=277	n=211	--	n=488
Positive	105 (36.1%)	101 (51.5%)	--	206(42.3%)
Negative	64 (22.0%)	23 (11.7%)	--	87 (17.9%)
Uknown	122 (41.9%)	72 (36.7%)	--	194(39.8%)

∗ only married or divorced patients

where more women were affected, probably due to the practice of reusing syringes in informal health care settings.[5,10]

The majority of men in our study gave a history of sex with commercial sex workers. A recent study of HIV/AIDS surveillance project conducted across 12 major cities in Pakistan showed prevalence of HIV among female sex-workers was 0.8%, for male sex workers 3.6 %, and transgender sex workers 7.3%.[3] It was found that majority of injection drug users (IDU) and men who have sex with men in Pakistan are married and therefore act as a bridge for spread to their wives.[3,14,15] The women gave history of either an HIV positive spouse, or described clinical features suggestive of advanced AIDS in a spouse who had died. It was not possible to obtain any history of extramarital sexual relationships from women because of social and cultural taboos. An emerging risk factor for acquiring HIV in the country is among injection drug use. A national survey on drug addiction and AIDS shows progressive increase of HIV among IDUs from 0.4 in 2003 to almost 37% in 2010.[3]

Another important finding was that prior to 2000, majority of our male patients gave history of travel and a stay longer than one month abroad, particularly in the Gulf States, where many admitted to having had sex with female sex workers. After 2001, we observed a changing trend of more patients with no history of travel abroad being diagnosed with HIV acquired indigenously. This finding is consistent with several other studies from Pakistan. Shah *et al* in their study reported that 73% of HIV positive patients who presented at the AIDS control program between 1996 to 1998 gave history of working in Gulf countries.[4] Other researchers have also seen similar trends. Rai *et al* in their study had tracked the origin of HIV endemicity among IV drug abusers in Karachi to overseas workers who had lived in Middle East for extended periods and admitted to having contacts with commercial sex workers.[16] Many of our male patients also admitted to possible acquisition of infection in South East Asia and South Africa.

The most common clinical manifestations such as significant weight loss and diarrhea were similar to other reports. In a study conducted in a tertiary care hospital in Karachi, the three most common clinical manifestations were fever, diarrhea and significant weight loss.[10] Similar findings were reported in studies from Bangladesh, Nigeria and other developing countries.[17-19]

Though TB was the single most common co-infection in our

Table 3: Clinical profile of HIV infected patients prior to ART therapy

Characteristics n=308	Indus n=339	SKMCH&RC n=647	Total
WHO Clinical Staging of HIV Disease at Presentation			
Stage	I92 (29.9%)	60 (19.5%)	152 (24.7%)
Stage II	40 (13.0%)	81 (26.3%)	121 (19.6%)
Stage III	65 (21.1%)	140 (45.5%)	205 (33.3%)
Stage IV	111 (36.0%)	27 (8.8%)	138 (22.4%)
Medical Conditions			
AIDS-associated conditions			
Weight loss	186 (86.5%)	158 (53.2%)	344 (67.2%)
Candidiasis	87 (40.5%)	76 (26.9%)	163 (32.7%)
Chronic diarrhea	162 (75.3%)	116 (41.0%)	278 (55.8%)
Fever	92 (43.0%)	200 (70.7%)	292 (58.8%)
Pneumocystis pneumonia	54 (25.1%)	27 (9.5%)	81 (16.3%)
Cryptococcal meningitis	9 (4.2%)	4 (1.3%)	13 (2.5%)
Cytomegalovirus	7 (3.3%)	4 (2.1%)	13 (2.6%)
Toxoplasmosis	8 (3.7%)	3 (1.0%)	11 (2.1%)
Herpes zoster	11 (5.1%)	37 (13.1%)	48 (9.7%)
Other medical conditions			
Anemia	28 (13.0%)	110 (37.0%)	138 (27.0%)
Tuberculosis			
Pulmonary	48 (15.6%)	50 (14.8%)	98 (15.2%)
Extra pulmonary	35 (11.4%)	24 (7.1%)	59 (9.1%)

study, it was considerably less than some other Asian studies where the TB rates in the adult population were much higher. A study from Thailand reported 45.5% prevalence of TB among HIV positive patients, whereas in another study from India, TB was seen in 68% of HIV positive patients.[13,20-22] The difference in rates of TB and HIV co infection could be because HIV is an established epidemic in these countries while in Pakistan it is seen in high risk groups only.

Some of the opportunistic infections in our case series were diagnosed on the basis of clinical presentation and response to therapy and not confirmed microbiologically due to lack of specific tests available in the country, so it was possible that some opportunistic diseases were either under diagnosed or over diagnosed. The reported prevalence of *Pneumocystis jiroveci* from India is very low (0.7 to 7%) but it was seen at a higher rate in our patients cohort.[21] It is possible, though, that pneumocystis pneumonia may mimic other bacterial pneumonias and the treatment success seen with cotrimoxazole therapy may have been because of its efficacy against other bacterial agents as well.

We did not diagnose any cases of disseminated fungal infection, and identified only two cases of HIV associated malignancy.

Table 4: Management of HIV infected patient with ART and preventive care

	Indus n=339	**SKMCH&RC n=647**	**Total n=348**
Pre ART CD4, cells/uL			
μ± SD	154.9 ±120.5	184.3 ± 151.4	177.2 ± 146.9
Median (IQR)	110 (556)	154 (1044)	149 (1044)
ART regimen for treatment naive			
3TC, AZT, NVP	42 (40.0%)	22 (7.1%)	64 (15.5%)
3TC, AZT, EFV	42 (40.0%)	16 (5.2%)	58 (14.0%)
3TC, D4T, NVP	4 (3.8%)	163 (52.8%)	167 (40.3%)
3TC, TDF, EFV	6 (5.7%)	38 (12.3%)	44 (10.6%)
3TC, AZT, LPV/r	0	3 (1.0%)	3 (.7%)
3TC, AZT, D4T	0	1 (.3%)	1 (.2%)
TDF, DDI, LPV/r	11 (10.5%)	66 (21.4%)	77 (18.6%)
Adherence on ART			
Good	63 (58.3%)	174 (70.7%)	237 (66.9%)
Poor	14 (13.0%)	67 (27.2%)	81 (22.9%)
Unknown	31 (28.7%)	5 (2.0%)	36 (10.2%)
Preventive Care			
Primary INH prophylaxis	21 (7.4%)	1 (.3%)	22 (3.5%)
HBV vaccination	75 (26.6%)	12 (3.6%)	87 (14.0%)
Pneumococcal vaccination	78 (27.7%)	118 (34.9%)	196 (31.6%)
Cotrimoxazole	109 (38.7%)	93 (27.5%)	202 (32.6%)
Azithromycin	58 (20.6%)	0	58 (9.4%)
Fluconazole	30 (10.6%)	13 (3.8%)	43 (6.9%)
Clinical outcome			
Not on ART	n=203	n=93	n=296
Being observed at same centre	17 (8.4%)	58 (62.4%)	75(25.3%)
Died	57 (28.1%)	8 (6.7%)	65 (22.0%)
Defaulted	1 (.5%)	3 (3.2%)	4 (1.4%)
Transferred out	5 (2.5%)	3 (3.2%)	8 (2.7%)
Lost to follow up	123 (60.6%)	21 (22.6%)	144 (48.6%)
On ART	n=105	n=243	n=348
Receiving ARV at same centre	43 (41.0%)	136 (56.0%)	179 (51.4%)
Died	28 (26.7%)	22 (9.1%)	50 (14.4%)
Defaulted	5 (4.8%)	8 (3.3%)	13 (3.7%)
Transferred out	18 (17.1%)	13 (5.3%)	31 (8.9%)
Lost to follow up	11 (10.5%)	64 (26.3)	75 (21.6%)

These manifestations have also been reported rarely from other South Asian countries.[17,20.21]

Nearly one fourth of patients presented in WHO stage 1, i.e. asymptomatic. These were generally those deported from other countries on basis of positive antibody tests, as well as asymptomatic wives of HIV infected men.

Most deaths, defaults and loss to follow-ups were among patients who did not receive ART prior to their free availability through the NACP. The benefits of receiving free ART have been enormous. The patients who were started on ART and have continued to adhere to therapy have shown good clinical, immunological and virological responses as reflected by improved quality of life, absence of opportunistic infections and return to their occupations, along with undetectable virus. Mortality was higher in those who presented at advanced clinical stages despite receiving ART. Although HIV testing and ART is provided free of cost by the NACP, testing is still not carried out effectively in health care settings. This is probably because of reluctance of health care workers to discuss high risk behaviors with their patients, but it is hoped that awareness and knowledge regarding HIV prevalence and manifestations will improve, and along with better services for diagnostics, treatment, care and prevention will result in HIV control.

Limitations of our study: Prior to 2006, CD4 and viral load testing were done irregularly and erratically, and only a few patients could afford tests and ART privately. Hence, pre 2006, CD4 counts were not available for all the patients. Another study limitation was that diagnoses of most opportunistic infections were made clinically, so there may be some opportunistic infections that we have not been able to ascertain.

Pakistan can still avert a crisis through large scale awareness campaigns for the masses, especially to propagate needle safety and avoidance of sexual transmission of the disease. Increase in the number of IDUs is the result of increasing unemployment and poverty; hence attention should be focused on the growing number of IDUs. Public hospitals at district levels should be provided diagnostic facilities and easy referrals to tertiary care centers. WHO and UNAIDS have outlined a new strategy for HIV treatment called “Treatment 2.0” to simplify HIV treatment, increase community involvement and increase global access to antiretrovirals. If ART can be provided to all HIV positive patients who need treatment, this strategy can globally prevent 1 million new HIV infections each year and reduce 10 million AIDS related deaths by year 2025. Pakistan should be in a position to adopt “Treatment 2.0” to attain this goal.[23]

Conclusions

Although the overall prevalence of HIV/AIDS is presently low in Pakistan, it is evolving rapidly as more spouses and children add to the pool through infected men who repatriated from abroad. There is also a rising epidemic among IDUs that is likely to spread to the general population through the reuse of needles, unscreened blood transfusions, as well as their sexual contacts. Provision of free ART especially in early stages of the disease has shown success, and encourages other HIV patients to seek treatment.

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Conflict of Interest

The authors have no conflict of interest and declare that they do not have any financial nor personal relationships with other people or organizations that could inappropriately influence (bias) their work.

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ORIGINAL ARTICLE

Anti-dengue Antibodies in Renal Transplant Recipients Diagnosed with Dengue Viral Infection

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Abstract

Background
Dengue serology is the most common method employed for laboratory diagnosis of dengue. We studied dengue antibody production in the immunocompromised renal transplant recipient.

Methods
All renal transplant recipients tested positive for anti-dengue IgM antibodies, and had a repeat serology sample between January 1st 2009 to December 31st 2010 at the Sindh Institute of Urology & Transplantation in Karachi, Pakistan were included in the study. Dengue IgG and IgM titers were measured in both samples.

Results
We studied 52 patients, of whom 24 had primary and 28 had secondary dengue viral infection. IgM antibodies on repeat sampling remained detectable in 32(61.5%) patients at 12.5±9.9 week. In 12 (23%), IgM persisted beyond 12 weeks.Ten patients with primary infection did not seroconvert and IgG antibodies were undetectable at 14.6±11.8 weeks. Of these 10, more patients had HCV infection (70% Vs 38%, p=0.087).

Conclusion
In transplant patients, dengue antibody kinetics is different from that in the general population. Serological diagnosis of dengue is challenging and a combination of tests will increase the sensitivity and specificity for the diagnosis of dengue viral infection in transplant recipients.

Key words
Dengue, Immunoglobulin M, Immunoglobulin G, Renal Transplantation.

Introduction

Dengue viral infection has become a major health problem in Pakistan.[1] Circulation of multiple serotypes and presence of severe disease in the population has been reported.[2-4] Clinically dengue can present from non-specific illness to severe disease. Diagnosis based on clinical symptoms is unreliable. Since dengue infection can progress rapidly to severe disease and even death, reliable laboratory confirmation is essential for timely intervention and management.[5]

Renal transplantation has been expanding in Pakistan. It is known that transplant recipients may not mount a febrile response to infection.[6] Moreover, they can have co-morbidities that mimic dengue viral infection.

Dengue diagnosis can be made by virus isolation, antigen detection or serology. Serological tests are commonly used in endemic countries as immunoglobulins are stable at tropical room temperatures.[5] Since transplant recipients are receiving immunosuppressive therapy, it is possible that their antibody response to dengue infection may not follow the kinetics described in the general population. Humoral immune response may be altered due to inhibition of T-cell activation.[6] Therefore, not only is the clinical but the serological diagnosis may also be unreliable in transplant recipients.

We sought to study the pattern of dengue antibody production in renal transplant patients and compared our findings to published literature.

Material & Methods

The study was conducted from January 1st 2009 to December 31st 2010 at the Sindh Institute of Urology and Transplantation (SIUT), Karachi, Pakistan.

Adult and pediatric renal transplant recipients that tested positive for anti-dengue IgM antibodies and had repeat serology test done during the study period of interest were included. The SIUT laboratory was instructed to inform study investigators when a patient from the transplant ward or transplant outpatient department (OPD) was tested positive for dengue IgM antibodies. The medical records were reviewed. Concomitant co-morbidities like thrombocytopenia, neutropenia and/or fever such as hepatitis C virus (HCV), cytomegalovirus (CMV), bacteremia with sepsis, and malaria at the time of presentation were recorded. We documented the immunosuppressive drugs that study patients were receiving, which included either cyclosporine (CSA), azathioprine (AZA), tacrolimus (TAC) or mycophenolatemofetil (MMF) in combination with low dose (<7.5 mg/day) or high dose (≥ 7.5 mg/day) oral steroids. Detailed anti-dengue IgG antibody response in relation to immunosuppressive drugs, comorbidities and clinical features are shown in table 1.

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A second sample for dengue serology was required not earlier than 2 weeks from the initial sample. This was either obtained during admission, or at the transplant OPD when patients returned for their routine visit. The list of patients with positive dengue serology on admission was provided to the OPD clerical staff and they were requested to repeat dengue serology when any of these patients came for their follow-up visits. Patients were not asked to return for repeat serology sampling expressly for study purposes since many patients lived at long distances from SIUT.

Initial and repeat samples were both tested for anti-dengue IgM and IgG antibodies by enzyme linked immunosorbant assay (ELISA) using a commercially available kit (Nova Tec Immunodiagnostics, Germany). The values were calculated in Novatec units (NTU) by the formula: optical density (OD) of sample/ OD of cut off control x 10. NTU < 9 was considered negative, while NTU ≥ 11 was positive according to the manufacturer's instructions. NTU values with two fold increase or decrease in the repeat sample was considered as an increase or decrease in titer, or no change if the variation in NTU values was less than two fold. Also, a fourfold increase in NTU values was considered fourfold rise in titers. The ratio of anti-Dengue IgM to IgG was calculated to distinguish between primary and secondary infection.

Dengue antibody kinetics, as occurs in the general population, has been described in a comprehensive WHO publication.[5]

Acute Dengue viral infection

Acute dengue infection is considered highly suggestive if serum

Table 1: Anti-dengue IgG antibody response in relation to immunosuppressive drugs, comorbidities and clinical features

	Anti-dengue IgG negative on repeat sample (No seroconversion) n=10	Anti-Dengue IgG positive in initial and/or repeat sample n= 42	*p*-value
Immunosuppression			
CSA[1]+ AZA[2]+ delta[3]	6 (60)	18 (43)	0.483
CSA+ MMF[4]+ delta	0	4 (9.5)	0.576
Tac[5]+ AZA+ delta	1(10)	7 (17)	1.000
Tac+ MMF+ delta	2(10)	3 (7.1)	0.242
CSA+ delta	0	2 (4.8)	1.000
AZA+ delta	0	3(7.1)	1.000
MMF+ delta	1(10)	2(4.8)	0.487
Delta (alone)	0	3 (7.1)	1.000
MTOR[6] inhibitor	1(10)	3(7.1)	1.000
delta = 7.5 mg/day	4(10)	15 (36)	1.000
delta < 7.5 mg/day	6(10)	21 (64)	
Comorbids			
HCV[7] infection	7 (70)	16 (38)	0.087
Malaria	1 (10)	0	0.192
CMV[8] disease	0	4 (9.5)	0.576
Pulmonary TB[9]	0	1 (2.4)	1.000
Chicken pox	1 (10)	1 (2.4)	0.351
Bacterial infection	2 (20)	9 (21.4)	1.000
Fungal infection	1 (10)	0	0.192
Primary infection	10 (100)	14 (33)	
Secondary infection	0	28 (67)	0.000
Dengue associated Clinical and Laboratory Features			
Fever	4(40)	33(78.6)	0.024*
Thrombocytopenia	9 (90)	39 (93)	1.000

Footnote: [1]= cyclosporine, [2]= azathioprine, [3]= deltacortil, [4]= mycophenolatemofetil, [5]= tacrolimus, [6]= mammalian target of rapamycin, [7]=hepatitis C virus, [8]=cytomegalovirus, [9]= tuberculosis, *= significant *P* value

IgM is positive in a single serum sample. It is confirmed if there is IgM seroconversion to IgG or if there is a four-fold or greater increase in IgG titers, observed in paired sera.

Serology of primary infection
During a primary dengue infection (infection in a person who has not previously been infected with dengue) IgM antibodies are the first immunoglobulin to appear and are detectable in 50-80% of patients by days 3-5 after onset of illness, peaking at two weeks and then declining to undetectable levels by 12 weeks. Serum anti-dengue IgG is detectable at low titers at the end of the first week of illness, slowly reaching a peak and then declining. However, they remain detectable after several months, and usually for life.

Serology of secondary infection
During a secondary dengue infection (infection in a host that has previously been infected by a dengue virus), the dominant immunoglobulin isotope is IgG which rises rapidly and is detectable at high levels, even in the acute phase, and persists for periods lasting from 10 months to life. Early convalescent stage IgM levels are significantly lower in secondary infection as compared with a primary infection.

Ratio of IgM to IgG to distinguish between primary and secondary dengue infection
When both anti-dengue IgM and IgG are positive, ratio of IgM to IgG is used to distinguish between primary and secondary infection.

IgM/IgG ratio > 1.2 is considered to be a primary infection IgM/IgG ratio < 1.2 is considered to be a secondary infection Data analysis was done using SPSS version 17. The numerical data is expressed as mean ± SD. Chi-square tests and student's *t*-test was applied to determine the significant differences in categorical and numerical data respectively. $P \leq 0.05$ was considered significant.

Results
Fifty two renal transplant recipients were enrolled in the study over a 2 year period. There were 38 males and 14 females with a mean age of 29 ± 10.2 years.

Repeat dengue serology was obtained at an average of 13.4 weeks (range 2-32 weeks) as shown in figure 1. All patients were asymptomatic at the time of repeat sampling. Of 52 patients, 24(46%) had primary dengue viral infection. Twenty one of 24 (87.5%) had only IgM antibodies and three (12.5%) had both IgM and IgG antibodies in their initial samples.

Out of 21 who were only IgM positive, 11(52.3%) tested positive for IgG on repeat sampling. Ten patients (47.6%) were negative for IgG antibodies at an average of 14.6±11.8 weeks. Out of three patients who were both IgM and IgG positive, two had increase in IgG titers of which in one, a fourfold rise on repeat sample was documented. One had a decrease in IgG titers from baseline measured at >12 weeks.

Of 52, 28 (53.8%) patients had secondary infection all of whom had both IgM and IgG positive on the initial sample. Of 28 patients, 13(46.4%) had increase in IgG titers of which a fourfold increase occurred in 12(42.8%) patients. In 11 (39.2%) there was no rise in titers measured at an average of 15.5±11weeks. In 4 (14.2%), there was a decrease in titers from baseline at an average of 6.6±2.8weeks.

Out of 52, in 20 (38.5%) patients IgM had become undetectable on repeat sampling. In 32 (61.5%), IgM antibodies remained detectable measured at an average of 12.5±9.9 week. In 12 (23%), IgM persisted at high titers beyond 12 weeks.

Clinical features, co-morbidities and immunosuppressive drug regimen in those without an IgG response as compared with those that had an IgG antibody response are shown in table 1. We performed an analysis of the 10 patients in whom IgG was not detectable on repeat sampling as compared with those that had IgG antibodies detectable in either the first or repeat sample.

In those that failed to develop IgG antibodies, a higher number of patients had HCV infection (70%vs 38%, p=0.087). Of 10, IgM became undetectable in 4 patients. In 3 patients IgM remained detectable at high titers at an average of 16.6±13.6 weeks. Of the 6 patients with detectable IgM, 4 were HCV positive. Fever was absent in a significantly higher number of patients who did not seroconvert (60%Vs 21.4%, p=0.024).

Discussion
Dengue viral infection is hyper endemic in Pakistan.[1,3] Over half of our study patients had secondary infection. In immunocompetent patients, IgM begins declining after 2 weeks and generally becomes undetectable by12 weeks.[5] We observed that more than half of our immunocompromised patients had prolonged IgM positivity and in almost one quarter of them, IgM was detectable beyond 12 weeks. Persistence of IgM could be due to delayed clearance in an immunosuppressed population. Further studies are therefore warranted on rate of clearance of antibody titers after an acute infection in transplant recipients.

Although IgG antibodies should be detectable by the end of the first week of illness in primary infection, ten of our patients did not seroconvert and IgG antibodies remained undetectable in repeat samples drawn at greater than 2 weeks. In these patients, IgM reactivity on initial sample may represent a false positive result. It is known that flaviviruses can give false positive antibody results due to cross reactive antigenic epitopes.[7] HCV is a flavivirus and since 7 (70%) of the patients who did not seroconvert also had concomitant HCV infection, it is conceivable that these patients had a false positive result.
Persistence of IgM antibody levels in 3 of 10 patients, all of whom were HCV positive, further raises the suspicion of HCV

cross reactivity. However, previous studies do not support cross reactive antigenic epitopes between HCV and dengue virus.[8] Further more, an immunomodulating effect of HCV has been reported in patients on hemodialysis and renal transplant recipients which may have played a role in failure to mount IgG antibody response.[9,10] Our sample size was small and further studies are required to evaluate cross reactivity between HCV and dengue virus.

In secondary infection, a fourfold rise in IgG titers was demonstrated in over two-fifths of our patients. However, in 11 patients there was no increase in titers noted at all. It is possible that these patients presented late and both samples were drawn when IgG titers had already reached a plateau. Interestingly,

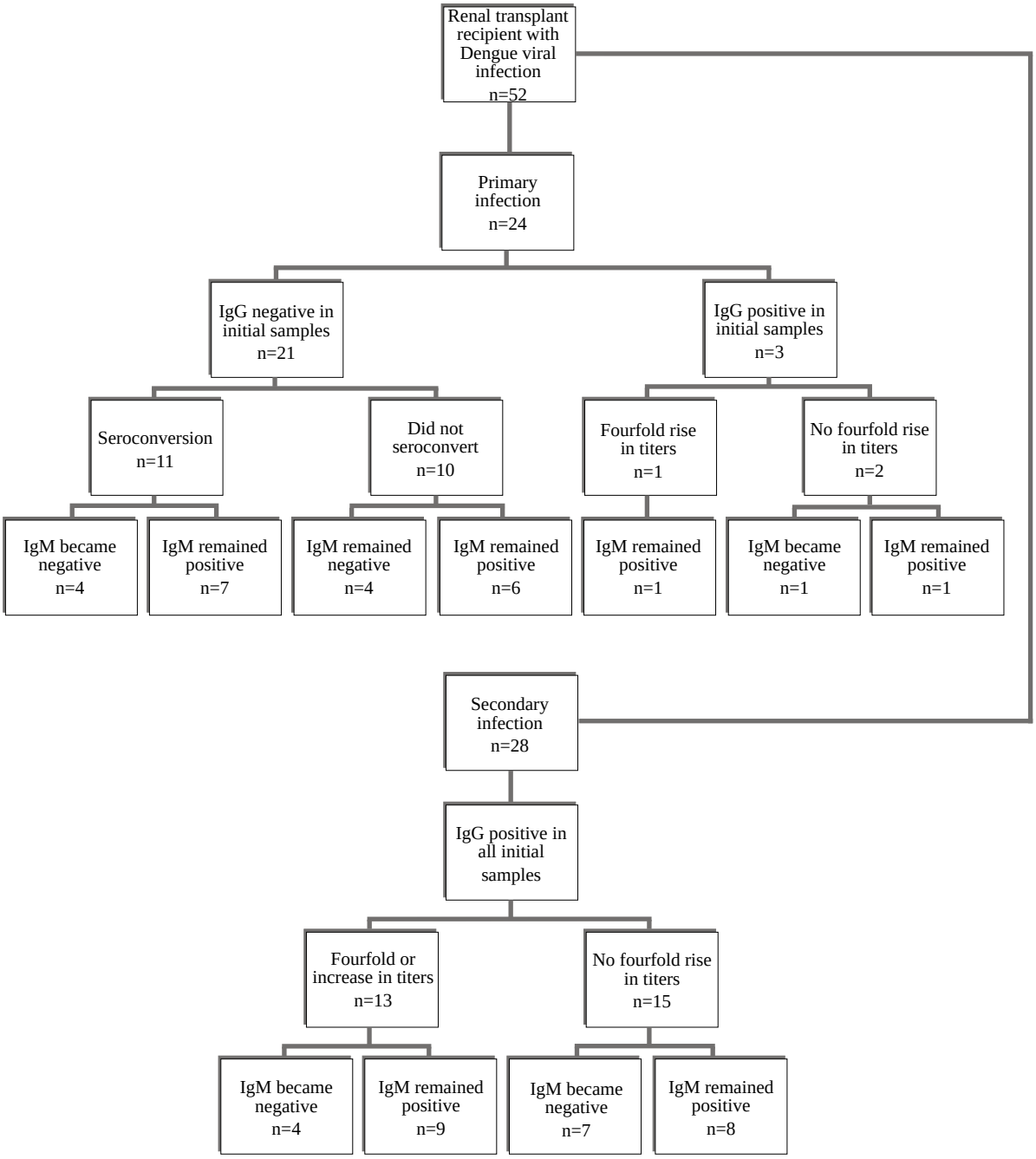

Fig 1. Serum anti-dengue antibodies at presentation and on repeat sampling at an average of 13.4 (2-32) weeks in renal transplant recipients diagnosed with dengue viral infection

4 patients actually had a decline in IgG titers from baseline.

The abnormal IgG response could also be attributed to iatrogenic immunosuppression, where down regulation of both cellular and humoral immune response occur. It has been shown that vaccination with tetanus toxoid or keyhole limpet hemocyanin during the post-transplantation period failed to produce the desired IgG response in a majority of patients.[6,11] However, a larger sample size is required to elucidate the effect of various immunosuppressive drugs on anti-dengue antibody formation.

Our study had several limitations. The diagnosis was based on IgM positivity alone, which is not confirmatory. We did not have availability of viral antigen and RNA detection tests which would confirm and identify patients with possible false positive results. A major limitation was that we did not get the second sample for dengue serologyat a uniform interval from initial presentation. Samples drawn at fixed intervals would have allowed more accurate plotting of IgM and IgG titers. Instead, we relied on samples drawn at the convenience of the patients. The reason being many of our patients live outside Karachi, were financially constrained and could notafford travel cost. Therefore, repeat sampling was obtained at varying intervals across a wide time frame as a result of which peaks and troughs of antibody titers migh thave been missed.

Conclusion

In transplant patients, dengue antibody kinetics is different from that in the general population. Serological diagnosis of dengue is challenging and a combination of tests will increase the sensitivity and specificity for the diagnosis of dengue viral infection in transplant recipients. Further studies of anti-dengue antibody kinetics in this population are needed.

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ORIGINAL ARTICLE

Head Trauma Associated with Poor Outcome in Neurosurgical Meningitis

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Abstract

Objective
To assess factors associated with mortality in neurosurgical meningitis.

Methods
This case control study was undertaken at the Aga Khan University Hospital from June 2010 to July 2011 to look for factors associated with mortality in neurosurgical meningitis. Neurosurgical meningitis was defined as using Briggs *et al* and Centre for Disease Control & Prevention (CDC) surveillance definitions. Cases were defined as those who died and controls who survived meningitis.

Results
A total of 24 patients were included in the study; 7 cases and 17 controls. Amongst 24, eight had craniotomies for tumor resection; seven aneurysm clipping and five underwent decompression craniotomies. Ten patients underwent shunt procedure of which only two had hydrocephalus as their primary diagnoses and the rest needed shunt after primary intervention. Most of the procedures (60%) were done in emergency. Patients were equally distributed in gram positive, gram negative and culture negative meningitis (n=7). Mortality was highest with head trauma (*p*-value 0.038) and gram negative meningitis (58%).

Key words
Neurosurgical Meningitis, Trauma Head.

Introduction

Neurosurgical meningitis is a serious procedure related complication with attributable mortality of 3-12%.[1] Although, the incidence of meningitis depends on the type of neurosurgical procedure undertaken, the rate of such infection is reported to be highest after shunt surgery.[2]

Due to increasing antimicrobial resistance amongst hospital acquired pathogens, therapeutic options are often limited. Additionally, inadequate drug delivery and penetration into the meninges and brain tissue make such infections extremely difficult to treat resulting in prolong and costly hospitals stay with higher risk of failure.

A number of studies have looked at risk factors for developing post operative meningitis, but there is no data that has looked at factors associated with poor outcomes[3, 4]. We undertook this study to look at factors associated with mortality in patients with neurosurgical meningitis for early risk stratification, monitoring and intervention.

Material and Methods

A case control study was done to identify factors associated with adverse outcomes in patients who developed meningitis after neurosurgical procedure at the Aga Khan University Hospital, Karachi. Patients who had undergone neurosurgical procedure from June 2010 to July 2011 were screened for meningitis using electronic medical records for cerebrospinal fluid (CSF) analysis and culture. Hospital charts were reviewed for verification and data collection. Patients diagnosed with meningitis during the study period were included and stratified into cases and controls based on mortality. Cases were defined as those who died.

Case Definition

Neurosurgical Meningitis was diagnosed within two months of neurosurgical intervention, if at least *three* of the following criteria were fulfilled: [1, 5]

(1) CSF culture positive;
(2) CSF glucose < 45mg/dl;
(3) CSF white cell count >10cells/mm^3;
(4) CSF Protein > 45 mg/dl;
(5) Diagnosis of meningitis by infectious diseases consultant.

Statistical Analysis

Data was entered and analyzed on IBM SPSS© version 19. Ratios, percentages, mean and standard deviations (SD) were calculated using standard methods. *P-value* was calculated using Fisher's exact test for categorical and Mann-Whitney U test for continuous variables. Exact *p-values* were reported and a *p-value <0.05* was considered to be significant.

Results

A total of 24 patients were found to have developed meningitis after neurosurgical intervention during the study period, of

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which there were seven cases and seventeen controls (2.5 controls per case).

Table 1 shows baseline characteristics of the subjects enrolled. The most common diagnosis was brain tumor (38%). Mortality in patients with meningitis after head trauma was found to be statistically significant with *p-value 0.038 (OR=10, CI: 1.22-81).*

Table 2 describes important surgical variables. Although, none were statistically significant but the most common surgical procedure was shunt placement (n=10). Majority of the procedures were done in emergency (60%), even than had no impact on outcome.

Table 3 shows CSF analyses and microbiological data of our study population. Although, none of the parameters in CSF analysis predicted the outcome, but more deaths occurred in patients with gram negative meningitis (n=58%). No mortality was seen in patients with gram positive meningitis.

Table 1: Varialbles of neurosurgical intervention undertaken

Variable	Case (n=7)	Control (n=17)	*p*-value
Age in years (mean+SD)	38 (17)	38 (15)	0.86
Gender			
Female	2	12	0.075
Diagnosis on admission			
Brain tumor	3	5	0.64
Head trauma	4	2	0.038
- Road traffic accident	3	2	
- Gunshot injury	1	0	
Intracranial hemorrhage	0	8	0.06
Hydrocephalus	2	0	0.6

Table 2: Types of neurosurgical procedure and duration

Variable	Case	Control	*p*-value
Neurosurgical procedure			
Decompressive craniotomy	3	2	0.12
Shunt surgery	3	7	0.6
Tumor craniotomy	3	5	0.6
Clipping of aneurysm	0	7	0.06
Type of surgery			
Elective surgeries	3	7	>1
Duration of surgery in hours (mean+SD)	4.2(2.7)	4(3)	0.71
Interval (in days) between surgery & meningitis	17(16)	15(14)	0.57

Table 3: Comparison of cerebrospinal fluid analysis and cultures

Variable	Case	Control	*p*-value
CSF Analysis			
CSF glucose mg/dl (mean+SD)	22(16)	28(20)	0.58
CSF proteins gm/dl (mean+SD)	263(230)	580(1364)	> 1
CSF leucocytes cells/cmm	652(700)	386(450)	0.23
CSF Culture			
Gram positive cocci	0	7	0.065
Methicillin resistant *Staphylococcus aureus*	0	5	
Coagulase negative staphylococcus	0	2	
Gram negative bacilli	4	3	0.13
Eneterobacteriaceae			
Klebsiella pneumoniae	1	1	
Eneterobacter spp.	1	0	
Non-enterobacteriaceae			
Pseudomonas aeruginosa	0	1	
Acinetobacter spp.	2	1	
Polymicrobial	1	2	> 1
Culture negative	2	5	> 1

Discussion

Post operative infections come in all sizes and shapes and depending on their severity add to undue morbidity or mortality. Neurosurgical meningitis is a severe form of post operative infection with significant morbidity and relatively high mortality. Knowledge of factors associated with poor outcome will help in modifying them.

Our study on neurosurgical meningitis showed higher mortality with head trauma and gram negative meningitis. Head trauma may be trivial or severe. Severe head injuries may cause intra parenchymal bleed, skull fractures or a combination of complex head injuries that may cause CSF leak and breach in blood brain barrier, predisposing to infections.

Head trauma by itself could have a devastating outcome and with CNS co-infections the outcomes could be total dismal. CNS infections acquired in hospital milieu are fairly antimicrobial resistant and pose therapeutic challenge. Only a handful of antimicrobials when given intravenously have good CNS penetration including 3rd generation cephalosporin (ceftriaxone and cefotaxime), but due to surge in extended

spectrum beta-lactamase (ESBL) producing gram negative organisms, this class of antimicrobials has largely been lost. Ineffective drug delivery into the meninges and high virulence of gram negatives makes gram negative meningitis difficult to treat with adverse outcomes, as also seen in our study. Also interesting to note is that none of the CSF parameters predicted the outcome. Although, CSF glucose is a good indicator of disease severity and is frequently monitored to assess response, but our study did not show any association of low glucose with mortality in neurosurgical meningitis.

Most data published on neurosurgical meningitis is descriptive and studies looking at factors associated with poor outcomes are scarce. Previous studies on neurosurgical meningitis found delay in antimicrobials, concomitant sepsis and presence of intra-ventricular drain for longer duration to be associated with mortality, none of these factors were looked at in our study.[6,7] Baltas *et al* in a study on post traumatic meningitis reported no mortality which differs in mortality quoted by Lu and Kim *et al*.[6, 8,9] This variation between the centers could likely be due to the difference in the level of trauma being handled.

Our studies foremost limitation was its small sample size. Since, this was a retrospective study hence only variables documented in hospital charts could be analyzed. Our study did not use any functional scoring system to assess disease or trauma severity that may have affected our results. But this study has identified an important area to be looked at in more detail for precise risk stratification.

Conclusion

Post traumatic head injuries with meningitis are associated with poor outcomes and needs close monitoring for early recognition of infection and intervention. Also gram negative meningitis is associated with higher mortality and with the dearth of antimicrobials; ensuring strict adherence to infection control measures to prevent transmission of such infections is pivotal in managing such patients.

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ORIGINAL ARTICLE

In vitro Susceptibility Testing of Urinary Tract Isolates to Fosfomycin

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Abstract

The aim of this study was to evaluate the *in vitro* activity of fosfomycin against various urinary isolates and their comparison with antimicrobial agents commonly used for the treatment of urinary tract infections such as, fluoroquinolones (ciprofloxacin) and nitrofurantoin.

Methodology
The study was carried out in the Department of Microbiology, The Indus hospital, Karachi from April 2012 to August 2012. Various isolates were included in our study; for selection of isolates no specific criteria was set, all clinical isolates, both in and out patients were included in this study.

Results
A total of 524 bacterial urinary isolates (*Escherichia coli, Klebsiella spp., Pseudomonas spp., Acinetobacter spp., Proteus spp.*, others members of the enterobacteriaceae, and *Enterococcus spp.*) were tested for *in vitro* activity of ciprofloxacin, nitrofurantoin and fosfomycin. The resistance pattern was 54%, 42% and 11% to ciprofloxacin, nitrofurantoin and fosfomycin respectively.

Conclusion
Fosfomycin-trometamol was found to be the most effective antimicrobial agent *in vitro* against various urinary isolates. *E.coli*, the most prevalent uropathogen was responsible showed only 4% resistance for fosfomycin and may be used as an empirical therapy in urinary tract infections.

Key Words
Fosfomycin, Urinary Tract Infection, Uropathogens.

Introduction
Urinary tract infections (UTIs) are the most common type of infections and the knowledge on antimicrobial susceptibility pattern is important to choose an appropriate antimicrobial agent.

UTIs are often treated with different antimicrobial agents such as beta-lactams (ampicillin, amoxicillin), and trimethoprim-sulfamethoxazole.[1] However, by the increasing rate of resistance to these antimicrobial agents, treatment shifted to fluoroquinolones.[2,3] Now remarkably high resistance to fluoroquinolones has been documented which is a great concern worldwide because fluoroquinolones (ciprofloxacin) are used as first line empiric treatment in UTIs and its increased resistance makes the treatment difficult.[4-8] Additionally, *Escherichia coli*, the most prevalent uropathogen exhibits high level of resistance against fluoroquinolones due to the production of extended spectrum beta lactamase (ESBLs).[9, 10]

Nitrofurantoin, an antibiotic consisting of a nitro group joined to a heterocyclic ring, is also used for the treatment of UTIs. Nitrofurantoin has limited spectrum of activity.[1] Increased resistance of nitrofurantoin has been reported in various urinary isolates in recent studies.[7,11,12] Therefore, there is a great need of an antimicrobial agent for the treatment of UTIs which is effective, has low level of resistance and can be use as an empirical treatment for uncomplicated UTIs.

The aim of our study was to obtain data on susceptibility pattern of antibiotics commonly used for the treatment of UTIs against various urinary isolates and their comparison with fosfomycin.

Methodology
This retrospective descriptive study was carried out at the Department of Microbiology, The Indus Hospital, Karachi, from April 2012 through August 2012. A total of 524 bacterial isolates from urine specimen were included, from both in and out patients.

All 524, urine specimens were collected in a wide mouth, leak proof sterile containers. The urine sample was centrifuged and examined microscopically, sample having ≥ 4-6 pus cells/HPF was inoculated on CLED (Oxoid, UK) by quantitative method using calibrated wire loop of 0.0001 ml. Plates were incubated at 35-37ºC for 24-48 hours aerobically. More than 10^5 colony forming units/ml of a single pathogen were considered as significant bacteriuria.

Identification of isolates was done by colony morphology, gram stain, and conventional biochemical test; reaction on triple sugar iron (TSI), citrate utilization, indole and urease production, oxidase test, motility, bile esculin.

Antimicrobial susceptibility testing was performed on Muller Hinton agar (Oxoid, UK) by disk diffusion method according to the Clinical and Laboratory Standard Institute (CLSI) guidelines 2012. Disks (Oxoid, UK) were ciprofloxacin (5 µg),

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nitrofurantoin (300 µg), and fosfomycin (50 µg). Fosfomycin is less acknowledge by CLSI and included in group "O", CLSI proposes breakpoints only for *Enterococcus* spp. and *E. coli*. Therefore, fosfomycin breakpoints for other negative isolates were followed.

Results

We examined 524 positive urine samples of both in and out patients. The results showed that *E. coli* was the most prevalent uropathogen (64%). Detail of isolates is as per table 1. Resistance to ciprofloxacin was remarkably high especially in *E. coli* (60%), followed by other organisms (Figure 1).

Nitrofurantoin also showed increased resistance rate as compare to fosfomycin.

The cumulative resistance of various urinary isolates is shown in table 2.

Discussion

UTIs are one of the most common types of infection and due to increased antimicrobial resistance worldwide selection of appropriate antibiotic has an increasing concern. UTIs are mostly treated empirically especially in developing countries, therefore the knowledge on susceptibility patterns of uropathogens is very important to prescribe an appropriate antimicrobial agent.[7]

Table 1: Microorganisms isolated from urine samples (n= 524)

Organisms	n	%
Escherichia coli	337	64
Klebsiella spp.	92	18
Pseudomonas spp.	26	5
Acinetobacter spp.	15	3
Proteus spp.	13	2
Others	21	4
Enterococcus spp.	20	4

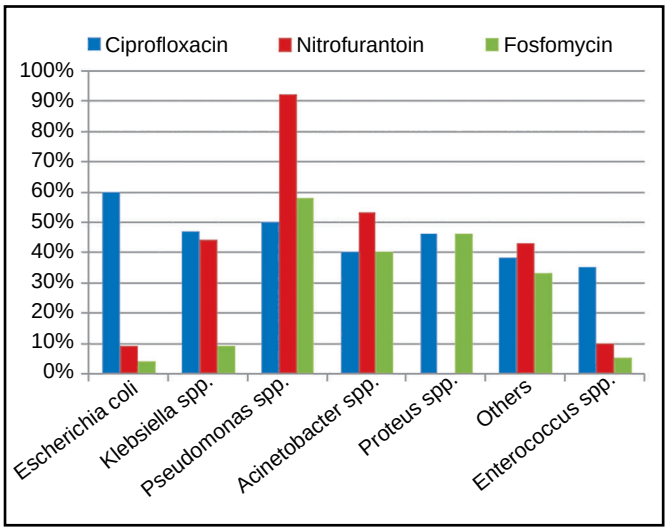

Fig 1. *In vitro* **resistance pattern of different urinary tract isolates to ciprofloxacin, nitrofurantoin and fosfomycin (n= 524)**

Table 2: Cumulative susceptibility rate of various urinary tract (n= 524)

Antimicrobial Agents	% Resistant	% Sensitive
Ciprofloxacin	54 %	46%
Nitrofurantoin	42%	48%
Fosfomycin	11%	89%

In Pakistan, ciprofloxacin is one of the most frequently prescribed antibiotic in UTIs.[13] However, it has been observed that ciprofloxacin resistance has been increasingly reported, which is a great concern to used it as a first line empiric treatment for UTI.[14]

Fosfomycin, was discovered in 1969, originally named as phosphonomycin, attained from cultures of the *Steptomyces* spp. It is a broad spectrum bactericidal antibiotic and used against most gram positive and gram negative organisms. It targets the mucopeptide synthesis by inhibiting the phosphoenolpyruvate, during the first step in synthesis of peptidoglycan.[15] Initially fosfomycin was used to treat severe infections like sepsis and soft tissue infections and was administered intravenously. Fosfomycin trometamol, an oral formulation, used to treat uncomplicated UTIs and also effective in multi-drug resistant UTIs.[16,17] Fosfomycin shows very low resistance worldwide among *Escherichia coli*, and in other urinary isolates.[18-20]

The increased resistance of ciprofloxacin may be due to that it is self prescribed, extensive use, inadequate doses, and prescribing without antimicrobial sensitivity testing. Our results also showed increased resistance to ciprofloxacin; with cumulative resistance of 54%. Other studies also showed similar results.[21,22] Nitrofurantoin is also used for the treatment of UTI, its resistance is also reported in various recent studies.[7] In our results, nitrofurantoin was 42% resistant against various urinary isolates. Therefore, there is a need to use new options for the treatment of UTI which shows high sensitivity for all urinary isolates and can be used as an empirical treatment. In our study, *E. coli*, was the most prevalent uropathogen and showed maximal susceptibility for fosfomycin, the cumulative resistance of fosfomycin was low; other studies also showed the similar results.[21,22] We found that fosfomycin was less sensitive against *Pseudomonas spp.*(42%), which was responsible for only 5% of urinary tract infection. Fosfomycin exhibited maximum antimicrobial activity *in vitro* among all three antimicrobials tested against various urinary isolates.

Conclusion

Fosfomycin was found to be most effective *in vitro* antimicrobial agent for various urinary isolates, therefore it could be an alternative treatment option for UTIs particularly against uropathogens resistant to routinely prescribed antimicrobials.

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In Vitro Sensitivity of Chloramphenicol against Extended Spectrum Beta Lactamase Producing Gram Negative Bacteria

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Abstract

Objective
To determine *in vitro* sensitivity of chloramphenicol against ESBL producing Gram negative organisms.

Study design
Cross sectional study.

Methodology
One hundred and twenty isolates of ESBL producing Gram negative organisms detected by combination disc synergy method were included in this study. Minimum inhibitory concentration (MIC) of chloramphenicol against these isolates was determined by E-strip. Clinical and Laboratory Standards Institute recommendations were followed; ≤ 8µg/ml sensitive, 16µg/ml intermediate and ≥ 32µg/ml resistant.

Results
Out of 120 ESBL producing Gram negative organisms, 86 (71.7%) were susceptible to chloramphenicol with MICs of ≤ 8µg/ml; 33 (27.5%) were resistant with MIC of ≥ 32µg/ml while 1 (0.8%) was in intermediate range with MIC of 16µg/ml.

Conclusion
Chloramphenicol has shown good *in vitro* activity against ESBL producing Gram negative bacteria and can play a key role in the treatment of infections caused by these organisms.

Key Words
Chloramphenicol, Extended Spectrum Beta Lactamases (ESBL), Gram Negative Rods, *In vitro* Susceptibility.

Introduction

Various groups of antimicrobials are used to treat infections caused by Gram negative rods (GNRs). These antibiotics include aminoglycosides, beta-lactams, co-trimoxazole, fluoroquinolones and nitrofurantoin. The injudicious use of these antibiotics has greatly contributed to the emergence of antimicrobial resistance in GNR.[1] One of the important mechanism of resistance among these bacteria is the production of extended spectrum beta lactamases (ESBL) enzymes. Theses enzymes, produced by various GNR, confer resistance to penicillins, cephalosporins and monobactams. The ESBL producing organisms can also develop co-resistance to other antimicrobials (fluoroquinolones, co-trimoxazole and aminoglycosides), frequently used to treat infections caused by these organisms.[2]

Chloramphenicol was introduced into clinical practice in 1949 and alongside tetracycline was considered to be prototypical broad spectrum antibiotic. Chloramphenicol is effective against most gram positive, anaerobes and Gram negative bacteria (including ESBL producing organisms).[3] The resistance and potential side effects of the drug have largely forced the clinicians to refrain from using the drug during 70's to 90's. With threat of evolving superbugs like MRSA, vancomycin resistance *Enterococci* (VRE) and ESBL producing organisms, this old antibiotic suddenly came out of wilderness with renewed interest and is being considered as an alternate to treat such infections.[4] Chloramphenicol has wide antimicrobial spectrum and excellent tissue penetration and is used empirically in the hospital setting for the treatment of patients with unknown sources of fever.[4] In the recent times, with an increasing trend of ESBL production among GNRs, the significance of broad spectrum antibiotics like chloramphenicol has further increased.[5] Unfortunately there is no current data available in Pakistan regarding the susceptibility pattern of ESBL producing pathogens to chloramphenicol. The objective of this study was to find out the *in vitro* susceptibility of chloramphenicol by doing minimum inhibitory concentration of antimicrobial against ESBL producing GNR.

Materials & Methods

This laboratory based cross-sectional study was carried out at the Department of Microbiology Armed Forces Institute of Pathology, Rawalpindi Pakistan, from May 2012 through November 2012. Approval from Institutional Ethical Committee was taken.

Sample size (n=120) was calculated by WHO sample size calculator using anticipated population proportion 0.40 %, absolute precision 0.1 % and confidence level of 95 %.[6]

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Sampling technique was non probability consecutive sampling. All ESBL producing GNRs, detected by combination disc synergy method were included in the study while isolates positive for ESBL production by double disk synergy but negative by combination disc synergy (confirmatory test) were excluded from the study.

Hospital identity number, age and gender of the patients were recorded. All clinical samples were collected in sterile leak proof container aseptically. Specimens for culture were collected before administration of antibiotics.

Clinical specimens were inoculated on appropriate culture media plates and incubated at 37°C for 24 to 48 h. Organisms were identified by standard microbiological methods and confirmed by biochemical characteristics. The screening of ESBL production was done with cefotaxime (30µg) disc along with routine first and second line antibiotics by Kirby-Bauer disc diffusion technique according to Clinical Laboratory Standard Institute (CLSI), guidelines (double disc synergy).[7] The isolates with cefotaxime zone diameter equal to or less than 25 mm were further confirmed for ESBL by phenotypic confirmatory test.

Prepared Mueller-Hinton agar (MAST Diagnostics, UK) was inoculated with the test organism (0.5 McFarland standard) to give a semi-confluent growth. *K. pneumoniae* ATCC 700603 and E. *coli* ATCC 25922 were used as control strains. Ceftazidime (30 µg) or cefotaxime (30µg) disc along with ceftazidime-clavulanic acid (30/10µg) or cefotaxime-clavulanic acid (30/10µg) combination disc were then placed at 20 to 25 mm distance from each other. Following overnight incubation in air at 35°C ± 2, an increase in a zone diameter ≥ 5 mm for either antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone confirmed the isolate as ESBL producer (combination disc synergy).[7]

Isolates of ESBL producing organisms were standardized using colony suspension method and was matched with 0.5 McFarland standard to give a resultant concentration of 1.5 x 10^8 cfu/ml. The antibiotic susceptibility testing was determined by swabbing the Mueller-Hinton agar (MHA) plates with the resultant saline suspension of bacterial isolates and then E-strip of chloramphenicol (AB Biodisk Sweden) was placed on the plate and MIC (µg/ml) was determined. MIC recommended interpretative standard (µg/mL) of CLSI with a zone of inhibition of ≤ 8 as sensitive, 16 as intermediate and ≥ 32 as resistant were followed.[7] *E. coli* ATCC 25922 was used as control strain. The petri dish containing the E-strip was allowed to stand for at least 30 minutes before incubating at 35°C ± 2 for 18-24 hours. Findings were recorded in the proforma designed for this purpose.

The data was entered in SPSS (version 17) software. Descriptive statistics were calculated for both qualitative & quantitative variables. For quantitative variables like age, mean±SD was calculated. For qualitative variables like gender, chloramphenicol susceptibility, frequency and percentages were calculated.

Results

During the period of study, a total of 120 ESBL producing isolates were isolated from different clinical specimens (Table 1). 64 (53%) specimens were from male patients while 47 % from female patients. Age range varied from 6 months to 90 years; mean 46.35 + 19.71 SD. Majority of ESBL producing isolates (60%) were recovered from urine specimens, followed by pus & pus swab (20.8%).

E.coli was the most common organism isolated (74.2%), followed by *K. pneumoniae* (12.5%). Percentages of other

Table 1: Isolation of ESBL producing isolates from different specimens (n=120)

Specimen type	n	%age
Urine	72	60%
Pus & pus swab	25	20.8%
Catheter tip	9	7.5%
Blood	4	3.3%
High vaginal swab	3	2.5%
Bronchoalveolar lavage	2	1.7%
Tissue	2	1.7%
CSF	2	1.7%
Sputum	1	0.8%

ESBL producing organisms isolated are depicted in figure 1. MICs of chloramphenicol against ESBL producing isolates revealed 86 isolates susceptible to chloramphenicol and 33 as resistant (Table 2).

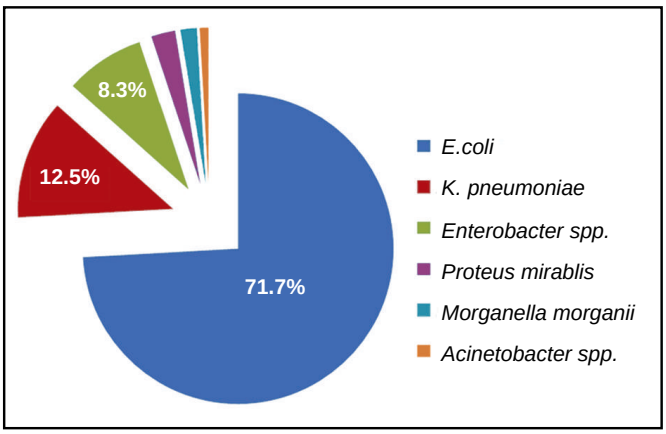

Fig 1. Percentages of ESBL producing isolates (n=120)

Among susceptible category, 62 isolates (72.1%) had MICs ≤ 4µg/ml while 24 (27.9%) were in the range of 6-8µg/ml. (Table 3).

Table 2: MICs of chloramphenicol against ESBL producing isolates (n=120)

MICs of Chloramphenicol		
≤ 8 Sensitive n (%)	16 Intermediate n (%)	≥ 32 Resistant n (%)
86 (71.7%)	1 (0.8%)	33 (27.5%)

Table 3: MIC Range of susceptible isolates (n=86)

MICs (µg/ml)	n	%
1	6	7%
1.5	8	9.3%
2	13	15.1%
3	17	19.8%
4	18	20.9%
6	11	12.8%
8	13	15.1%

Discussion

Increased antimicrobial resistance has emerged as one of the greatest threat of the 21st century.[3,8,9] Multidrug resistant bacteria have been reported from all over the world with varying antimicrobial susceptibility patterns.[8,10] In our study, antimicrobial susceptibility of ESBL isolates from various specimens against chloramphenicol was very encouraging. Our results were in concordance with the study conducted in Spain (2005), whereby 71% ESBL producing GNRs were susceptible to chloremphenicol.[11] Another study conducted in Turkey in 2009, showed that 82% of all the ESBL producing *K. pneumoniae* were susceptible to chloramphenicol.[12] It was reported in India in 2010, that 50% of ESBL producing salmonella species were susceptible to chloramphenicol as compared to non-ESBL producing species, in which the susceptibility was more than 70%.[13] Study conducted in Tanzania in 2005, revealed that 55% of ESBL producing organisms isolated from different clinical specimens were susceptible to this antibiotic.[14] So the *in vitro* efficacy of chloramphenicol against ESBL producing microorganisms tested in various countries of Asia, Europe and Africa has been encouraging.

Another very important finding in our study was that 70% of the isolates which were sensitive to chloramphenicol had a MIC of ≤ 4µg/ml which is lesser than the CLSI recommended susceptibility value. The reason is simple as the antibiotic has not been used in clinical practice for a while. A study carried out in United Kingdom revealed similar efficacy of chloramphenicol against carbapenem resistant GNRs as well.[15] These results should encourage the microbiology labs to use chloramphenicol for *in vitro* testing both against ESBL and carbapenem resistant organisms.

Another study conducted at Rawalpindi during 2011, revealed that urine (45 %) was the most common specimen which yielded ESBL producing GNR, followed by pus specimen (30%).[16] Similarly, *E.coli* and *K.pneumoniae* were the most common ESBL producing GNR isolated in most of the studies conducted on ESBL producing organisms.[16-18] Similar to our study, many studies have shown that ESBL producing GNRs were isolated from patients with a mean age of above 45 years.[16,19,20-22]

Good *in vitro* efficacy coupled with the low price and oral availability can make chloramphenicol a very good alternative choice for a wide variety of infections caused by ESBL producing GNRs, especially when other options are limited or are too expensive.

Conclusion

Chloramphenicol has shown reasonably good *in vitro* susceptibility against ESBL producing GNRs. This antibiotic can play a key role in the infections caused by these super bugs which are also resistant to many other antibiotic groups.

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ORIGINAL ARTICLE

Non-beta-lactam Antimicrobials versus Extended Spectrum Beta-lactamase Producing Gram Negative Bacteria: *In vitro* Susceptibility Study

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Abstract

Objective
This study was performed to know the *in vitro* susceptibility of the ESBL positive gram negative bacteria to the non-beta-lactam antimicrobials so that these can be considered as alternate for the treatment of infections caused by such bacteria.

Study design
Descriptive observational

Place and duration of study
This study was performed at the department of pathology, Dr Sulaiman Al Habib Hospital, Olaya Medical Complex, Riyadh, Kingdom of Saudi Arabia during Septmber 2010 to December 2011.

Patients and Methods
A total of 204 ESBL producing gram negative bacteria were isolated during the study period from the specimens sent from in patients (108) and out patients (96). The specimens processed were, urine (124), wound/pus (40), vaginal swab (12), blood (10), tracheal aspirates (10), sputum (07) and prostatic secretions (01). The isolates included: *Escherichia coli* (133), *Klebsiella pneumoniae* (41), *Proteus mirabilis* (14), *Enterobacter cloacae* (6), *Enterobacter aerogenes* (06), *Morganella morganii* (2), *Citrobacter freundii* (1) and *Pseudomonas aeruginosa* (1). The antibiotic susceptibility testing and ESBL detection was performed by Vitek 2 automated system. ESBL positivity was also confirmed by double disk synergy test. Appropriate control organisms were included to maintain the quality control.

Results
Susceptibility of these ESBL producing isolates to various non-beta-lactam antimicrobials was as follows: co-trimoxazole 31.38%, quinolones 39.22%, gentamicin 46.08%, nitrofurantoin 57.25%, tobramycin 58.44%, amikacin 93.14%, tigecycline 91.66% and colistin 99.5%.

Conclusion
As per the susceptibility pattern, amikacin, tigecycline and colistin proved good alternates to carbapenems for the treatment of infections caused by ESBL producing gram negative bacteria.

Key words
Extended Spectrum Beta-Lactamases, Non Beta-lactams.

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Introduction
Increasing antimicrobial resistance is not a new phenomenon as the rate of multidrug resistant bacteria is continuously rising since long. Unfortunately the possibility of overcoming this problem with newer antimicrobials is becoming smaller.[1] Bacteria are evolving various mechanisms to become resistant to the available antimicrobials. Production of beta lactamase with extended spectrum beta lactamase (ESBL) is now quite common amongst gram negative bacteria isolated from the hospitalized as well as the out patients. After discovery in 1990s for the first time, now there are more than 170 TEM, 120 SHV and over 90 CTX-M ESBLs known.[2] These enzymes are produced by enterobacteriaceae mostly including *Escherichia coli* and *Klebsiella pneumoniae* but other gram negative bacilli such as *Proteus* species, *Salmonella* species and *Pseudomonas aeruginosa* have also been found positive for these.[3,4] The infections caused by these ESBL positive bacteria are leading to high mortality rates in addition to heavy burden on hospital resources.[5] These isolates are resistant to all the broad spectrum penicillins, cepahlosporins and the monobactems. These are susceptible to cephamycins and carbapenems only, which make the treatment of infected cases a real challenge.[6] In addition, the ESBL positive isolates exhibit co-resistance to many other classes of antimicrobials but a few of these still remain effective.[7]

There is a need to do the surveillance of alternate antimicrobial susceptibility against ESBL positive isolates so that these can be considered for treatment if so required. Keeping in view the same we performed this retrospective study on the ESBL positive isolates from our hospital so that some alternate antimicrobials can be considered for treatment of such cases in addition to an existing option of carbapenems.

Material & Methods
This study was carried out from September 2010 through December 2011 at Dr Sulaiman Al Habib Hospital. All the positive culture reports for various specimens sent to the microbiology section of the laboratory department were

reviewed. Those positive for gram negative isolates having ESBL production were included in this study. Antimicrobial susceptibility of the isolates and ESBL detection was performed by VITEK 2 system (bioMerieux, Durham NC) and interpreted according to CLSI guidelines[8]. Confirmatory testing for ESBL presence was performed by disk diffusion using ceftazidime and cofotaxime disk with and without clavulanic acid (CA). Isolates showing a CA effect, defined as an increase in zone diameter of ≥ 5mm in the presence of CA in addition to being classified as ESBL positive by the VITEK 2, were considered to be positive for ESBL. ESBL negative *Escherichia coli* (ATCC 25922) and ESBL positive Escherichia *coli* (ATCC 36218) were included as negative and positive control strains respectively.

Results

A total of 204 ESBL positive gram negative bacilli were isolated during the study period from various specimens (Fig 1). Out of the total isolates, 128 (62.7%) were from female patients and the rest 76 (37.3%) from male patients; 108 (52.94%) from inpatients and 96(47.06%) from the out patients. The age range of the patients was from a few months to 82 years.

Various ESBL producing gram negative bacilli isolated are shown in table 1. Susceptibility of these ESBL producing isolates to various non-beta-lactam antimicrobials are shown in figure 2. Colistin (99.5%) was the most sensitive while Co-trimoxazole (31.38%) was least.

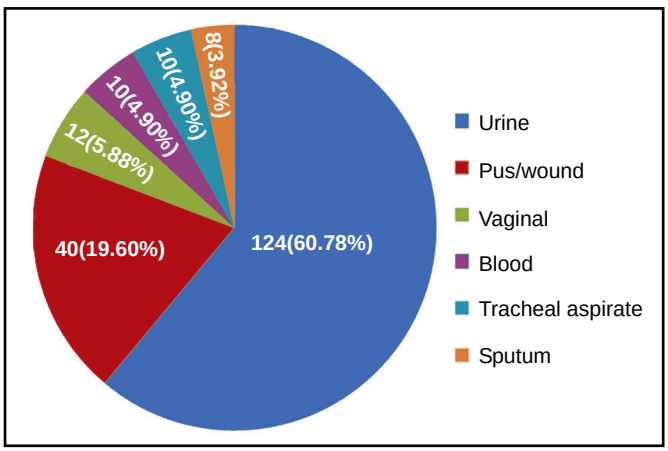

Fig 1. Various specimens yielding ESBL producing gram negative bacilli (n = 204)

Discussion

The last twenty years have witnessed extensive use of extended spectrum cephalosporins both as empiric and prophylactic therapy especially in the hospitalized patients.[9] Before this, penicillinase resistant penicillins, broad spectrum penicillins and first generation cephalosporins were the main defense against gram negative bacterial infections.[10] Over use of the cephalosporins has basically led to the emergence and spread of ESBL bacteria.[11] In this study we have found 65% of the

Table 1: Various ESBL producing gram negative bacilli isolated

Isolate	n	%
Escherichia coli	133	65.19
Klebsiella pneumoniae	41	20.09
Proteus mirabilis	14	6.86
Enterobacter cloacae	06	2.94
Enterobacter aerogenes	06	2.94
Morganella morganii	02	0.83
Citrobacter freundii	01	0.49
Pseudomonas aeruginosa	01	0.49

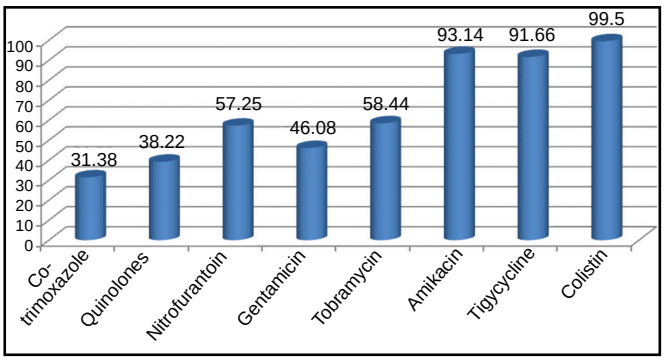

Fig 2. Percent susceptibility of the ESBL producing gram negative bacilli to various non-beta lactam antimicrobials

ESBL producing isolates to be *Escherichia coli* and 20% *Klebsiella pneumoniae*. Kader *et al* reported very near figures of 58% for *Escherichia coli* and 16.6% for *Klebsiella pneumoniae* from Saudi Arabia.[12]

ESBL bacteria have grave consequences for the infected patients. Mortality amongst those infected has been found to be four times greater than that of the patients infected with non-ESBL producing strains.[13] The treatment options for these bacterial infections are quite limited. Out of the beta lactam antimicrobials, carbapenems are the only ones uniformly active against ESBL producing bacteria.[14] But, due to the high cost and having parenteral use only, these cannot be administered to all the patients especially the out patients. More over about 60% of our ESBL producing isolates were from urine, so we need some antibiotics other than carbapenems to treat urinary tract infections. Quinolones and co-trimoxazole can be considered as good oral alternates but low sensitivity of our isolates to quinolones and co-trimoxazole limit their usage. This susceptibility is much lower to that reported by Schwaber *et al* from Israel, but higher than the reported by Roshan *et al* from Pakistan and Grandesso *et al* from Italy.[15-17]

Amongst the injectable non-beta-lactams, tobramycin was having an intermediate level of susceptibility while amikacin, tigycycline and colistin had good susceptibility. This corresponds

to other studies.[16] Tigecycline and colistin are the only antibiotics currently available for carbapenamase positive bacteria which are resistant to most of the other antimicrobials.[18] These can be used for the treatment of ESBL positive bacteria also if there is some contraindication to the other antimicrobials.

Nitrofurantoin also had an intermediate level of susceptibility for the ESBL producing urinary isolates. Babypadmini and Appalaraju found 89% of the ESBL positive isolates to be susceptible to it and have recommended it as an alternate.[19]

There is a need to control the emergence and spread of multi-resistant bacteria through appropriate and careful use of antimicrobials. Surveillance for antimicrobial resistance should be undertaken continuously so that the treating physicians can be given guidelines regarding their appropriate use.

Conclusion

Co-trimoxazole, quinolones and gentamicin have limited value due to low susceptibility, tobramycin and nitrofurantoin have an intermediate position while amikacin, tigycycline and colistin due to their high susceptibility, are good alternates to the carbapenems for the treatment of infections caused by ESBL positive gram negative bacteria.

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1st Joint PAP Meeting including IDSP

A unique meeting "*1st Joint Conference of PAP" by* Pakistan Association of Pathologists and other Societies was held on 7th-9th December 2012 at AFIP Rawalpindi and at the Convention Centre, Islamabad. IDSP was invited as participant. A number of different sub-specialties from pathology were taking part and IDSP was honored to have been invited at the august forum.

On Day 1, there were many workshops; IDSP organized two workshops. The 1st Workshop *"Quality management in Clinical Microbiology Laboratory"* was conducted by Prof Afia Zafar, Dr Kausar Jabeen, Dr. Naima Fasih and Dr Joveria Farooqi. The 2nd Workshop *"Biosafety – Concepts & Management"* was with Pakistan Biological Safety Association (PBSA) and conducted by Dr Erum Khan and Col Aamer Ikram. Both of these interactive workshops were very well attended.

The inauguration ceremony was held at the Convention Centre, Islamabad on same day. President IDSP spoke on the occasion and highlighted ID issues in Pakistan and urged everyone to join hands with IDSP activities. He congratulated the organizers for holding such an excellent and splendid conference.

On Day 2, combined plenary session was followed by IDSP plenary within one of the Convention Center's halls. The speakers included: Dr Altaf Ahmad from Indus Hospital (*WHO approved methods of diagnosing TB and its implementation in our patients*), Dr Asim Beg from Aga Khan University (*Challenges and opportunities for malaria in Pakistan 2012*), Brig Babar Cheema from Military Hospital (*Clinical perspective for TB diagnostics*) and Dr Ejaz Khan from Shifa International Hospital, Islamabad (*Why, when, where not to use antibiotics?*). The hall was packed with audience and there was a lively Q&A at the end.

In the afternoon there were free paper sessions. A total of 15 papers were read covering various topics including TB, antibiotic resistance, malaria etc. Many ID-related posters were presented. An IDSP desk was placed and lot of material was distributed among the audience. In brief it was a very fruitful day for IDSP as it received an overwhelming exposure.

2nd Symposium; Pediatric Infectious Diseases – An Update

Infectious Disease Group, Pakistan Pediatric Association and Infectious Disease Society, Pakistan (IDSP) held a **Pediatric ID Symposium** on 6th October 20 12 at Quaid E Azam International Hospital, Islamabad.

This interactive meeting was aimed to overview some important preventive and therapeutic aspects of some current issues in pediatric ID and help promoting ID awareness and encourage such academic activities. It was a well-attended session with around 100 participants including pediatricians, general practitioners and microbiologists and other physicians and students from the twin city of Rawalpindi-Islamabad.

Dr. Haseeb Khawaja, ER Physician from Shifa International Hospital gave *"An update on Rabies"* and spoke about problems in Pakistan and highlighted some key management issues.

This was followed by a talk by Col. Dr M Afzal, Head of Pediatrics, Quaid E Azam International Hospital, who shared ongoing research *"New vaccines-an update"*. Dr. Ejaz Khan then lamented poor antibiotic prescriptions and how to avoid them in common infections in his presentation, "*Bad antibiotic prescriptions: a rejoinder"*. Lastly Prof. Ashraf Sultan, Chairman Infectious Disease Group, PPA from Lahore spoke about the threat and how to deal with dengue in *"Dengue fever in children"*.

There was active participation from the enthusiastic audience in the Q & A session. CEO, Quaid E Azam International Hospital Dr Shaukat Ali Bangash gave a brief about the new hospital and thanked the participants. Overall the symposium was regarded as a great success.

Curbside Consultation

An attempt at humor in infectious diseases

I am getting old but good to know that I can spread some pearls of wisdom (if someone will take them). The other fact is that I am not good at writing humor! But I see so much of it around that sometimes I laugh and cry (and cringe, you will see what I mean). I coined this "blog" as "*CURBSIDE CONSULTATION.*" In simple terms it is defined as taking advantage of someone when they are vulnerable. The medical dictionary describes it as "An informal and unofficial consultation obtained from a health professional by either a lay person or a fellow health care professional…". It has many benefits if used for simple ailments. As physicians and pediatricians in particular, we see this a number of times and we hate it! Many a times we are asked for quick remedies or queries by colleagues, friends and strangers alike in odd places, occasions and times! "Doc I have a headache (so what)"; "You saw my son last year and he is running a fever now…what can I give him? (paracetamol please!); "Can you please tell me what to do with a 2 year old with rash and fever (show him!)…" Here I will attempt to give curbside consults using my clinical (ID mostly) experiences that I feel will help highlight some of the blunders that are so common among us like Shakespeare's "A comedy of errors" pales in front of these follies!

Infectious diseases are rampant around in this pure land and we are mistreating it with abundance and I decided long time ago to bring it to everybody's attention (as if they don't know it yet). My stories relate to my pediatric patients that I see and every day, there is something to be learned (and ashamed). Many errors are obvious to my aging brain and mostly occur in diagnosis, interpretation and (mis) management in infectious diseases! Here I will start with my first blog and hope to bring in more similar stories in future (and hope I succeed). Please bear this new piece of blog (aptly called "A comedy of errors") in our journal in this new digital age it will be mostly short sentences (sometimes long), new acronyms, loose talk, no attempt to use proper grammar, use of local language and lots of exclamations!!!!!

Blog 1: A comedy of errors

One of my jobs is to see patients and manage them accordingly. Often I spend a lot of time knowing what happened to them before. Many times this exercise makes me arrive at an accurate diagnosis or reveals the horrors that they went through! If it is likely that I am dealing with an infection then I revel in digging out details and sequence of events. My teachers have told me that an event no matter how small or subtle in history, tell tale physical signs or labs are enough to be a golden clue. Last week patients are enough to prove my point!

A 4-year female came with her distraught mother with recurrent urinary tract infection (UTI) since last 6 months. Going through her illness details it was more of URI symptoms since last 6 months. After 3 days of fever (which can happen in childhood URIs) she had a urine analysis, which showed WBC 4-10/HPF with lot of epithelial cells but no culture was done. The child was given an antibiotic (cefixime) for *UTI* for 10 days and a repeat urine analysis after 2 weeks showed 10-20 WBC/HPF (again low and behold no culture was done). More important to note was that the child had no symptoms at all. I don't know what was the doctor thinking (more infection, resistance?) but he was happy to dole out more antibiotics). His prescription (I was excited and took a snap to show at many of my future meetings against antibiotic use) showed another course of cefixime (another brand of course) for next 2 months! The poor patient took the antibiotic diligently and then after 2 months a repeat urine analysis showed numerous pus cells but no culture was done and was asymptomatic with no urinary complaints. Thinking (if there was any!) this was persistent or recurrent the doc gave another (not again!) course of cefixime (another brand of course) for 2 months!

The only workup was a normal ultrasound abdomen. And if that was not enough cefixime was with double dose for 2 months. So this poor patient came after 4 months course (that's what the mother was told by the doc!) of cefixime because of some pus cells in the urine! It behooves me to think that one would label someone with UTI just because of some pus cells with no symptoms and no culture (happened to witness that a lot). But makes me pull my hair (whatever left) to see for real that this child was given oral cefixime for more than 4 months with no UTI as far as I am concerned. Ever since cefixime has been introduced, its use has been exponential. Reason: oral, third generation (must be very potent then?) and once-twice a day dosing. So thinking it as a magic potion I have seen umpteenth times (I mean literally) being given from headache to toe infections! It cannot get more scandalous! But the curbside question is, what must I do if there are pus cells in the urine? My answers are ensure correct method of urine collection that is immediately processed and follow it with urine culture if clinically indicated or urine is abnormal.

The orthopedic surgeon calls me for a consult about a 10-year-old girl with swollen knee since 2 weeks after an apparent trauma and fever next day. She is able to walk and knee is warm, swollen and doughy but has good range of motion. The local orthopedic had done 6 aspirations!! I don't know for what but each time was either unsuccessful or had some blood only. No culture was ordered at all. Looking at the prescription first antibiotic was oral cefixime (I would easily strangle those who invented this antibiotic for being used so senselessly!), followed by oral augment and then IV cefuroxime and gentamicin since last few days. So the poor patient was not worked up properly, had too many invasive taps, no cultures done and then given antibiotic that was inappropriate with wrong route! I wanted to cry! The curbside question is, which bug and what antibiotic should I use for bone and joint infections? Easy answer! always (and usually only) cover *Staph aureus* with either an anti-staph

penicillin or first generation cephalosporin.

Then worried parents brought a 13-year-old girl, as she was the only child who has been sick with recurrent fevers (actually 2-3 brief episodes/year) and was each time labeled as *typhoid* over last 4 years! Multiple tests had been done that were normal except numerous Typhidot tests (there were 6 that I counted) that were positive or borderline. Each time she took a course of antibiotic. Examination was normal and the girl was thriving like anything! Now this is not the first time that I have seen Widal or Typhidot test being done for fevers and a positive test always taken as a confirmation for typhoid! I feel disgusted at seeing heaps of these tests being done left right and even patients proudly claim it even if there is no clinical evidence (some of them have low grade fever of not more than 99ºF). They have only one doctor who has been (mis) treating her and when I explained along with my students chipping in that she probably never had typhoid their amazement was exciting to watch. I felt like going and giving that doc my heart out on diagnosis of typhoid and obsoleteness of these serological tests in this day and age! But I spared him my wrath that day (and fumed at my helplessness!). So the curbside question here is, should I rely on these dubious and obsolete serological tests for diagnosis of typhoid? The answer is a big fat no and as I tell my youngsters throw these tests in the dustbin! You usually have more reliable ways such as clinical picture, normal or low WBC and of course the blood culture.

Lastly, as I was leaving my office the other day, my secretary said a certain pharmaceutical person wants to meet me. Two gentlemen tried to convince me that lincomycin should be used for upper respiratory tract infections with some of their ads! I have hardly recommended the drug ever in my life but know for sure that it is a favorite antibiotic among our medics in the streets! It is not the first line drug anyway for any major or minor infections (on top, it has serious side effects such as pseudomembranous colitis as well). I explained that firstly most URTI are viral and no antibiotics are needed! The senior pharma was amazed and wanted to know why antibiotics cannot be given for viral URIs! Now I could not take that! I said that if I have to use an antibiotic for bacterial URTIs (otitis media, sinusitis, pharyngitis) then I have simple drugs such as amoxicillin that is recommended in most situations and works! More recent studies have shown and recommended that antibiotics are less useful even for otitis media, sinusitis and bronchitis. Mostly strategies used to combat the increasing use of antibiotics have proven very effective such as no antibiotic in common viral infections, deferring an antibiotic prescription or a close follow up.

"Patient, clinician, and system factors influence unnecessary antibiotic prescribing for viral acute respiratory infections (ARIs). When patients seek care for ARIs, they use a variety of tactics to obtain antibiotic prescriptions. Both clinicians and patients tend to overemphasize the importance of purulent secretions, whether from the nose or lung, in deciding whether an antibiotic is needed. Antibiotic prescribing increases with clinician age and number of years in practice, and antibiotics are more likely to be prescribed for ARIs in urban and nonteaching practice settings.……."

As we continue to do blunders as briefly narrated here I see little hope in educating simple facts. Patients must be seen as a whole with emphasis on getting the facts right, know our basic medical knowledge (microbiology for sure), when antibiotics work, which tests to order and how to interpret them correctly! I just hope small efforts from all of us will some day make a change but continue we must. Be simple and keep to the basics!

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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.

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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 Editor, Infectious Diseases Journal of Pakistan, Department of Pathology and Microbiology, The Aga Khan University, Stadium Road, P.O. Box 3500, Karachi 74800, Pakistan.** An electronic copy of the manuscript must also be sent to maahin1@yahoo.com and 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 andFigures 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