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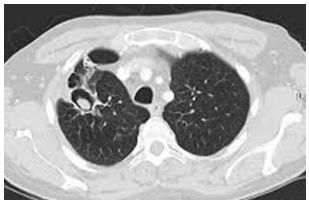

CT scan image of a patient showing soft tissue lesion within a lung cavity surrounded by rim of air.

Courtesy: Dr. Ghulam Murtaza, Aga Khan University, Karachi.

The Case for Viral Hemorrhagic Fever (VHF) Epidemic Preparedness: A priority health issue in Pakistan

Growing concern over hemorrhagic fevers especially after the West African Ebola epidemic has forced many international public health authorities to trigger epidemic response training throughout the world.[1] The World Health Organization (WHO) has initiated training of country-specific rapid response teams in Ebola preparedness in its AFRO, EMRO, WPRO, PAHO, and SEARO regions. The probability of encountering Ebola in a number of these regions with the exception of the AFRO region, however, remains low.[2] The overreaching benefit though is the emergency preparedness these trainings inculcate for other similar and currently prevalent hemorrhagic fevers in tropical and subtropical regions: dengue, yellow fever, Lassa fever, and Crimean-Congo hemorrhagic fever (CCHF).

Recurrent outbreaks of dengue and CCHF have occurred in Pakistan relentlessly.[3, 4, 5] The most recent outbreak of both dengue and CCHF is currently ongoing in Sindh, Punjab, and KPK. The true magnitude is difficult to glean from the reports of hospital deaths as these do not account for either those deaths that occur at grassroots level, nor for the morbidity resulting from these infections and the resulting loss of QALYs. The economic impact is magnified by co-occurring epidemic illnesses such as influenza, measles, and varicella. Specifically for Pakistan, natural disasters such as floods and earthquakes may enlarge this effect even further or may in fact be the triggering event. Such vicious cause-and-effect balances make even smaller-scale VHF outbreaks disproportionately difficult to manage in the context of our weak health system. As much as this is true of any infectious disease, the public anxiety and lack of awareness associated with VHFs, compounded by the alarm triggered by the Ebola epidemic, make control of these fevers a gargantuan but futile exercise for public health professionals.

A two-pronged approach of prevention and epidemic response training is usually employed to mitigate the effects of such outbreaks.[6, 7] There are also several levels at which preparedness efforts should take place: national, provincial, healthcare facilities, and the grassroots. Physicians and healthcare workers and epidemiologists from each level serve as prime targets of training to facilitate rapid and efficient response to early warning reports of hemorrhagic fevers. Central to this effort, and concurrent to the training, is laboratory capacity development to confirm early diagnoses. This issue of the Infectious Disease Journal of Pakistan includes a photo essay by participants presenting one such effort by the Aga Khan University in collaboration with the NIH-Fogarty's Global Infectious Disease training and capacity building grant to train physicians and epidemiologists in prevention, control, and management of VHFs in Pakistan.[8] Such trainings impact not only physician training and development but also indirectly contribute towards impacting public policy through advocacy and education of the masses through the several physician-patient interactions where doctors aware of the epidemiological impact of such illnesses influence public awareness and opinion.

As a next step to these workshops it is important to engage policy-makers to initiate preventive efforts at the provincial level. This can be planned through assembly appearances and public messages delivered through the MMIDSP platform to the government as well as the public, respectively. This is in fact the essential step required to enforce policy-making and implementation. As knowledge of the effectiveness of preventive efforts increases through formative research, it is becoming increasingly important to consider these educational and engagement activities to successfully prevent and control VHFs. It is hoped that through such initial efforts, we will be able to create a forum of advocacy for control of many such infectious diseases with outbreak potential.

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ORIGINAL ARTICLE

Fecal Carriage of "Extended Spectrum βeta-lactamases" and Ampc producing Gram-negative Bacilli in Hospitalized Patients

Maliha Ansari, Tehmina Munir, Nadia Saad, Amira Hafeez, Sabahat Rehman, Sara Najeeb, Mahwish Latif, Mehreen Gilani

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Abstract

Background
Emergence of ESBL and AmpC producers as disease agents demand an unceasing attention as it has led to increase in the rate of morbidity and mortalitydue toineffectiveness of empiric antibiotic therapy. Here, we aim to determine fecal carriage of ESBL and AmpC producing gram negative bacilli as normal flora in stool samples of hospitalized patients.

Methodology
This cross sectional study was conducted at the department of Microbiology, Army Medical College, National University of Sciences and Technology from January 1st, 2014 to May 1st, 2014.Hundred random stool samples, collected from hospitalized patients irrespective of age and gender, were included in the study. Stool samples were inoculated onto Mac Conkey and XLD media and incubated at 37°C for 24 hrs. The isolates were identified by colony morphology and by applying API 20 E. Isolates identified as *Salmonella* spp, *Shigella* spp. and Enteropathogenic *E.coli*were excluded from the study. Phenotypic detection of ESBL was carried out by applying antibiotic discs (Oxoid,UK) of aztreonam 30 ug and ceftriaxone 30 ug placed at a distance of 25 mm from the combined disc of amoxicillin /clavulanic acid (30 µg) and incubated 37°C aerobically for 24 hrs. ESBL production was identified by observing the enhanced zones of inhibition between any of the beta lactum disc (aztreonam and ceftrioxone) and the disc of amoxicillin /clavulanic acid. Phenotypic detection of AmpC beta lactamases was carried out by applying cefoxitin(30 µg) disc. Strains were labelled as AmpC producers when the zone diameter around cefoxitin disc demonstrated was less than 18 mm. (standard disk diffusion breakpoint forcefoxitin zone diameter <18 mm according to CLSI).

Results
Out of 100 fecal samples that were collected 75 were found to be ESBL producers. Among those ESBL *E.coli* were 72% (n=54) ,*K.pneumoniae* were 17%(n=13) , *Enterobacter cloacae* 2.6% (n=2) , *Klebsiella oxytoca n=2(2.6%) , Citrobacter freundii 1.3%(n=1), Proteus* mirabilis 4%(n=3). AmpC production among these collected samples were (n=16)16%. Out of these, 62% (n=10) were E.coli, 31.75%(n=5) were *Klebsiella pneumoniae* and 6.25 % (n=1) were *Enterobacter cloacae.*

Conclusion
Escherichia coli and *Klebsiellapneumoniae* were found to be the most prevalent ESBL and AmpC producing Gram negative organisms inhabiting the gastrointestinal tract of hospitalized patients.

Key Words
Enterobacteriaceae, *Escherichia coli, Klebsiellapneumoniae*

Introduction

Extended spectrum beta lactamases (ESBL) are the plasmid mediated enzymes that are produced by some strains of microorganisms. These enzymes hydrolyze oxi-amino beta lactams and make the microorganisms resistant or less susceptible to narrow and broad spectrumcephalosporins.[1]

However, there is no effect of these enzymes on carbapenems and cephamycins.[2] ESBL producers are extremely sensitive to inhibition by clavulanic acid and tazobactam.[1,2] Another class of beta lactamases is Ambler class C (AmpC). AmpC producers are resistant to β-lactamase inhibitors and resistant to all third generation cephalosporins, cephamycins and monobactams. They show susceptibility to fourth generation cephaloporins (cefepime and cefpirome). Genes for AmpC beta-lactamases are located on either chromosomes or plasmids.[3]A study on ICU pathogens, conducted at several intensive care units in Germany, revealed the presence of ESBL strains among enterobacterial isolates for the first time.[3] It was also detected that the expression of this enzyme is transferrable by conjugation to *Escherichia coli.*[4] The ESBL producing strains of *Kp* were also reported from different hospitals in France.[3,6]

*Kp and Escherichia coli*are among the most important causes of severe bacterial infections in hospitalized patients.[6] The enhancement in resistance of Gram-negative bacteria is primarily

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due to portable genes on plasmids that can easily disseminate through bacterial populations.[6] Much of this propagation is concealed as the resistant clones are carried in the normal human flora and manifest only when they are the cause of endogenous infections.[7]

The increase in the proportion of carriers aggravates the hazard that other individuals will become carriers as a result of human-to-human transmission of resistant bacteria.Transmission of such bacteria usually ensuesthrough fecal-oral route, both directly and indirectlyvia hand contact with healthcare staff, and is alsofacilitated by overcrowding.[8] Thereis serious concern regarding the increased prevalence of spontaneous bacterial peritonitis in hospitalized patients of liver cirrhosis.[8] In a study conducted by Shiekhbhi S *et al* the most possible cause of infection detected was the haematogenous dissemination or translocation of the intestinal flora. The over growth of intestinal bacteria can also worsen the condition. Studies have shown that Gram-negative Enterobacteriaceae such as *Escherichia coli* (*E. coli*) were the most common isolated organisms in spontaneous bacterial peritonitis in patients of liver cirrhosis.[8]

Another critical issue is the difficulty in the treatment of appendicitis complicated with perforation and peritonitis. The main cause of peritonitis in appendicitis are resistant strains of fecal flora that infect the peritoneum after perforation. Studies have shown that mostly isolated bacteria are ESBL producing gram negative bacilli. The occurrence of such cases has resulted in increased mortality rates due to lack of effective treatment against these resistant bacteria.[9]

This study was undertaken to identify ESBL and AmpC producing strains in order to reduce the associated risk factors and also to design a treatment strategy in the earlier stages of disease to prevent fatal consequences.

Objective

To determine the frequency of ESBL and AmpC producing Gram negative bacilli present as normal flora in stools of hospitalized patients.

Methodology

It was a cross sectional study, carried out in the Department of Microbiology, Army Medical College, Rawalpindi, National University of Sciences and Technology, Islamabad, affiliated with the Military Hospital, Rawalpindi, an eleven hundred bedded tertiary care facility. Random stool samples, collected from hospitalized patients, irrespective of age and gender, were included in the study. Isolates identified as *Salmonella,Shigella* and Enteropathogenic *E.coli* were excluded from the study**.** Stool samples were inoculated on to Mac Conkey and XLD agar and incubated at 37ºC for up to 24 hrs.Identification of organisms was done by using API 20E. (Bacterial suspensions of isolates equivalent to 0.5 McFarland's turbidity standards were prepared and inoculated on to Mueller- Hinton agar plates. Phenotypic detection of ESBL production was carried out by double-disc diffusion (DD) testing method. The sensitivity discs of aztreonam (30 µg) and ceftriaxone (30 µg) placed at a distance of 25 mm from the combined disc of amoxicillin/clavulanate disc (20/10 µg) and incubated at 37ºC aerobically for 24 hrs. ESBL production was identified by observing the enhanced zones of inhibition between any of the beta lactam disc (aztreonam, ceftriaxone) and the combined disc of amoxicillin/clavulanate disc.Strains producing ESBL were defined as those showing zone enhancement of 5 mm between amoxicillin/clavulanate and either of ceftriaxone or aztreonam.Phenotypic detection of AmpC beta lactamases was carried out by applying cefoxitin(30 µg) disc. Strains were labelled as AmpC producers when the zone diameter around cefoxitin disc demonstrated was less than 18 mm. (standard disk diffusion breakpoint forcefoxitin zone diameter <18 mm. according to CLSI).

Table 1. Frequency of AmpC Producers in stool samples

	Total (n=100)	%
Amp C producers	16	100
E. coli	10	62
K. pneumoniae	5	31
Enterobacter cloacae	1	6

Results

Among the 100 fecal samples that were collected 75 were found to be ESBLs. Among those ESBL *E.coli* were 72% (n=54),*K.pneumoniae* were 17%(n=13), *Enterobacter cloacae 2.6%(* n=2) , *Klebsiella oxytoca 2.6%* (n=2) , *Citrobacter freundii (1.3% n=1), Proteus* mirabilis 4% (n=3).AmpC production among these collected samples were (n=16)16%. Out of these, 62% (n=10) were E.coli, 31.75%(n=5) were *Klebsiella pneumonia* and 6.25 % (n=1) were *Enterobacter cloacae.*

Discussion

There is an increasing trend in the feacal carriage of ESBL producing Enterobacteriaceae in our set up as can be seen in the study done by Anjum R *et al*. in 2012.[10] The study was conducted at the same tertiary care hospital of Rawalpindi. There is a significant increase in the fecal carriage of ESBL *E.coli* within a period of less than one and a half year. In 2012, the prevalence of *E.coli* among the hospitalized patient was 69% whereas in my study conducted in 2014, it has increased to 72%. This rapid rise in ESBL carriage is quite worrying. The concerned hospital setup was informedabout necessary precautions to be taken to avoid further spread.

Presence of AmpC producers among normal feacal flora is alsoalarming. In this study the prevalence of AmpCand ESBL production among the fecal samples collected was relatively

higher as compared to study done by Ahmed, S. F *et al* in Libyan community in 2013.[11] In her study, AmpC producing enterobcateriaceae were 6.7% and ESBL producers were 13.8%

The rate of fecal carriage of ESBL-producing *Enterobacteriaceae* in this study is similar to that reported in Indonesia by Severin Ja *et al.*[12] In this study, 107 ESBL-positive strains included n= 68 (62%) *E. coli*,*K.pneumoniae* n=35 (32%) and four other Enterobacteriaceae (7%). Faecal carriage of ESBL-producing Enterobacteriaceae as dominant flora in Indonesia was almost exclusively hospital-associated at the time of study.[12] The results from this study are almost parallel to the results of the study done in our setup. Thus, the significance of detecting the presence of ESBL producing strains among normal flora has become evident, as it will help to direct the treatment strategy towards specific cause.

A very important risk factor regarding fecal carriage of ESBL producing *E. coli* is the presence of strain 104:04 that inhabit the human intestine.[13] This particular strain had been a major cause of a deadly outbreak in Central Europe in 2011(enemy within us) as described by Helge Karch *et al* in their study.[13] It was found out that the causative agent of this outbreak was isolated from the fecal samples of the patients not from food. According to the data collected by Helge Karch *et al* approximately 4000 people were infected in Germany only. Out of 4000, 900 cases led to haemolytic uremic syndrome (HUS) ensuing death in 54 patients.[13] In southwest France, another outbreak of bloody diarrohea was reported .This outbreak led to 15 cases of bloody diarrohea. 9 out of which developed HUS.[14] Similar outbreaks had been reported on prior occasions, two in Germany (both in 2001), two in France (2004 and 2009) and one each in Korea (2005), Georgia (2009) Italy (2009),and Finland (2010)[15]. This data puts emphasis on the importance of detection of such resistant strains that are present as gut flora, in order to decrease further chances of outbreaks in near future.[14,16] These findings demand extensive follow-up evaluations in future in order to identify the changing trends of the antibiotic resistance in the community and clinical settings, and in due course predict the identification of microorganisms with the ability to cause pandemics in the future.[16] It has been observed and reported that outbreaks are often caused by ESBL-

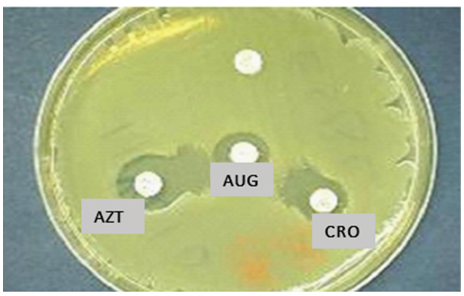

Fig 1. Key-hole phenomenon showing phenotypic presentation of extended spectrum β–lactamase production

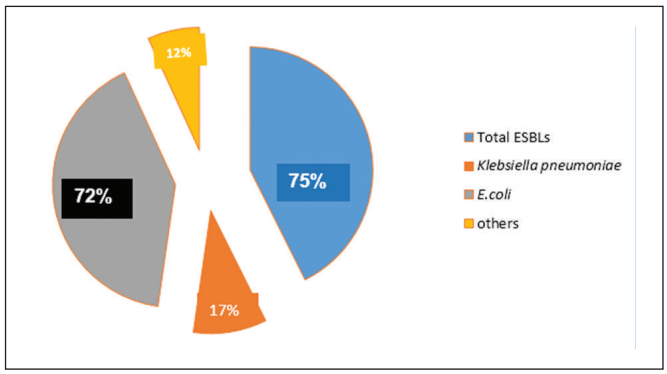

Fig 2. Frequency of fecal carriage of ESBL producers among normal intestinal flora

producing *K. pneumoniae* than by ESBL-producing *E. coli* [17], and this suggests that health care worker has to take extra precautions around patients with ESBL-producing *K. pneumoniae* in the hospital settings.

Sankar S *et al* conducted a study in Tamil Nadu , India, and demonstrated the same results as ours with the increasing frequency of ESBLs in normal gut flora (50.29%)[17] and in a study by Azim. A *et al*, the prevalence was 92%.[18] However, in the United Kingdom, in a study by Wickramasinghe, N *et al*, there has been a decrease (11.3%) in the prevalence of ESBLs in *E. coli*. A possible justification for this could be the considerable changes in prescribing antibiotics that have been made in order to lessen *Clostridium difficile* infections.[19]

The results of all the studies mentioned, show a significant inclination towards the spread of ESBL and AmpCproducing bacteria, especially in the hospitals of Asia. During the period of 2005-2007 Kaier *et al*.[20] observed that hand disinfection has a significant role in preventing infections with ESBL-producing bacteria, and it was also found out that the use of alcohol-based hand sanitizers had resulted in lowering the incidence of such strains.[20] Another important factor is probably the call for establishing a proper sewage systems mainly in the regions of Asia and Africa. It should be ensured that people in developing countries are supplied with food and water without fecal contamination as it would solve many problems.[15,16]

This study reveals the emergence and spread of ESBL and AmpC producing *E.coli* and *K. pneumoniae* in patients in our setup. A limitation of our study is that the samples were collected from patients at a single tertiary-care medical center in Rawalpindi, Pakistan. Investigations of ESBL and AmpC producing Enterobactericeae on advanced level should be done from other regions of Pakistan to elucidate the epidemiology and health effects of emergence of this specific pattern of resistance in community and clinical settings. It should be made mandatory in all laboratories to detect and report suspected or proven ESBL and AmpC producers. The report must also include a note that such resistant strains may result in therapeutic failure

with antimicrobials such as penicillin, aztreonam and all cephalosporins, irrespective of their in vitro susceptibility. Thus, it is advised that the empirical treatment strategies and antibiotic policies should be carefully formulated for patient management in such clinical settings. Carbapenems (doripenem, imipenem and meropenem) currently are the only effective treatment.

Conclusion

The rate of fecal carriage of ESBL andAmpC producing bacilli has substantially increased among hospitalized patients since 2012. Predominant species found were *Escherichia.coli* and *Kp.* These resistant fecal commensals may be a major cause of cross infection in hospitalized patients.

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ORIGINAL ARTICLE

Optimization of Protocol for Disc Diffusion of Antifungal Drugs Directly from Blood Culture Bottles Positive for *Candida* Species

Haresh Kumar, Joveria Farooqui, Raunaq Mahboob, Kauser Jabeen, Afia Zafar

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Abstract

Background
Candidemia has emerged as major concern in hospitalized patients on broad spectrum antibiotics, underlying gut disease, central venous lines and immunosuppressed states. To ensure this, antifungal susceptibilities have to be made available as early as possible. A study was designed to optimize the method of performing direct antifungal susceptibilities by disc diffusion from blood culture vials positive for yeasts to obtain susceptibility results at least 24h earlier. Two methods, centrifuged and uncentrifuged, were compared to the standard approved method of disc diffusion from isolated colonies.

Methods
Twenty-four blood culture bottles positive for yeasts from 18 patients and two simulated blood cultures with control strains were included in the study. Most of the isolates were *C. albicans*, *C. parapsilosis* and *C. tropicalis*. 100µl of centrifuged and uncentrifuged blood culture broth were spread on Mueller-Hinton agar with dextrose and methylene blue to test voriconazole, fluconazole and amphotericin by disc diffusion. Zone diameters obtained from the two direct methods at 24 hours were compared with those obtained by recommended method from isolated colonies.

Results
Paired t-test results showed there was no significant difference from standard for any antifungal by either method. However the variance of amphotericin zone diameters obtained by centrifuged method was unequal to that of standard methodology (F-test p-value=0.007). In addition, centrifuged method resulted in one very major and two minor categorical errors compared to only one minor error by uncentrifuged method.

Conclusion
Uncentrifuged blood culture broth may be used to perform antifungal susceptibility testing from vials positive for yeasts as it resulted in fewer reporting errors. This method can now be used to evaluate the performance of direct antifungal susceptibilities on a larger scale.

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Key words
Antifungal, Blood Culture, Candida, Disc Diffusion Antimicrobial Tests, Direct Disc Diffusion test
Words: 285

Introduction

Invasive *Candida* infections are important causes of morbidity and mortality in immunompromised and hospitalized patients.[1] *Candida* species are reported to account for 9%-13% of nosocomial infections, with high mortality.[2] Although fluconazole susceptible *C. albicans* and *C. tropicalis* are the most frequently reported species responsible for candidemia,emergence of both inherently and acquired fluconazole resistant non-albicans *Candida* species is of concern.[3] As delay in initiation of appropriate antifungal therapy in patients with candidemia have a higher risk of hospital mortality, early reporting of susceptibility results are essential for management of patients.[4, 5]

Conventional disc diffusion antifungal susceptibility testing requires growth of *Candida species* on solid agar which requires specimen inoculation and at least 24 hours of incubation. The growth is then used to prepare inoculum for susceptibility testing with further incubation of 24 hours.[6] This delay could lead to poor outcomes if *Candida* species is resistant to the initial empirical therapy and an alternate method for early susceptibility testing is required.[7]

Direct susceptibility testing using disc diffusion from smear positive specimens for bacteria is a standard practice in clinical microbiology laboratories.[8] This practice for yeasts has been reported in a few studies using E-strips with good results.[9, 10] However in a resource limited setting use of E-strips for susceptibility testing is very expensive and therefore direct susceptibility testing of *Candida* species using disc diffusion technique will be more feasible.

In order to achieve that objective we conducted a study to evaluate direct disc diffusion testing from positive blood culture bottle showing growth of yeasts with a conventional disc diffusion method from isolated colony. This study was conducted in two phases: first phase was to optimize protocol for disc

diffusion directly from blood culture bottles positive for *Candida* species. In this phase, a pilot study was conducted using direct inoculation of positive blood culture specimens after centrifugation and without centrifugation. The second phase was post-optimization on a large sample size. In this study we are reporting results of firstphase of the study.

Methods

The study took place in the Clinical Microbiology Section of Aga Khan University (AKU) Clinical Laboratories from January to April 2012, as part of an umbrella project on improving diagnosis of fungal infections in Pakistan, reviewed and approved by the Ethical Research Committee of Aga Khan University (1373-Path-ERC-09). A cross-sectional study was planned in phase one to estimate agreement between standard disc diffusion protocol and two new techniques of performing antifungal susceptibilities directly from blood culture broth: i.e. centrifuged and uncentrifuged inoculation. The results would determine which method is preferable for performing direct sensitivities from blood culture vials.

Blood cultures sent to AKU Laboratory were processed in BACTEC9240 continuous monitoring system. Vials that flagged positive were taken out and gram stain smears were made from the blood culture bottles. Cultures showing yeasts on smear and yielding a pure growth of *Candida species* were selected for the study. A total of 24 blood cultures fulfilled the eligibility criteria and were subcultured on Sheep blood, Sabouraud's and chocolate agar. They were processed for direct and indirect antifungal susceptibilities and identification of the *Candida species*. Two aerobic blood culture vials were also inoculated with a 100 µl of a light suspension of the control strains (*C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258) and incubated in BACTEC9240 system and further processed in the same way.

Identification of *Candida species*

Identification of *Candida species* was essential for interpreting the zone diameters according to the established clinical breakpoints for various candida species. Yeasts were identified on the basis of colony morphology on blood agar, chocolate and BiGGY® agar, germ tube production, growth in the presence of cycloheximide, urea hydrolysis, microscopic morphology on thin layer Cornmeal Tween80 agar and API 20C AUX profile.

Conventional antifungal disc diffusion

Standard (indirect) antifungal disc diffusion was performed as described in CLSI M44-S2 and used as gold standard.[11] A single colony of yeast was suspended in 2-3 ml distilled water to get turbidity standard of 0.5 McFarland. A sterile cotton swab was immersed in this suspension and after draining excess fluid lawn was made on Mueller Hinton Agar supplemented with 2% dextrose and 0.5 µg/mL methylene blue (MHA-MB). Fluconazole (25µg), voriconazole (25µg) and amphotericin B (10µg) discs were placed on the agar surface and plates incubated for 20-24 hours at 35±2°C in ambient air.

Direct antifungal disc diffusion using centrifuged and uncentrifuged inoculum

Direct antifungal disc diffusion was performed using two different methodologies: after centrifugation and uncentrifuged. For uncentrifuged, 100 µl of blood culture broth was withdrawn from the positive blood culture vial and poured onto the surface of MHA-MB. In parallel, 1 ml of the broth was centrifuged for 15 minutes at 3000 revolutions per minute and then 100 µl of the pellet was poured onto MHA-MB surface. With a sterile cotton swab, lawn was made on each plate and fluconazole, voriconazole and amphotericin discs were placed on the agar surface and incubated for 20-24 hours at 35±2°C.

Zone diameters were read in millimeters using the same transparent centimeter scale. For each blood culture, fluconazole, voriconazole and amphotericin zone diameters for all three methods were recorded and interpreted according to CLSI M44-S2.[11]

Statistical analysis

Mean and standard deviations of the zone diameters obtained by the three methods for each antifungal were calculated. Paired t-test was used to compare the means of centrifuged and uncentrifuged methods with the standard method. Variance ratio test (F-test) was applied to detect inequality in the variances of each test and standard methods. Minor errors (defined as labeling an isolate intermediately susceptible when either susceptible or resistant and vice versa), major errors (labeling an isolate resistant when susceptible) and very major errors (labeling an isolate susceptible when actually resistant) were calculated for each method.[12]

Results

A total of 26 blood cultures positive for *Candida species* were evaluated. In cases where both bottles were positive, only aerobic bottle was processed. In addition to 24 clinical samples, two simulated aerobic blood culture bottles spiked with ATCC strains recommended as controls for antifungal susceptibility, *C. parapsilosis* (ATCC 22019) and *C. krusei* (ATCC 6258). Ten aerobic, one anaerobic and 13 paediatric bottles from 18 patients were included; the mean age of the patients was 25.7 years (SD=27.5) and male to female ratio was 1.7. Of the 24 yeasts isolated, 11 (44%) were *C. albicans*, 7 (28%) were *C. parapsilosis*, 4 (16%) were *C. tropicalis* and one each of *C. lusitaniae* and *C. pelliculosa*. Antifungal susceptibility revealed only one *C. parapsilosis* strain which was resistant to fluconazole and Susceptible-Dose Dependent (S-DD) to voriconazole; the rest of the clinical strains were susceptible to all three drugs. The zone diameters of the control strains were all within range.

The means and variances of the zone diameters obtained by each new method were compared to the standard method using paired t-test and F-test (Table 1). Fluconazole and voriconazole

by both methods did not differ significantly from the standard methodology. However, for amphotericin B, although the mean diameters by either method did not differ from the standard, the variances, and hence standard deviation, of the centrifuged and standard method were found to be unequal (p=0.007).

Categorical errors observed by the two methods are shown in Table 2. One minor error was seen with uncentrifuged method while with centrifuged method there were two minor and one very major error. Hence the centrifuged method for direct susceptibility testing had a greater number of categorical errors, and variance of zone diameter also differed significantly from the standard for at least one antifungal.

Discussion

The results of this optimization study showed that inoculation of 100 µl of uncentrifuged blood from positive blood culture bottles for direct antifungal susceptibility was similar to conventional disc diffusion method with only one minor error. This was in comparison to centrifuged inoculum that although had similar results to that of conventional disc diffusion method but had one very major and two minor errors.

Use of centrifugation requires an additional step in processing of specimens and is prone to errors such as contamination and labeling errors etc. Better results with uncentrifuged inoculum in our study were encouraging as this extra step in processing was not required,leading to reduced processing time and cost.

In addition, a study using 39 positive blood culture bottles for yeastreported that cell count was in the range of 10^5-10^8 CFU/mL.[9] Additionally 34/39 bottles had a cell count of 10^6-10^7 CFU/mL that is exactly the count in 0.5 McFarland standards that is recommended for disc diffusion technique for *Candida species*.[6] These results also support our findings that a step of centrifugation is not required.

Conclusion

In conclusion, the results of this study support use of uncentrifuged blood for direct disc diffusion methodology for *Candida s*pecies. These results will be used in the second phase

Table 1: Results of Paired t-test and variance ratio test (F-test) used to compare mean and variances of zone diameters obtained by each test method to standard method to see if they are similar.

Antifungal (n)	Standard ZD Mean (CI)	Standard ZD SD	Test Method	Test ZD Mean (CI)	Test ZD SD	T-test p-value	F-test p-value
Fluconazole (26)	27.73 (24.56-30.90)	7.86	Uncentrifuged	27.19 (24.18-30.20)	7.45	0.362	0.791
			Centrifuged	26.35 (23.37-29.32)	7.37	0.158	0.752
Voriconazole (26)	30.96 (28.76-33.16)	5.45	Uncentrifuged	30.96 (28.91-33.01)	5.95	1.000	0.721
			Centrifuged	28.96 (26.56-31.36)	5.07	0.102	0.665
Amphotericin (23)	22.69 (22.00-23.39)	1.61	Uncentrifuged	22.35 (21.56-23.14)	1.82	0.411	0.559
			Centrifuged	21.87 (20.60-23.14)	2.93	0.231	0.007*

Note: CI = confidence interval, SD = standard deviation, ZD = zone diameter.
*Statistically significant at 5% level of significance.

Table 2: Categorical errors observed by the two new methodologies compared to the standard antifungal disk diffusion testing methodology.

Test method	Antifungal	Minor errors	Major errors	Very major errors
Uncentrifuged	Fluconazole	0	0	0
	Voriconazole	1	0	0
	Amphotericin	0	0	0
	Total	**1**	**0**	**0**
Centrifuged	Fluconazole	1	0	1
	Voriconazole	0	0	0
	Amphotericin	1	0	0
	Total	**2**	**0**	**1**

of study to evaluate agreement between direct and standard disc diffusion methodology for Candida species on a large sample size.

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ORIGINAL ARTICLE

Frequency of Pathogenic Stool Parasites in Patients from a Tertiary Care Hospital in Karachi

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Abstract

Background
Intestinal parasitic infections have a significant impact on the health of the community. This study was aimed to estimating the frequency of intestinal parasites among patients presenting at Liaquat National Hospital between Jan 2009- Dec 2013.

Material and Methods
We performed this cross sectional study on stool specimens received at Liaquat National Hospital from Jan 2009 – Dec 2013. Stool samples were examined macroscopically to assess their consistency,presence of blood, mucous, and parasites. Microscopic examination was carried out using normal saline and lugol's Iodine. Concentration method was used where appropriate.

Results
Out of 9646 samples examined 15% (1460 out of 9646) were positive for intestinal parasites. All ages and both the sexes were affected. Male to female ratio was 1.2:1. 89 out of 1460 positive samples i.e. 6% had more than one parasite. 29% of the isolates were from children = 10 years, 50% between 11-40 years and 31% from patients' = 40 years of age. *Entamoeba histolytica* (n= 552)38%, *Blastocystis hominis*(n= 411) 28% and *Giardia lamblia* (n= 342)23% were found to be the most prevalent parasites.

Conclusion
Entamoeba histolytica was the most prevalent intestinal parasite in our study. There is a high prevalence of intestinal parasites in the community which requires improving water quality, proper sanitation and health education on urgent basis.

Key words
Intestinal parasites, *Protozoa, Helminths*

Introduction

Intestinal parasitic infections have a significant impact on the health of the community. Studies done in different parts of the world reveal a high prevalence (52-54%) of these infections.[1, 2] Poor sanitation, lack of access to safe drinking water and improper hygiene are linked to parasitic infections. Studies indicate that a large majority of these infections are asymptomatic and do not report to the laboratories.[3] The true prevalence is difficult to be calculated and a large number of carriers remain a serious threat to the community.[4] There is a large variation 18-81% of intestinal parasite prevalence reported in various published literatures of Pakistan.[5-7]

Parasitic infections, caused by intestinal helminths are more prevalent in developing countries whereas, in developed countries, protozoan parasites are a more common cause of gastrointestinal infections.[3] People with poor water and sanitation conditions of all ages in underdeveloped countries are affected by the prevalent parasitic infections however, children are the worst affected.[9] The consequences of these parasitic infections result in malnutrition, anemia, cognitive impairment and increased susceptibility to other infections.[10] This study was aimed at estimating the frequency of intestinal parasites among patients presenting at LNH.

Material and Methods

We performed this cross sectional study at Microbiology department of Liaquat National Hospital between Jan 2009-Dec 2013. All stool samples were first examined macroscopically to assess whether watery, loose or formed. pH, presence of mucus, blood and parasites was also assessed.

The stool smears were examined under light microscope using normal saline and lugol's iodine solution. Modified formal ether concentration method was used where appropriate.

Study variables such as age, gender, macroscopic examination and presence of stool parasites was recorded. Data was collected and statistically analyzed using Statistical Package for Social Sciences version 16 (SPSS 16). Percentage prevalence of each type of parasite was calculated.

Result

A total of 9646 samples were received in the study period and 15% (1460) were positive for intestinal parasites. The overall prevalence of parasitic infections in our study was estimated to be 15 % (1460 out of 9646) were positive for various intestinal parasites. 6% of the positive samples contained multiple parasites.

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Male to female ratio was 1.2: 1. 29% of the isolates were from children ≤ 10 years, 40% between 11-40 years and 31 % from patient's ≥40 years of age. 82% of the samples with intestinal parasites were positive for pus cells, representing infection and 21 % were also positive for RBCs. (Figure 1) Seasonal variation of stool parasites was also observed. Although, greater number of positive cases were seen in May and October, cases appeared throughout the year.

Entamoeba histolytica being the most common intestinal parasite, was present in 37.81% samples (552 of 1460), *Blastocystis hominis* in 28.15 % (411 out of 1460) *Giardia lamblia* 23.42%(342 of 1460) followed by *Ascaris lumbricoides* present in 6.16 % (90 out of 1460) samples, *Hymenolepis nana* in 3.56% (52 of 1460) samples. *Taenia* in 0.48% (7 out of 1460) samples, *Ankylostoma duodenale* in , 0.2% (3 out of 1460) samples, *Enterobius vermicularis* in 0.1% (2 out of 1460) samples and *Trichurus trichura* in 0.06% (1 out of 1460) samples was identified. (Table 1)

Discussion

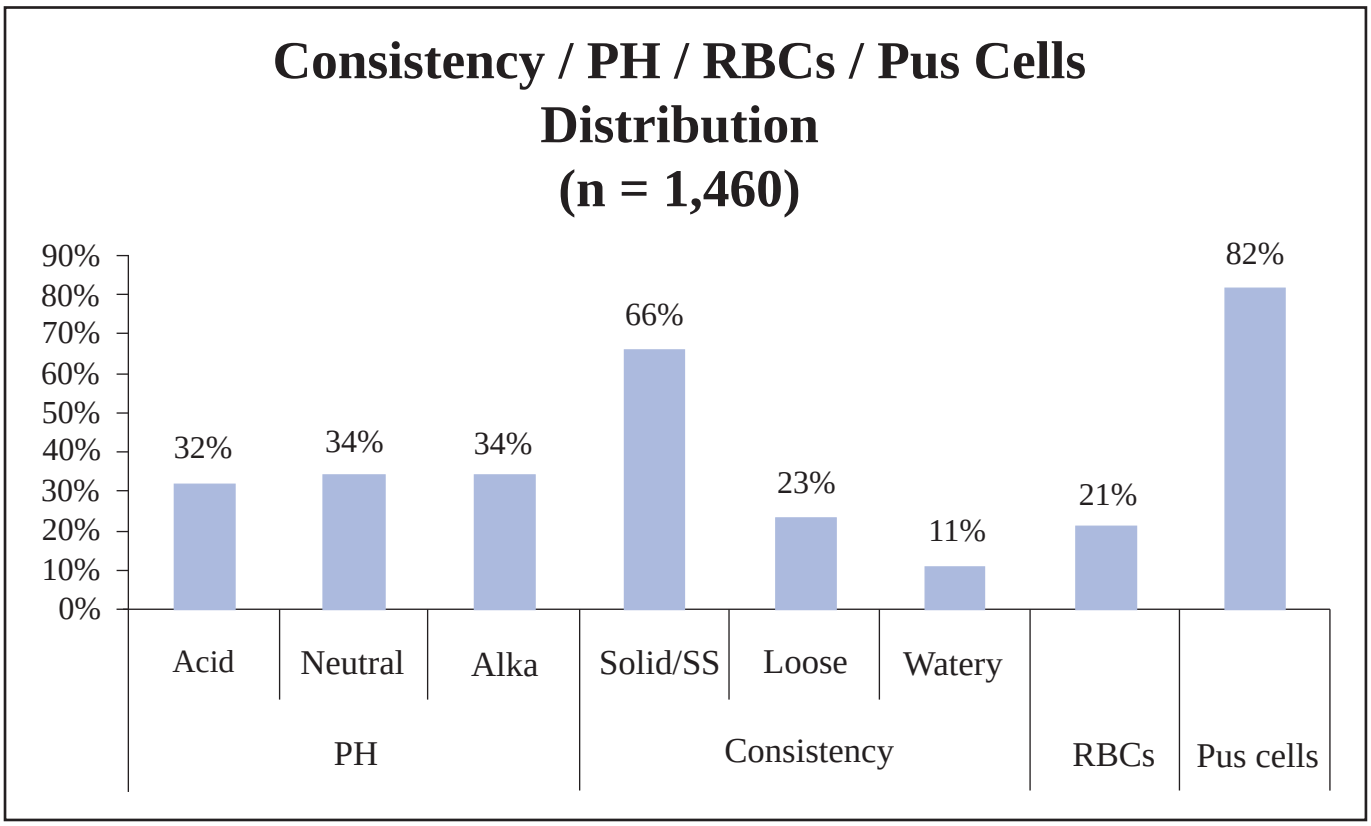
A high prevalence of parasitic infections is reported from various studies carried out in different parts of the world. Studies from India show a prevalence of 71 % and 46%.[11, 12] Those from Mexico and Turkey show 57% and 31% prevalence respectively.[10, 13] Various studies carried out in Pakistan also show a high prevalence of 69% and 47%.[4, 14]

The data collected in our study is hospital based, so represents a segment of patients presenting to our hospital and not the community in general. Community based surveys need to be conducted in order to find the exact prevalence in the community. Varied percentages of helminthic infections were found in studies carried out in different areas of Pakistan. In our study 10.62 % helminth and 89.38% protozoa were isolated. Studies from Abottabad, Skardu and Azad Kashmir report *Asacaris Lumbricoides and Giardia lamblia* as the most common parasites isolated.[5,6,7, 15]

The intestinal parasites namely *Giardia lamblia*, *Ascaris lumbricoides*, *Blastocystis hominis*, *Hymenolepis nana*, *Endolimax nana*, *Entamoeba coli* and *Iodoamoeba butschlii* were identified from the stool samples in a study in urban slums of Karachi.[16]

In other studies carried out in Karachi *Giardia lamblia and E.histolytica* were the most common parasites with a prevalence of protozoan parasites *Giardia lamblia* 25% and *E. histolytica* 19 %.[4, 14]

An increasing protozoa to helminth ratio on moving from plains to coastal areas has been observed on reviewing various studies carried out in Pakistan.[4] This can be attributed to the hot climatic conditions which are not favorable for survival of the helminth eggs in the soil. This observation is consistent with our study



Macroscopic appearance of stool samples.

Table 1: Gender, age distribution and prevalance percentage of stool parasites.

Variable	No.	%age
Gender:		
Female	657	45%
Male	803	55%
Total	1460	100%
Age:		
≤ 10 yrs.	423	29%
11 yrs. ~ 20 yrs.	190	13%
21 yrs. ~ 30 yrs.	219	15%
31 yrs. ~ 40 yrs.	161	11%
≥ 41 yrs.	467	32%
Total	1460	100%
Parasites:		
Entamoeba histolytica	552	38%
Blastocystis hominis	411	28 %
Giardia lamblia	342	23 %
Ascaris lumbricoides	90	6 %
Hymenolepis nana	52	4 %
Taenia	7	0.5 %
Ankylostoma duodenale	3	0.2 %
Enterobius vermicularis	2	0.2 %
Trichurus trichura	1	0.1%
Total	1460	100%
Ratios:		
Protozoa	1305	89.38%
Helminths	155	10.62%
Total	1460	100%

carried out in Karachi which shows a higher protozoa to helminth ratio.

The high prevalent parasites were mainly *E. histolytica*, *Blastocytis hominis* and *G.lamblia* indicating poor water quality, hygiene and sanitation. Self medication with easily available over the counter antihelminths can also be a reason for the low prevalence of these infections. The trend of taking prophylactic anti-helminths for various other gastrointestinal symptoms can also account for a lower percentage of detection of helminth infections. In our study all age groups i.e. children and adults and both the sexes were found to be affected.

Thus, a high rate of intestinal parasitic infections and also a large number of asymptomatic carriers are present in the community. This can be attributed to unsafe water supply and poor sanitary conditions and lack of awareness within the community about the mode of transmission and prevention of the parasitic infections.

Conclusion

Entamoeba histolytica was the most prevalent intestinal parasite in our study. Community based surveys need to be conducted in order to find the exact prevalence of parasitic infections in the community. There is an urgent need of improving the quality of water, proper toilet facilities and health education of the community for decreasing the prevalence of both helminth and protozoan infections.

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Diabetic Foot Infections: a microbilogical study

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Abstract

Objectives
To identify the pathogenic microorganisms and their antibiotic susceptibilities in patients developed diabetic foot infections (DFIs) at a tertiary care setting of Pakistan.

Methods
We performed this retrospective cross sectional study during 2006-11 at inpatient and outpatient department of Shifa international hospital. The microbiological data of all patients with DFIs was collected. Data on demographics, clinical, laboratory features such as cultures and susceptibility were analyzed with SPSS version 17 (SPSS Inc., Chicago, USA).

Results
A total of 165 organisms were isolated from 108 patients. Single organism was isolated in 68 (63%) patients, and 40 (37%) had poly-microbial infections. There were 74 (45%) gram-positive organisms and 86 (52%) gram-negatives isolates. Five fungi (3%) were also isolated. *Staphylococcus aureus* [29 (17%)] and *Escherichia coli* [33 (19%)] were the most frequent gram positive and gram-negative organisms respectively including multidrug-resistant organisms [80 (74%)].

Conclusion
The etiological microorganisms in DFIs in a tertiary care setting may be diverse and resistant to a broader range of antibiotics, which necessitates immediate culture and sensitivity for appropriate antibiotic selection. The empirical antibiotic treatment of DFIs without culture and sensitivity can have disastrous outcome in these patients.

Key words
Diabetic Foot infection, Microbiology, Multidrug-resistant organisms, Infection.

Introduction

Diabetes Mellitus (DM) is a common worldwide illness which is associated with neuropathic, vascular, ophthalmic, renal and other serious systemic complications. Infections are one of these major complications mainly aggravated by uncontrolled diabetes. It was estimated that in 2011, approximately 366 million people had diabetes, 7.0% of the world's population. Around 80% of these people live in developing countries. By 2030, the global estimate is expected to rise to 552 million, 8.3% of the adult population.[1]

One of the serious complications of DM is diabetic foot, the pathogenesis of which is complex, including neuropathic, vascular and infective mechanisms. The severity of this condition increases with poor control of DM. Every year, more than 1 million people with diabetes loose a leg as a consequence of this disease.[1]

Diabetic foot infections (DFIs) usually start from small superficial wounds but frequently develop into severe necrotizing infections, which are difficult to treat and may end in amputations of toes, feet and even limbs and not infrequent, it leads to systemic spread of sepsis and death. In Pakistan studies have shown the high morbidity and mortality in diabetic foot infections including amputation rates in more than one third of these patients.[2-5] Most likely poor diabetes control, late referrals, poor medical care, and decreased awareness regarding foot care are risk factors. These studies have shown high prevalence of infections due to diverse etiologic agents with *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas* as common isolates.[4, 5] We aim to further study the etiological microorganisms and their changing susceptibilities pattern in DFIs in a tertiary care setting of Pakistan.

Materials and Methods

This was a retrospective chart and microbiological data review. Institutional Review Board of Shifa International Hospital approved the protocol, which is a tertiary care teaching hospital with fully developed microbiology laboratory and diabetic care specialists. The data of all patients with diabetic foot infections from 2006 to 2011 was collected on predesigned proforma, both from inpatient and outpatient departments. The criteria for DFI was according to classification by Infectious Diseases Society of America and International Working Group on the Diabetic Foot (IDSA).[6]

Patients were enrolled if they have DFIs, and presented in outpatient department or admitted in hospital and whose specimens were sent for microbiological testing either in outpatient department before admission or within 48 hours of admission. The exclusion criteria were the patients, whose microbiological data could not be retrieved or whose specimen

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was taken after 48 hours of admission in order to exclude the possibility of hospital acquired infections (HAIs).[7] All the patients who had history of any hospitalization in last three months were also excluded in order to reduce the chances of HAIs.

Multi-drug resistant organisms (MDROs) were defined as microorganisms, predominantly bacteria, that are resistant to one or more classes of antimicrobial agents.[8, 9] The culture specimens were sent in the form of either deep swab after cleaning superficial necrotic material, pus or debrided tissue. The Gram stain and culture for bacteria were performed on all samples sent for culture. The culture and sensitivity for anaerobic organisms and fungi were performed either on demand of physician or recommendation of microbiologist only. Cultures were done according to the guidelines of the Clinical and Laboratory Standards Institute and antibiotic susceptibility assessed by Kirby-Bauer technique.

The data was entered in SPSS version 17 (SPSS Inc., Chicago, USA). For continuous variables (i.e., age), mean ± SD was reported. Descriptive statistics (frequencies) of categorical variables i.e., gender, mode of presentation (outpatient or inpatient), year, type of diabetes (I or II), treatment of diabetes (oral, insulin or both), glycemic control (poor or good), severity of infection (mild, moderate, severe), culture (done or not), micro-organisms isolated, antibiotic susceptibility, type of growth (single or poly-microbial), type of resistance (multidrug-resistance or not) and type of organism (gram negative, gram positive, fungi).

Results

A total of 272 patients of DFI were seen from year 2006 to 2011. The culture and sensitivity of 132 (48%) patients with DFIs was either not done at all, sent after many days of admission when patient was not responding to empirical therapy or had history of previous hospital admission within period defined in protocol. These patients were excluded from analysis. The rest of the 140 patients had cultures done and were included in final analysis. The minimum age for the patients was 13 years and maximum was 100 years [median (IQR) = 59 (52-65)]. Other demographic characteristics of included patients are shown in Table 1. Most patients had poor glycemic control.

Cultures were negative in samples of 32 (23%) patients. Out of remaining 108 patients, 68 (63%) patients had single organism whereas 40 (37%) patients had poly-microbial infections.

A total of 165 organisms were cultured from 108 patients with gram-positive organisms [74 (45%)] and gram-negative organisms [86 (52%)]. Anaerobic cultures were requested in only 10 patients and were negative. Five fungi (3%) were also cultured.

The most frequently isolated gram-positive organism was *Staphylococcus aureus* [29 (17%)] followed by *Enterococcus* [19 (11%)], whereas *Escherichia coli* was most frequent [33 (19%)] gram-negative organism followed by *Proteus mirabilis* [13 (8%)]. MRSA was in more than 21% of *Staphylococcus aureus* isolates.

Other bacterial isolates were also seen (Table 2). *Candida albicans* was the only isolated fungus. The resistance pattern of gram-positive (Table 3) and gram-negative (Table 4) microorganisms for various antibiotics showed variable susceptibilities. A very high proportion of patients [80 (74%)] had infection with MDROs. The sensitivity of fungi was not available in hospital during this period. There were no significant differences in type and sensitivity of microorganisms among mild, moderate or severe infections.

Table 1: Characteristics of patients (n=140) with diabetic foot infections

Characteristic	Total n (%)
Age [Median (IQR)]	59 (52-65)
Sex	
Male	97 (69%)
Female	43 (31%)
Inpatient	102 (73%)
Outpatient	38 (27%)
Year	
2006	3 (2%)
2007	3 (2%)
2008	18 (13%)
2009	27 (19%)
2010	46 (33%)
2011	43 (31%)
Diabetes Mellitus	
Type I	4 (3%)
Type II	136 (97%)
Previous Treatment	
Insulin	69 (49%)
Oral Hypoglycemics	60 (43%)
Both	11 (8%)
Glycemic Control	
Good	46 (33%)
Poor	94 (67%)
Severity of Infection	
Mild	49 (26%)
Moderate	63 (46%)
Severe	28 (20 %)
Culture	
Positive	108 (77%)
Negative	32 (23%)

Table 2: Micro-organisms isolated from diabetic foot infections in 108 patients

Micro-organisms	Total
Gram Positive	
Staphylococcus aureus	29 (17%)
Enterococcus	19 (11%)
Methicillin-resistant *Staphylococcus aureus* (MRSA)	8 (5%)
Staphylococcus epidermidis	7 (4%)
Group B *Streptococcus*	4 (2%)
Streptococcus Spp.	3 (2%)
Group A *Streptococcus*	3 (2%)
Group C *Streptococcus*	1 (1%)
Gram Negative	
Escherichia coli	33 (19%)
Proteus mirabilis	13 (8%)
Pseudomonas	11 (6%)
Proteus vulgaris	8 (5%)
Morganella morganii	7 (5%)
Enterobacter	4 (3%)
Acinetobacter spp.	4 (3%)
Klebsiella pneumoniae	3 (1%)
Klebsiella spp.	2 (3%)
Citrobacter Freundii	1 (1%)
Fungi	
Candida albicans	5 (3%)
Total	**165**

Discussion

The majority of patients with DFIs in Pakistan are treated by local wound care, glycemic control and empirical antibiotics.[2, 3, 5] Majority of our patients had poor glycemic control and had moderate to severe infections. Culture and sensitivity of infections are seldom used or used only in the case of deteriorating conditions. The reasons for not using microbiologic methods are: (1) Non availability of culture and sensitivity facilities in almost all primary healthcare settings and in majority of secondary healthcare facilities. These facilities are usually available in tertiary care settings, but because of inadequate quality assurance, are not used or are less helpful and (2) Cost for routine and even special testing is prohibitive. Also majority of adequate facilities for culture and sensitivity are available in private sector only. This lack of data leads to (1) lack of appropriate local guidelines for management of DFIs and (2) lack of awareness in healthcare professionals about diversity and resistance pattern of etiological organisms in DFIs except in specialist centers.

Additional hurdles are prevalent in Pakistan. This includes lack of effective legislation regarding quality control and use of medicines, particularly antibiotics. This leads to inappropriate and injudicious use of antibiotics by healthcare professionals.[10]

In addition, availability of common antibiotics over the counter and quackery also play a significant role in inappropriate use of antibiotics. In these circumstances the resistance to common antibiotics emerges manifolds. Majority of patients had already

Table 3: Antibiotic resistance (percentages only) for gram positive organisms in diabetic foot infections.

Drug Name	*Staphylococcus aureus*	*Enterococcus*	*Methicillin resistant Staphylococcus aureus (MRSA)*	*Staphylococcus epidermidis*	*Group B Streptococcus*	*Streptococcus*	*Group A Streptococcus*	*Group C Streptoccoccus*
Methicillin	0[1]	-[2]	100	0	-	-	-	-
Piperacillin + Tazobactam	4	-	88	0	-	-	-	-
Doxycycline	0	50	63	0	50	33	33	-
Ampicillin	88	25	100	50	0	0	0	0
Moxifloxocin	0	-	63	0	0	0	0	0
Penicillin	92	6	100	50	0	0	0	0
Vancomycin	0	0	0	0	-	-	-	-
Amoxycillin + Clavulonic Acid	0	13	100	0	0	0	0	0
Cefpirom	0	-	100	0	-	-	-	-
Ceftriaxone	0	-	100	0	0	0	0	0
Chloramphenicol	0	0	0	0	-	-	-	-
Co-Trimaxozole	52	-	75	80	-	-	-	-
Fuscidic Acid	0	-	0	-	-	-	-	-
Levofloxocin	0	-	75	0	0	33	0	0
Teicoplanin	0	0	0	17	-	-	-	-
Cephazolin	4	-	88	17	-	-	-	-
Cloxacillin	0	-	100	17	-	-	-	-
Rifampicin	-	31	-	-	-	-	-	-
Cefexime	-	-	-	-	0	0	0	-
Clarithromycin	-	-	-	-	0	33	67	0

0[1] -No resistance seen -[2]-Not tested for this drug

Table 4: Antibiotic resistance (percentages only) for gram negative organisms in diabetic foot infections.

Drug Name	*Escherichia coli*	*Proteus mirabili s*	*Pseudomonas*	*Proteus vulgaris*	*Morganella morganii*	*Enterobacter*
Amikacin	0	9	11	0	0	0
Gentamycin	40	55	22	25	43	25
Piperacillin + Tazobactam	13	0	0	0	0	25
Cefoperazone + Sulbactam	13	0	22	0	0	25
Imipenem	0	0	0	0	0	25
Ciprofloxocin	77	27	45	13	14	25
Cefexime	84	55	-	38	86	100
Cephalexin	84	64	-	88	100	100
Ceftazidime	70	36	44	13	14	100
Doxycycline	93	86	-	66	100	-
Ampicillin + Sulbactam	95	33	-	57	-	75
Ampicillin	93	90	-	100	100	100
Moxifloxocin	86	-	-	33	-	-
Amoxycillin + Clavulonic Acid	80	46	-	75	100	75
Cefpirom	71	0	100	0	33	75
Ceftriaxone	80	36	-	14	14	75
Co-Trimaxozole	91	75	-	100	40	50
Levofloxocin	38	66	-	25	0	75

used first line antibiotics before coming to tertiary care hospital.

The microbiology of DFIs from different studies has shown variable proportion of MDROs. Diane *et al*, reported much broader range of micro-organisms with anaerobic bacteriology but with lower susceptibilities than in our study.[11] The reasons maybe proper use of antibiotic guidelines for DFIs and better microbiologic facilities. Khalifa *et al* from Kuwait, [12] Ozer *et al* from Turkey, [13] Abdul Razaq *et al* from Adan[14] and Hefni *et al* from Egypt[15] have also reported the microbiology of DFIs from their regions. The results of these studies have shown similar data but they have reported lower number of pathogens and better susceptibilities than our study.

Studies on DFIs in literature from Pakistan have shown similar results. Studies that focused primarily on surgical management of DFIs show that complications of diabetes including involvement of limbs and foot are common. A variety of surgical interventions including different amputations may be required in up to 35% of these patients.[2,3,5,16,17] Some of these studies primarily on bacteriology of DFI.[4,14] Some others reported etiologic agents without susceptibility pattern.[16,17] These studies have shown high prevalence of infections due to *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas*. Overall resistance rates were up to 65%.[4] The present study describes the resistance to a broad range of antimicrobials, which are not used routinely for DFIs and are usually reserved as second line drugs for resistant cases only. Such a broader resistance pattern has rarely been reported in previous studies. Our study represents an area, where broader empiric and uncontrolled antibiotics are used for treatment of DFIs with no microbiologic backup and if cultures are seldom done and that also after a poor or suboptimal response. Most common organisms seen in our cohort were *Staphylococcus aureus*, *Enterococcus*, *Escherichia coli* and *Proteus mirabilis.*

Our results will help healthcare professionals to be aware the grim reality of such serious MDRO infections and choose optimal therapy options for DFIs.

Most of our DFIs patients, including those with moderate and severe infections, did not have culture and sensitivity and were treated by empirical antibiotics only. Such practices can have serious outcome especially those with infection due to MDROs. This study emphasizes the importance of early culture to establish infecting organism(s), polymicrobial nature if any and susceptibility and thus guide therapy. Multidrug therapy may be needed in some cases and the use of empirical treatment without culture backup for longer duration may lead to further resistance.[18, 19] The limitations of our study were single center experience, retrospective nature of the study and non-availability of anaerobic and fugal culture susceptibility in all patients.

Conclusion

Etiological microorganisms in DFIs in tertiary care settings may be diverse and antibiotic resistance is common. It is important to have appropriate early cultures with good microbiology laboratory to have optimal therapy and outcome.

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Pulmonary Aspergillosis: are we still afraid of surgery

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Abstract

Background
There is limited literature available on the clinical features and postoperative outcomes of patients with pulmonary aspergilloma. This study presents the outcome of patients managed surgically over nine years at a tertiary care hospital of a developing country.

Methods
This is a retrospective review of patients who underwent surgery for pulmonary aspergilloma over a period of nine years (from July 2001 to July 2010). Cases of aspergilloma were diagnosed on the basis of CT scan chest. Outcomes were morbidity, mortality and recurrence of aspergilloma.

Results
A total of 56 patients who underwent surgery for pulmonary aspergilloma were included. All patients presented with massive hemoptysis. Unilateral aspergilloma was found in 52 (92.8%). 49 patients (87.5%) had past history of pulmonary tuberculosis. Most common surgical procedure performed was lobectomy (53 patients) with median intraoperative blood loss of 400 ml (IQR: 300 ml to 587 ml).

Postoperative morbidity was recorded in 10 patients (17.8%). Complications includedprolonged air leak [8, 14.2%)], atelectasis requiring bronchoscopy [2, 3.6%)], renal dysfunction [1, 1.7%)] and prolonged ventilation [1, 1.7%)]. One patient expired within 30 days due to hospital acquired Acinetobacter pneumonia. With minimum follow up of one year, none of the patients had recurrent hemoptysis or aspergilloma.

Conclusion
Surgery, being safe and effective, should be treatment of choice in patients with pulmonary aspergilloma especially in the life-threatening situation of massive hemoptysis.

Keywords
PulmonaryAspergilloma, Lobectomy, morbidity, mortality, hemoptysis

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Introduction
Aspergillomais a fungal infection caused by several species of Aspergillum; however, most common of all is A. fumigatus, followed by A. flavus.[1] Hemoptysis is the commonest and gravest of all symptoms;[1, 5] the main purpose of any therapeutic intervention against aspergilloma is to prevent or treat hemoptysis. Surgical treatment remains the mainstay, while anti fungal agents (oral, parenteral or intra-cavitary) are adjuncts to it.[1, 4] Surgical management of aspergilloma commenced half a century ago;[6] however, it has been deferred due to high morbidity and mortality.[1] Mortality of surgical treatment of aspergilloma reported in literature ranged from 0-23%.[7- 14] Moreover, aspergilloma is encountered in patients with existing disease having compromised lungs, further perplexing the situation. So, the critical question remains, "should we operate?"

There has been gradual reduction in post-surgical morbidity and mortality of aspergilloma due to better patient selection and advanced postoperative care. Therefore, early surgical treatment of symptomatic as well as asymptomatic aspergilloma is advised.[5] However, there is paucity of literature from our country. We have conducted this retrospective review of patients with pulmonary aspergilloma, managed over period of nine years at a tertiary care hospital of a developing country.

Patients and Methods
This is a retrospective cohort study of patients who underwent surgery for pulmonary aspergilloma over a period of nine years (from July 2001 to July 2010) at our institute with minimum one-year follow up. Patients with missing data were excluded. Patients were identified through ICD code.

Cases of aspergilloma were diagnosed on the basis of typical CT scan finding of *a soft tissue density lesion inside a cavity surrounded by a rim of air* in all the patients and culture of bronchial lavage in few cases. Extent of resection was decided on the basis of lesion seen on CT scan chest preoperatively and on the extent of lesion intra-operatively. Tissues were sent for histopathology, fungal culture and Acid Fast Bacilli culture. Initial follow up was at one week after being discharged from hospital and then regularly up to at least one year. All the patients were kept on oral Itraconazole for three months from surgery.

Data was collected on a proforma to document demographic, clinical and outcome variables. Outcomes measured were morbidity, mortality and recurrence of aspergillosis. Statistical analysis is done on SPSS (version 19). Continuous variables i.e. age, duration of stay are analyzed as means and standard deviation for data with normal distribution and medians with interquartile range (IQR) for data with skewed distribution. Categorical variables i.e. morbidity, mortality are analyzed as proportions and percentages.

Results

A total of 56 cases of aspergilloma were included and reviewed. The median age was 35 years and most of the patients were in 3rd and 4th decade of life. Of these, 45 (80.3%) were males. All of them presented with massive hemoptysis and 49 patients (87.5%) had past history of pulmonary tuberculosis (Table 1). Unilateral aspergilloma was found in 52 (92.8%).

Most common surgical procedure performed was lobectomy (53 patients) (Table 2) and median intraoperative blood loss was 400 ml (IQR: 300 ml to 587 ml). The median length of stay in hospital was 5 days (IQR: 5-7). Chest tubes were removed within a week in 45 patients, and the longest duration was six weeks in one patient. Aspergillus species grew in tissue specimen of all the patients. AFB culture was negative in all the patients; however, tissue specimen sent for histopathology did reveal chronic granulomatous inflammation in three patients, highly suggestive of tuberculosis. Therefore, these three patients were kept on anti-tuberculosis therapy along with antifungal therapy. Postoperative morbidity was recorded in 10 patients (17.8%). Complications included prolonged air leak [8 (14.2%)], atelectasis requiring bronchoscopy [2 (3.4%)], renal dysfunction [1 (1.7%)] and prolonged ventilation [1 (1.7%)]. None had empyema postoperatively. One patient (1.7%) expired within 30 days due to hospital acquired Acinetobacter pneumonia. One patient expired after four months of surgery due to cachexia. With minimum follow up of one year, none of the patients had recurrence of hemoptysis or aspergilloma.

Table 1: Demographic data & baseline characteristics

Patient Characteristics	N (%)
Males	45 (80.4)
History of pulmonary tuberculosis	49 (87.5)
Intra-operative blood loss(ml), *median (IQR)*	400 (300-587)
Location:	
Left upper lobe	26 (46.4)
Right upper lobe	26 (46.4)
Bilateral	02 (3.4)
Left mid zone	01 (1.7)
Right middle lobe	01 (1.7)
Operative Procedure:	
Lobectomy	53 (94.5)
B/L Lobectomy	02 (3.4)
Pneumonectomy	01(1.7)

Table 2: Post-operative complications of patients undergoing surgical treatment in patients with aspergilloma.

Complications	N (%)
Prolonged air leaks	08 (14.2)
Atelectasis requiring bronchoscopy	02 (3.4)
Renal dysfunction	01 (1.7)
Prolonged ventilation	01(1.7)

Discussion

This is a retrospective review of patients with pulmonary aspergilloma managed surgically. Most common indication for surgery was massive hemoptysis and operative morbidity and mortality was 17.8% and 1.7%, respectively. There are certain limitations of this study. Being retrospective review, biases are inherently attached to it, as the data recorded on medical records were for clinical purpose and not for research purpose. Secondly it was a single centre study and there is a need to conduct such study at wider level. With the number of cases over last 9 years at a single centre, it is proved that aspergilloma is not uncommon here. In order to have long term follow up of patients, we included patients with minimum of 1-year follow up and none of them had recurrent hemoptysis or aspergilloma.

An *aspergilloma* is usually a manifestation of saprophytic fungal infection of devitalized tissues or pre-existing chronic cavity i.e. old healed tuberculosis, sarcoidosis, histoplasmosis, bronchiectasis and emphysema.[1] Therefore, these are mostly found in the upper lobes or superior segment of the lower lobes. Majority of the patients had history of pulmonary tuberculosis, which is common in this part of the world. (figure 1)

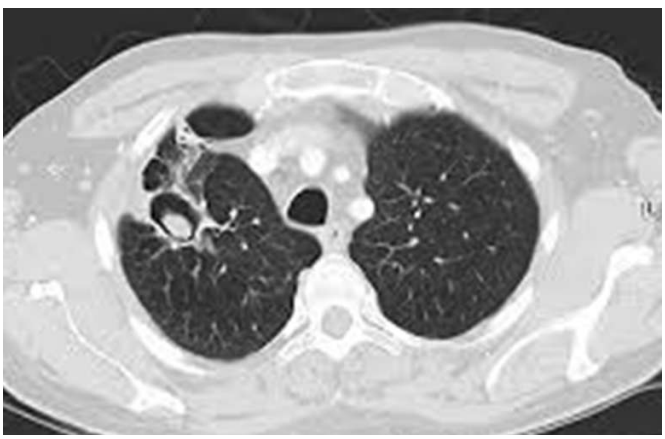

Fig 1. CT scan image of a patient showing soft tissue lesion within a lung cavity surrounded by rim of air.

The diagnosis is usually established by clinical evaluation and radiographic features[4] corroborated by the evidence of aspergillus species from biological specimen i.e. sputum, broncho-alveolar lavage, cavity tissue. Characteristic features on plain radiographs include a spherical or ovoid cavity consisting of a solid rounded mass of water density within it surrounding by an air space, which may be mobile or static. CT chest is the main modality of diagnosis. Most of the patients in our study were found to have disease affecting one lobe only.

Manifestations of pulmonary aspergilloma range from asymptomatic disease to life threatening massive hemoptysis.[3] Hemoptysis is the most common presenting symptom, reported in 50-80% of patients.[15] It is often intermittent and scanty but may be massive and life-threatening in upto 25% of cases. All of our patients presented with massive hemoptysis that prompted early surgical intervention. Hemoptysis usually occurs due to erosion of vascular cyst wall or surrounding vessels i.e. bronchial or intercostal arteries. Due to these extensions, hemoptysis does not cease with conservative management and early surgical intervention is warranted.

Most common and potentially fatal sequel of aspergilloma is hemoptysis.[1, 5] Angioembolization may act as a temporary measure to control the bleeding; whereas surgery provides the total cure of the disease. However, surgery had been deferred due to high morbidity and mortality[1] attributed to compromised lung functions. Hemoptysis may be fatal in 40-100% patients.[15] With advancement in peri-operative care, the mortality and morbidity has been reduced down to less than 5% and less than 20%, respectively. This is also evident from our cohort of patients. Thus surgery can safely be performed to cure the pulmonary aspergillomas rather than to let patient die of hemoptysis. Surgical treatment also offers certain benefits to the patients i.e. prevention and treatment of hemoptysis, prevention of growth of the pulmonary aspergilloma, preservation of the lung parenchyma; and eradication of the pyogenic component.[16] Therefore, based on our results and supporting literature, we conclude that surgical treatment remains the mainstay.[1, 4]

Conclusion

Surgery, being safe and effective, should be treatment of choice in patients with pulmonary aspergilloma especially in the life-threatening situation of massive hemoptysis.

Disclosure

Authors declare no conflict of interest or financial support from any authority

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ORIGINAL ARTICLE

Varying Faces and Presentations of Abdominal Tuberculosis in Children – A study from a tertiary care referral centre of a developing country

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Abstract

Tuberculosis is a common Childhood illness. Every eighth TB patient has extra-pulmonary disease; of which, abdominal involvement accounts for a significant proportion. Abdominal TB presents a diagnostic challenge owing to its variable presentation.

Objectives
Determine the frequency of different clinical presentations and utility of various investigations for diagnosis of abdominal tuberculosis in children.

Study Design
Prospective descriptive study.

Place and Duration of Study
The Department of Pediatric Gastroenterology & Hepatology at The Children's Hospital and The Institute of Child Health, Lahore, Pakistan. January 2014 till April 2015.

Material and Methods
Total number of 60 children of ages 1-15 years having abdominal tuberculosis were included. A detailed history was taken. Presence of BCG scar, abdominal guarding, rigidity and abdominal tenderness, Ascites, Hepatosplenomegaly were also noted. CBC, ESR, Montoux, CXR, Abdominal USG, Abdominal CT and Barium meal and follow through were done in all patients. T spot TB test, laparoscopy and colonoscopy were done in selected patients.

Results
A total of 60 children between 1-15 years of age were diagnosed with abdominal tuberculosis during study period. Male to female ratio was 1:2.16. Abdominal pain was observed in 88% of children, fever, diarrhea and weight loss in 70% each, abdominal tenderness and guarding in 80%, failure to thrive in 70%. Abdominal lymphadenopathy in 35% subjects and thickening of bowel loops was present in 95%, mesenteric lymphadenitis in 85% and ascites in 50% of patients. IGRA T spot TB test was performed in 20% subjects and was positive in all. Barium study, CT scan abdomen, ultrsonography were positive in 90%, 90% and 70% cases. Montoux test was positive in 50% subjects.

Conclusion
Common presentation of ATB is with abdominal pain & tenderness, fever and weight loss. Common findings on imaging studies are thickening of bowel, mesenteric lympadenopathy and ascites. Most useful diagnostic investigations are Ultrasound scan, CT scan, Barium meal and follow through and T Spot TB test.

Key Words
Abdominal Tuberculosis, Pediatrics, Diagnosis

Introduction

Abdominal tuberculosis has been known to be uncommon, especially in children. But, the patterns are changing now, especially with an association of HIV. 2 million people in India develop Tuberculosis each year, of which 0.87 million are infectious as per the WHO Core Clusters Committee for communicable diseases update of March 2009.[1] In a recent study by Wadhwa N *et al.*[2] It was reported that, among all the patients suffering from abdominal TB, 21.4% belonged to the pediatric age group. ATB can involve the gastrointestinal tract, peritoneum, and lymph nodes. Commonest cause is *Mycobacterium* tuberculosis. *Mycobacterium bovis* was an important cause of ATB in the past but incidence has decreased with boiling and pasteurization of milk, and eradication of infected cattle.[3]

Abdominal tuberculosis has two main common forms i-e peritoneal and second is intestinal tuberculosis.4 Almost all cases of ATB are due to hematogenous spread of *Mycobacterium tuberculosis* or due to swallowing sputum in patients with cavitary pulmonary disease.[4] Although ATB is common in adults,[5] only 1-5% cases of childhood Pulmonary Tuberculosis are complicated by ATB.[6] Signs and symptoms of ATB are usually mild in resulting in under-diagnosis. The actual frequency of ATB may be higher than reported.[6]

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Poverty, overcrowding and lack of access to health care facilities result in a high frequency of TB in developing countries. Tuberculosis is commonly accompanied with HIV infection, aging population or due to migration trans-globally.[7,8] One of every 8 TB case is extra pulmonary[9, 10], and 11-16% of these is accounted for by ATB.[11, 12] In children Diagnosis of tuberculosis poses technical and practical challenges, and it becomes more difficult in abdominal tuberculosis (ATB) because of the variable clinical manifestations.

Varying clinical manifestations mean that the diagnosis is often delayed leading to increased mortality and unnecessary surgery. The main diagnostic dilemma is to differentiate intestinal tuberculosis and Crohn's disease because a misdiagnosis can have dramatic consequences.

This study is planned to determine the frequency of different clinical presentations and utility of various investigations for diagnosis of abdominal tuberculosis in children.

Material and Methods

After informed consent; 60 newly diagnosed patients of abdominal tuberculosis, admitted in The Department of Pediatric Gastroenterology Hepatology Of The Children's Hospital, Lahore Pakistan; were entered in the study over a period of 16 months i.e from January 2014- April 2015. On admission each patient was thoroughly interviewed and all pertinent history points including history of BCG vaccination, housing, water supply, type of milk consumed, contact with Tuberculous patient, duration of fever, abdominal pain, bowel habits, weight loss etc were all noted. Each patient went through detailed physical examination including anthropometry, presence or absence of BCG scar, abdominal distension, fever, abdominal mass, abdominal pain/guarding/ tenderness , ascites etc were also noted in proforma. All investigational findings including CBC, ESR, Montoux, CXR, abdominal USG, T spot TB test (IGRA), barium series, CT scan abdomen or laparoscopy were done where needed and their findings were noted down. If present, ascitic fluid was taken for direct examination, protein content, cytology and mycobacterium tuberculosis culture.

We treated all our patients with normal liver function tests with combo of four drugs isozniazid (5 mg/kg), rifamapicin 10mg/kg), pyrazinamide (30mg/kg), ethambutol (15mg/kg) for total of nine months (Four drugs for two months and two drugs for 7 months). Patients who were sick enough to take orally or had deranged LFTs were started on second line ATT (IV Linezolid, Moxifloxacin, Amikin / Streptomycin, Klaricid) and quickly shifted back to first line as soon as liver functions returned to normal and or they started oral intake. Data was analysed using SPSS 20

Results

A total of 60 children were diagnosed with abdominal tuberculosis during study period. Females outnumbered males and we enrolled 19 male and 41 female children. Age range was between 1 – 15 years. The most common clinical feature was abdominal pain observed in 88% of children followed by fever in 70% and diarrhea and weight loss in 70% each. Duration of abdominal pain varied among subject and 50% of children had abdominal pain which persisted with varying degree for up to 6 months duration. Refer to Table 1 and Figure 1.

Most common Physical finding in children was abdominal tenderness and guarding which was present in 80% followed by failure to thrive in 70% and abdominal lymphadenopathy in 35% subjects. Refer to Table 2.

Mesenteric lymphadenitis seen in 85% was second most common type of tuberculosis after intestinal tuberculosis with thick walled bowel loops in 95%cases making it the most common form of abdominal tuberculosis in our study. Ascites was also very frequently seen in and was observed in 50% of our patients.

CT scan abdomen was very useful investigation and picked abnormalities in 95% of patients with abdominal tuberculosis as compared to abdominal ultrasound (70%). Refer to Figure 2.

IGRA T spot TB test was performed in only 20% subjects and it was positive in all. Colonoscopy and laparoscopy was also done in a select number of patients. Colonoscopy was done in 12 patients who presented with bleeding PR and were found to have colitis. Commonest colonoscopy findings were deep circumferential ulcers and polypoidal appearance. 3 (5%) had strictures; all patients with tuberculous colitis were less than 7 years of age and had equal sex distribution. 3 (5%) patients came with sub acute obstruction and were referred to surgeon after diagnosis and failure of conservative therapy. Laparoscopy was done in 6 (10%) patients and it was able to provide positive tissue diagnosis in 100% cases.

Table 1: Frequency of different clinical presentations in Abdominal Tuberculosis

Clinical Presentation	Numbers of Patients	Frequency %
Pulmonary TB	12	20
H/o contact	45	75
Lymphadenopathy	21	35
Ascites	30	50
Weight Loss	42	70
Blood in stools	6	10
Diarrhea	42	70
Constipation	18	30
Abd. Pain	53	88
Vomitings	24	40
Fever	48	80

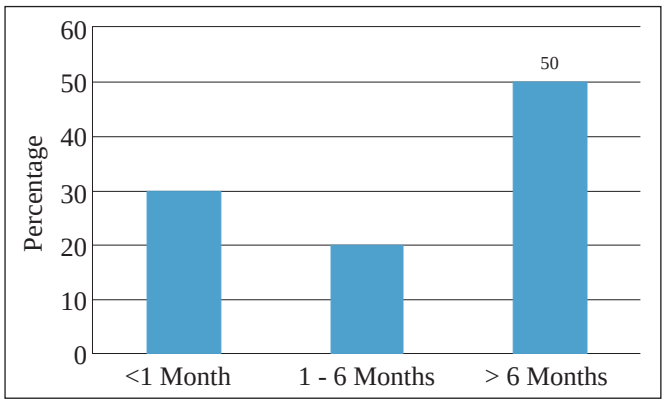

Fig 1. Duration of abdominal pain

Barium study, CT scan abdomen, ultrsonography were positive in 90%, 90% and 70% cases. Low Hb and raised ESR were observed in 80 & 85% respectively while Montoux test was positive in 50% subjects. Refer to Figure 3.

Treatment response was good in our study and only one patient expired of post-surgical complications.

Table 2: Frequency of different Clinical Signs in Abdominal TB

Physical Signs	Numbers of Patients	Frequency %
Abdominal mass	3	5
Pulmonary TB	12	20
Lymphadenopathy	21	35
Ascites	12	20
Failure to thrive	42	70
Guarding	48	80
Abdominal Tenderness	48	80

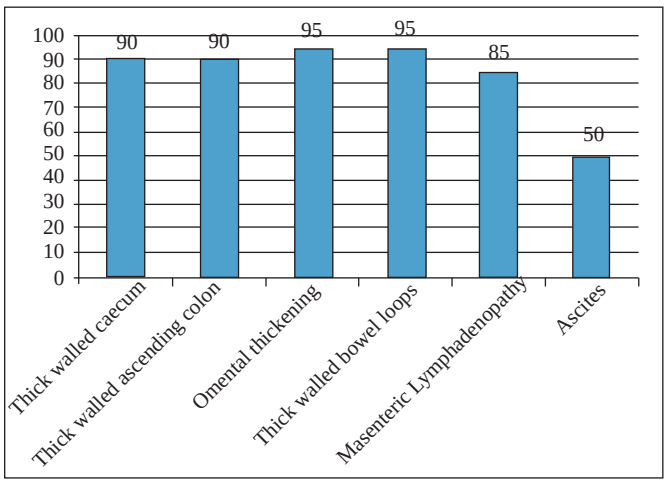

Fig 2. Frequency of CT Scan Findings

Discussion

In our study of 60 patients, females outnumbered males. This same female dominance: as a characteristic of abdominal tuberculosis has been shown in several studies in the past and also evident in our current series with females contributing to 68.33 %. However, a study from India showed 69.4% patients with ATB were male.[9, 13] Female predominance can be attributed to illiteracy, poor health facilities and possibly; direct extension from tuberculous salpingitis.[7, 14]

Diagnosis of abdominal tuberculosis is difficult because of variable clinical features and low yield of mycobacterium culture. Other limitation in diagnosis is difficulty in obtaining tissue for histopathology or culture.[6, 9] Supportive evidence of diagnosis is provided by imaging studies including like Imaging (ultrasound, barium X-Rays and CT scan) and Montoux test have only supportive value. In some cases, response to therapeutic trials of anti-Tuberculous drugs is the basis of diagnosis that may cause a delay in the diagnosis of other diseases which mimic abdominal tuberculosis e.g. Crohn's disease, abdominal lymphoma and malignancy of abdominal organs.[5] Therefore, diagnosis of abdominal tuberculosis is an ongoing challenge to the physicians, especially with limited resources.

The most common clinical feature of abdominal tuberculosis in our study was abdominal pain which was observed in 88% of children. It was also the main presenting clinical feature from another study from AKU Pakistan in which 93% patients presented with abdominal pain.[15]

Mid abdominal colicky pain represented intermittent small bowel obstruction and was seen in 90%-100% of patients in other studies as well.[14]

Second most common presentation of abdominal tuberculosis was fever, diarrhea and weight loss observed in 70% each. A study conducted in Taiwan in 2010 also shows that the most common clinical presentations of ATB included fever, abdominal pain, and weight loss in children.[16]

CT scan abdomen was very useful investigation and picked abnormalities in 95% of patients with abdominal tuberculosis in our series of patients. While ultrasound abdomen picked up abnormalities in up to 70% of patients. In our study only tuberculous changes in chest x ray were found in 20% of subjects. Radiological studies; x rays, Ultrasound, CT scan and contrast studies have been the main stay of diagnosis of ATB as described in other studies also.[14, 17] In our study, barium meal and follow through provided supportive evidence of ATB in 90% of patients. This is in comparison with other studies where barium series were able to support 60% of patients.[7]

The most common form of abdominal tuberculosis we encountered in our children was intestinal tuberculosis in 95%

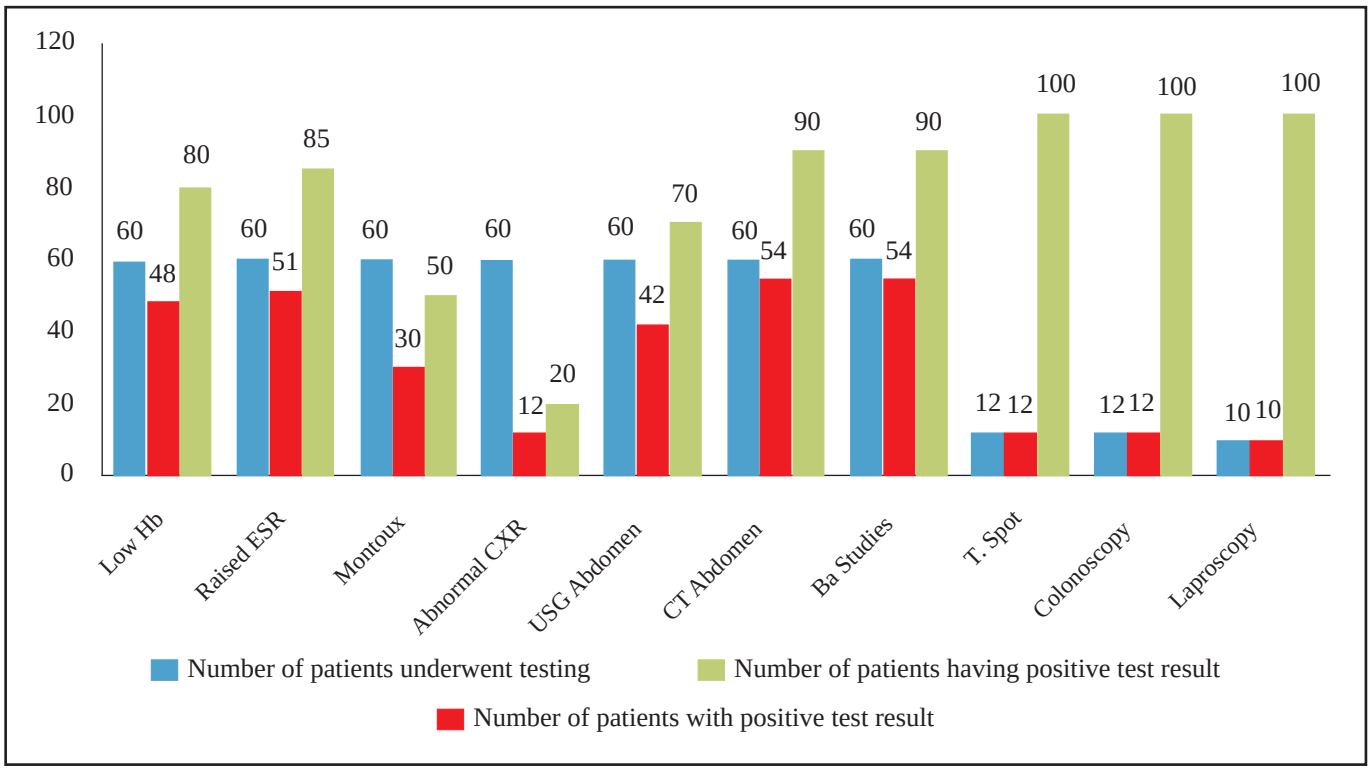

Fig 3. Percentage of utility (sensitivity) of different investigations

followed by lymph node involvement in 85%. The most common site of intestinal involvement was cecum and ascending colon in 90 % each. Colonoscopy was done in 12 patients and findings were positive in 100% of patients. Histopathology of colonic biopsy was positive for presence of granuloma. Commonest findings which were picked up on colonoscopy were deep circumferential ulcers and polypoidal appearance followed by stricture in two patients. Other study also show similar findings on colonoscopy in patients with abdominal tuberculosis and includes: ulcers, strictures, nodules, pseudo polyps, fibrous bands, fistulas, and/or deformed ileocecal valves.[18]

TB granulomas are often sub mucosal; Crohn Disease granulomas are typically mucosal, though sub mucosal granulomas may also be seen.[19] Therefore, deep endoscopic biopsies should be taken from ulcers and their margins. Care must be taken to avoid perforation in the setting of significant inflammation or deep ulcerations.

Laparoscopy was done in select number of patients' i-e 10% and was found to be 100% diagnostic. In comparison, laparoscopy was found helpful in the diagnosis up to 87%-92% of peritoneal tuberculosis, in other studies.[20, 21]

Among screening tests we found that IGRA the T spot TB test was positive in all 12 patients in whom it was performed. We couldn't do this test in all patients as it was very expensive and patients were non affording. Montoux test was positive in 50% of all cases most likely due to malnutrition and cutaneous T cell anergy.

IGRAs have specificity >95 percent for diagnosis of latent TB infection.[22,23] The sensitivity for T-SPOT TB appears to be higher than for QFT-GIT or TST (approximately 90, 80, and 80 percent, respectively.[23]

All of our patients made it and got better we lost only one patient post operative due to surgical complications.

Conclusion

Abdominal tuberculosis has many variable faces. Common presentation is with abdominal pain & tenderness, fever, weight loss. Common findings on imaging studies are thickening of bowel, mesenteric lympadenopathy and ascites. Most useful diagnostic investigations are Ultrasound scan, CT scan, Barium meal and follow through and T Spot TB test. With timely diagnosis, response to treatment is good.

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Campylobacter Lari Bacteremia in a Neutropenic Cancer Child; A case report

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Abstract

We report a case of *Campylobacter lari* bacteremia in a 3 year old neutropenic child with Pre-T-cell-Acute Lymphocytic Leukemia (ALL). *Campylobacter lari* (*C. lari)* is an infrequent cause of infections in humans; particularly bacteremia with this species is extremely rare. To our knowledge this is the first case in a child with ALL and chemotherapy induced neutropenia. She was treated successfully with Imipenem. This case revealed that *C. lari* can cause bacteremia in neutropenic patients and the outcome is excellent when treated with Carbapenems.

Key Words

Campylobacterlari, Neutropenia, Acute Lymphocytic Leukemia

Introduction

Most *Campylobacter* infections in humans are caused *by Campylobacter jejuni, Campylobacter coli and Campylobacter fetus*. *Campylobacter lari(C. lari)* is infrequently isolated from humans but is associated with enteritis and rarely causes bacteremia.Chemotherapy induced neutropenia has been associated with infections by various organisms but *C. lari* has not been reported in these patients so far. Due to the unavailability of locally published data in regards to treatment in such a scenario, this case report will assist in guiding optimal antibiotic therapy. It should also be noted that treatment failure has been reported even when antibiotic regimens were administered according to in vitro sensitivity results.

Case report

We describe a case of blood stream infection with *C. lari* in a 3 year old neutropenic female who was a known case of acute lymphocytic leukemia (ALL) and was on induction chemotherapy. She was admitted with complaints of diarrhea, 6-7 episodes per day, watery in consistency and associated with crampy abdominal pain. Empirical treatment with piperacillin-tazobactam was started considering neutropenic colitis. Stool examination for ova/parasites, Clostridium difficile toxin assay and cultures were negative. Later, metronidazole was added empirically to cover pseudomembranous colitis but her diarrhea persisted. At day six of admission the patient developed fever. Her complete blood count revealed absolute neutrophil count of zero. Her electrolytes, liver and renal function tests were normal. Piperacillin-tazobactamwasswitched to imipenem. A set of blood cultures was drawn and forwarded to the microbiology laboratory for incubation (Bactec plus Aerobic, Becton Dickinson, Sparks Maryland, MD), which were detected as positive within 48 hours of incubation. The initial gram stain revealed afaint staining, curved gram negative bacilli. The positive blood culture sample was then sub-cultured onto 5 % blood agar, MacConkey agar and chocolate agar plates and incubated at 35°C in a 5 % CO_2 environment. Additional blood agar plates were also incubated in an anaerobic environment as well as at42°Cin a microaerophilicenvironment. Growth of gray, flat and spreading colonies was seen after 48 and 72 hours on the 5% blood agar plates in the capnophilic and microaerophilic environmentrespectively. No growth was found on the plates incubated aerobically at 37°C. Gram stained smears from these colonies revealed the same curved, gram negative rods seen on the original smear. The colonies were found to be positive for production of both oxidase and catalase, hence giving a presumptive identification of *Campylobacter* species. A hippurate hydrolysis test and a urease test was also set up and both were found to be negative. Phenotypic identification was done through the application of nalidixic acid (30 µg) and cephalothin (30 µg) discs to identify the *Campylobacter* species. Both were found to be resistant. Based on these phenotypic characteristics, the bacteria were presumptively identified as *C. lari*. Antibiotic disk-diffusion susceptibility testingwas performed according to CLSI standards (Clinical Laboratory Standard Institute).[1]

Susceptibility results revealed sensitivity to ampicillin, imipenem, gentamicin, erythromycin and resistance to tetracycline, ciprofloxacin, and nalidixic acid. Her diarrhea and abdominal pain settled after 48 hours of Imipenem but the fever took 7 days to resolve. Her neutropenia recovered after 4 days of start of imipenem. Her repeat blood cultures at day 7 and 14 of start of imipenemwere negative.She was treated with imipenem for a total of 18 days.

Discussion

Campylobacters are gram negative rods, motile and curved. They are oxidase positive and micro-aerophilic. Most *Campylobacter* species cause gastroenteritis and are among the leading pathogens causing gastroenteritisin the developed world[2],however they are infrequently associated with blood stream infections.[3] *Campylobacter lari* was 1st described by

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Skirrows and Benjamin in 1980 who isolated the organism from animals, poultry and asymptomatic humans.

To our knowledge this is the first case report of *C. lari*bacteremia in a pediatric patient with ALL. A previous study from 1985-2007 has shown the incidence of *Campylobacter* bacteremiaas 0.24% of all positive blood cultures.[3] Paconowski in another study showed 183 cases of *Campylobacter* bacteremia from 2000-2004 among which there were only two cases of *C. lari* bacteremia.[4] In total there are 12 cases of *C.lari*associated blood stream infections reported in literature,[5,6] and no case has been reported from Pakistan so far.

Our described case had underlying diagnosis of ALL, and was neutropenic at the time of presentation. Literature has shown that most cases of *Campylobacter*bacteremia are associated with some underlying comorbid conditions like liver disease and malignancy.[3,4,7] The reported cases of *C.lari* bacteremia show that 9 out of 12 patients also had underlying conditions.[5,6]

In our patient *C. lari*was sensitive to imipenem, ampicillin, gentamycin and erythromycin and resistant to ciprofloxacin, nalidixic acid, and tetracycline. Literature shows overall resistance of *Campylobacter* to quinolones as 54%, ampicillin 2%, amoxicillin/clavulanate 4%, erythromycin 7%, gentamycin 0%, carbepanem 0%.[7] Regarding previous case reports we could find culture andsensitivity data of only four patients. All of these four strainswere resistant to cephalosporins, three were resistant to piperacillin-tazobactam, two were resistant to ciprofloxacin and amoxicillin.[5,6] All four strainswere sensitive to imipenem and three were sensitive to co-amoxiclav and aminoglycosides.[5,6] Therefore, culture andsensitivity results of our patient were consistent with the previous case reports regarding in vitro sensitivity to imipenem and aminoglycosides and resistance to quinolones.

There is no consensus on optimal treatment regimen and duration of treatment for *C.lari* bacteremia. We treated our patient with imipenem for 18 days with complete recovery. Initial treatment with piperacillin/tazobactam was not successful. Literature review of previous case reports showed that three patients received imipenem in combination with co-amoxiclav / erythromycin / gentamycin and all recovered.[5,6] Co- amoxiclav was given tothree patients, alone in one patient and in combination with aminoglycosides in two patients, all of three recovered.[5,6] One of the patients recovered with erythromycin while in another patient initial treatment with piperacillin-tazobactam was unsuccessful and he recovered with addition of erythromycin and co-trimoxazole.[5,6] We treated our patient for 18 days. In previous case reports available data showed two of the patients were treated for 2 weeks and another two for

Conclusion

*Campylobacter lari*can cause bacteremia in neutropenic cancer patients presenting with loose stools, although it is rare.*Campylobacters* are increasingly becoming resistant to quinolones and treatment with carbapenems has excellent outcome.

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Antibiotics Stewardship Activities

FAMILYCON

An Interactive session on *"Antibiotics Stewardship role of ID physician and medical microbiologist"* was held at PC Hotel, Lahore on 11th January 2015. This event was organized in collaboration with Pakistan Academy of Family Physicians at FAMILYCON 2015. The objective of this meeting was to highlight the role of family physician might play in reducing the antibiotic misuse and thus also reduce anti-microbial resistance and costs. The following speakers and topics were discussed.

i. The Menace of Drug Resistant Organisms: What Family Physician need to know, by Dr. Mateen Izhar, Microbiologist SZH.

ii. The Rational use of Antibiotics in Common Infections, by Prof. Sajid Maqbool, Prof. Emeritus Pediatrics

iii. Introducing Antibiotic Stewardship and the Role of Family Physicians, by Dr. Ejaz Khan, President MMIDSP

ASP within Pindi/Islamabad

After introduction of ASP activities for all major disciplines within Shifa International Hospital since May 2014 the ASP Multidisciplinary team has expanded to do advocacy and awareness within the twin cities of Rawalpindi and Islamabad. Major private and public hospitals are being targeted. So far the team has had sessions in AFIP, Railways Hospital, Children's Hospital and PIMS.

The following speakers and topics were discussed.

i. Antimicrobial Stewardship (ASP) in Public Hospitals, by Dr. Ejaz A. Khan

ii. Role of Microbiology-Knowing your bug, by Dr Usman Janjua

iii. Role of Pharmacy in ASP- Knowing your drug, by Ms Komal Fizza

iv. Role of Infection Control in Hospital settings, by Ms Ruth Samuel

The team is planning to cover all major hospitals in the coming months and will printed educational material prepared for this purpose with large main messages on Standees.

Instructions to Authors

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

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

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

Manuscript Categories

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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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.
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