EDITORIAL

Challenges in Diagnosing Childhood Tuberculosis
Thomas, Tania A

26

ORIGINAL ARTICLES

Is Thrombocytopenia Consistent with Specific Bacterial/Fungal Neonatal Sepsis?
Salman Javaid, Khawaja Irfan Ahmad Waheed, Mehmood Sheikh, Rafia Gul, Naiia Nizami, Syeda Tehseen Fatima

27

Assessment of Laboratory Bench Cleaning Protocols with Surface Cultures
Humaira Shafaq, Irfan Ali, Maqboola Dojki, Sadia Shakoore

31

Prevalence, Etiology and Predictors of Urinary Tract Infections in Febrile Children under the Age of Five Years
Tufail Soomro, Shiyam Sunder Tikmani

35

News & Views

40

INSTRUCTIONS FOR AUTHORS

41

1 year old boy with Multiple brain abscess. One large brain abscess with midline shift. Pus culture grew Pseudomonas Aeruginos, with Carbapenem Resistant (CRE)

Courtesy: Dr Ali Faisal Saleem, Aga Khan University, Karachi.
Challenges in Diagnosing Childhood Tuberculosis

*Mycobacterium tuberculosis* (Mt) is the leading global killer from a curable infectious agent yet the true scope of disease in children is not known. In 2014, estimates approximated 1 million cases of childhood TB and 136,000 deaths. However, the actual number of cases disclosed to national TB programs were vastly different: only 36% of estimated pediatric TB cases were reported. Reasons behind the under-recognition and under-reporting are multifactorial, but all speak to the urgent need for improved TB diagnostics for children.

Compared to adults, there are important differences in how children manifest with TB. The severity of disease at presentation often follows a bi-modal age distribution: young children, especially those who are less than two years of age, are at higher risk of developing severe disseminated forms of disease and adolescents are at higher risk of developing adult-type or cavitary disease; the remainder are more likely to present with intrathoracic disease with a predilection for isolated mediastinal lymph node involvement. These varied and often non-specific symptoms contribute to misdiagnosis.

Once TB is considered on the differential diagnosis, our diagnostic “tool-kit” is limited to non-specific tests. Immunologic biomarkers, including the century-old tuberculin skin test and newer interferon-gamma release assays, rely upon cell-mediated immunity which is conditionally impaired in those at highest risk of disease—children who are very young, malnourished, and/or HIV-infected. Furthermore, these tests fail to distinguish *Mt* infection from disease, making them less useful as a confirmatory test. Chest radiographs can aid in the determination of the presence and severity of active disease. However, the findings consistent with intrathoracic TB disease in a child can be heterogeneous and often times subtle at the earlier stages of progression, again lending a supportive but not confirmatory role.

“Gold standard” TB tests, including the acid-fast bacilli (AFB) smear, Mt culture, and Mt molecular assays, detect microorganisms from respiratory specimens. The difficulties in obtaining adequate respiratory specimens impart notable logistic challenges because children lack the tussive force and oromotor coordination to expectorate on command thereby calling for skilled procedures such as gastric lavage, sputum induction and/or nasopharyngeal aspiration. Once a specimen is obtained, the yield is hindered by the paucibacillary nature of childhood disease-- AFB smear, the most widely available TB diagnostic test worldwide, has a sensitivity of 10-15% among children with “probable TB”. The newer GeneXpert Mt/RIF assay has a yield of 65-76% among hospitalized children when testing two specimens, however this is in comparison to TB culture which also carries an unacceptably low yield of 30-40%. The successful detection of a child with TB represents the proverbial “tip of the iceberg.” Reliance on traditional microbiologic confirmation from respiratory specimens is insensitive, however reliance on clinical grounds alone is non-specific, resulting in increased morbidity and mortality. Further work is urgently needed to identify accurate biomarkers of disease, ideally from feasibly-obtained pediatric specimens. As we work towards a public health goal of “zero childhood deaths from TB,” we owe it to each child to think about TB early in the clinical presentation and use our current tools with knowledge of their limitations, while advocating for simple, accurate and pediatric-friendly diagnostics for TB.

**References**


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Is Thrombocytopenia Consistent with Specific Bacterial/Fungal Neonatal Sepsis?

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The Children’s Hospital & The Institute of Child Health, Lahore

Abstract

Background
Neonatal sepsis is a clinical syndrome with presence of both infection and systemic inflammatory response syndrome. Thrombocytopenia is a marker of neonatal sepsis. Present study was conducted to determine association of thrombocytopenia with any specific infection.

Methods
A total of 120 neonates were included in this cross sectional study conducted in the neonatal intensive care unit (NICU) of the Children’s Hospital, Lahore from January to July 2015. All neonates with presumed sepsis and positive blood cultures were included. The neonates who had received platelet/ blood transfusion or drugs known to cause thrombocytopenia were excluded. Data on age, gender, platelet count, microbiological culture and clinical outcome was collected on a proforma. Statistical analysis was performed using SPSS 20 to look for association between thrombocytopenia and different organisms (Gram positive, Gram negative or fungal) causing sepsis. Chi-square test and logistic regression methods were used for calculating significance. P-value of <0.05 was taken as significant.

Results
A total of 120 newborns were included with 81 males (68%) and 39 females (32%). Their mean age was 140.28 hours (±130.71 SD). Thrombocytopenia was detected in 29% (n=35, p=0.90). Out of these septic & thrombocytopenic babies, Gram negative organisms were found in 69% (n = 24, p =0.31) and Gram positive organisms in 31% (n = 11, p=0.44). No case of fungal sepsis had thrombocytopenia. Considering the individual microorganism, only Klebsiella pneumoniae (p=0.027) had a significant association with thrombocytopenia.

Conclusions
In neonatal sepsis, presence of thrombocytopenia may be an indicator of Klebsiella infection.

Key words
Neonatal sepsis, thrombocytopenia, blood culture, microorganism

Introduction
Neonatal sepsis is defined as the clinical syndrome with presence of both infection and systemic inflammatory response syndrome. Thrombocytopenia i.e. a platelet count of <100,000 is being used as a marker of neonatal sepsis.

The incidence of thrombocytopenia in well newborns is 1-5% while it is observed in 22-35% of neonates admitted in NICUs. According to a study 59.5% of septic neonates have been found to have low platelet count.

The underlying causes of thrombocytopenia include Infections, prematurity, birth asphyxia, IUGR, neonatal alloimmune / autoimmune thrombocytopenia and rare disorders like congenital amegakaryocytic thrombocytopenia. Bacterial, Viral and Fungal Infections can result in low platelet count through a number of mechanisms resulting in suppression of the bone marrow, immune-mediated destruction, DIC and platelet aggregation due to bacterial products adhesion to platelet membrane, structural changes in platelet membranes intravascular platelet aggregation, and decreased production from degeneration of platelet precursors in bone marrow.

Clinical manifestation of low platelet count can be prolonged bleeding from skin, bleeding from lungs, GIT or intraventricular hemorrhage while treatment options include management of underlying cause with platelet transfusion in some cases.

As sepsis is seen in one third of NICU cases and according to a study 59.5% of septic neonates have been found to have low platelet count, whether there is an association between low platelet count and type of microorganism responsible for sepsis is a debatable issue. Previous studies have shown conflicting results in this regard. Some suggesting thrombocytopenia as an early marker of Gram negative and fungal infections while other contradicting it. The aim of study was to determine potential role of platelets as an early marker for specific type of infection, thus modifying the choice of empirical antibiotics. This may help reduce the irrational use of the antibiotics, prevent emergence of resistance, check loss of finances, save the patient from
antibiotics associated side effects and result in early recovery.

Materials & Methods
This prospective cross sectional study was conducted from 1st January to 31st July 2015 in Neonatology Unit, the Children’s Hospital & the Institute of Child Health, Lahore. The study was initiated after obtaining permission from the IRB. Informed consent was obtained from the parents or guardians. A total of 120 neonates of either gender admitted in neonatal unit through neonatal OPD/ emergency room who presented with clinical sepsis or developed sepsis in hospital and their blood culture was found to be positive were included in the study. Detailed history and examination were carried out in all patients presenting with sepsis. Patients who had already received antibiotics known to cause thrombocytopenia or received platelet transfusion before blood culture or with incomplete data were excluded from the study.

Three milliliters of blood was collected under aseptic conditions in EDTA test tube (Improvacuator), while 1.5cc blood was collected in blood culture bottles containing 15 ml of BHI under sterile precautions. Samples were transported to respective laboratories for processing of platelet count and blood cultures. Platelet count was performed by automatic hematological cell counter (Sysmex KX-21) as part of complete blood count. Blood culture was performed after inoculation of sampled blood into blood culture bottles containing TSB with 0.025% sodium polyanethol sulfonate. Blood culture vials were incubated at 37°C for 7-days, during which subcultures were done on solid media - blood agar and MacConkey’s agar at 24 hours, 48hours and 6th day of incubation. Blood culture was reported sterile if no growth was seen on subculture after 6 days of incubation. If turbidity or hemolysis was seen earlier, subculture was done on respective day. Isolated bacterial organisms were identified according to the criteria laid down in Collee et al.10 Colony characteristics on blood and MacConkey’s agar were identified by examination of Gram stained smears and using the various biochemical tests including API.

Data on age, gender, platelet count, microbiological culture and clinical outcome was collected on a proforma. Statistical analysis was performed using SPSS v20 to look for association between thrombocytopenia and different (Gram positive, Gram negative or fungal) organisms causing sepsis. Chi-square test and logistic regression methods were used for calculating significance. P-value of ≤0.05 was taken as significant.

Results
A total of 127 babies were initially included. Out of these 07 were excluded and data of total of 120 babies was used for analysis. There were 81 male (68%) and 39 females (32%), with male: female of 2:1. The mean age of admission was 140 ± 131 hours. Mean birth weight was 2.44± 0.71 kg (0.8-4.5 kg) (Table 1).

A total of 47 cases (39%) of early onset sepsis (EOS) and 73 cases (61%) of late onset sepsis (LOS) were observed. Thrombocytopenia was observed in 35 (29%) cases (Table 2). Out of these septic thrombocytopenic babies EOS was present in (n=14, 29%, p=0.904) and LOS (n=21, 29%, p=0.90) both being statistically insignificant (Fig.1).

Table 1: Demographic data

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
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<td>39</td>
</tr>
<tr>
<td>Age &lt;72 hr</td>
<td>47</td>
<td>73</td>
</tr>
<tr>
<td>Birth Weight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1 kg</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>1.1 - 1.5 kg</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>1.6 - 2.5 kg</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>&gt;2.5 kg</td>
<td>78</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Association table

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Thrombocytopeni</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Gram +ve</td>
<td>11</td>
<td>33</td>
</tr>
<tr>
<td>Gram -ve</td>
<td>24</td>
<td>50</td>
</tr>
<tr>
<td>EOS</td>
<td>14</td>
<td>33</td>
</tr>
<tr>
<td>LOS</td>
<td>21</td>
<td>52</td>
</tr>
</tbody>
</table>

Fig. 1. Frequency of Thrombocytopenia in Bacterial/Fungal neonatal sepsis
Out of the thrombocytopenic babies, Gram negative organisms were found in 69% (n=24, p =0.31) and Gram positive organisms in 31% (n=11, p=0.44). No case of fungal sepsis had thrombocytopenia (Fig.2).

Considering individual microorganism, neonatal sepsis because of *Klebsiella pneumoniae* (p=0.027) had a significant association with thrombocytopenia. Other organisms found during study did not show significant association with thrombocytopenia (Fig.3).

A study conducted by Najeem in Peshawar found LOS to be more common than EOS which coincides with results of our study. The similarity may be because of same population and organisms’ common in our set-up. Haque has also described matching situation in his study done to find the pattern of culture-proven neonatal sepsis in the United Kingdom. The similarity may be because of same definitions of types of neonatal sepsis and cut-off limit i.e. 72 hours used in our studies. On the contrary the results of a study conducted by Ahmad in local population found EOS to be more common than LOS. The difference may be due to different cut-off limits i.e. 7-days to categorize EOS or LOS.

The studies performed by Sheikh et al, Waseem, Muhammad et al and Basheer et al found that Gram negative bacteria were more common than Gram positive in neonatal sepsis. We had similar observation which may be due to similar pattern of infections in our population. Benjamen in his study found a frequency of fungal sepsis in neonates to be 7% in western population. However, Basheer and Charoo found a frequency of 2% and 3% respectively in our population which is consistent with our results of 1.78%. The difference with study conducted by Benjamen can be because his study was conducted in a different population.

Jeremiah found that 63.7% of septic neonates had thrombocytopenia while Arif calculated a percentage of 33.8 in culture positive neonatal sepsis. Percentage of thrombocytopenia in neonatal sepsis was 29.2 in our study which is similar to study by Jeremiahs study conducted on very low birth weight and preterm babies revealed an association between thrombocytopenia with gram negative and fungal sepsis. The results are not in conformity with our study which may be because of different study population as our study included both preterm as well as term babies. However, in the study conducted by Alshorman, with similar objectives, no association was found between thrombocytopenia and bacterial/fungal organisms.

Many studies conducted in tertiary centers in developing world found that *Klebsiella* infection is significantly associated with making males more prone to sepsis. Muhammad, Sheikh and Tayeb have demonstrated in their studies that males are more susceptible to neonatal sepsis than females. Above studies support the results of our study that has revealed similar results with male to female distribution of 2:1.

**Discussion**

Thrombocytopenia is accepted as a non-specific marker of neonatal sepsis. Various studies done in different places aimed to find its association with specific causative organisms have shown variable results. Current study was aimed to find association of thrombocytopenia with a specific organism.

The male predilection of neonatal sepsis is a common observation. Hubacek observed an immunological difference between males and females claiming that the polymorphism in genes for Lysosomal Binding Protein is responsible for it. Marriot has shown that on males on account of cell surface receptors like TLR 4 trigger a different response than females in terms of inflammatory cytokines and acute phase reactants thus making males more prone to sepsis. Above studies support the results of our study that has revealed similar results with male to female distribution of 2:1.

Guida in his study conducted on very low birth weight and preterm babies revealed an association between thrombocytopenia with gram negative and fungal sepsis. The results are not in conformity with our study which may be because of different study population as our study included both preterm as well as term babies. However, in the study conducted by Alshorman, with similar objectives, no association was found between thrombocytopenia and bacterial/fungal organisms.
thrombocytopenia.

The results are in conformity with our study that may be because these studies have similar study design and have been performed in similar set-ups.

Conclusions

In neonatal sepsis, presence of thrombocytopenia may be taken as indicator of Klebsiella infection.

References

Assessment of Laboratory Bench Cleaning Protocols with Surface Cultures

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***Aga Khan University Medical College, Karachi, Pakistan

Abstract

Background
Laboratory-acquired infections are a serious concern for the clinical microbiology laboratory worker. Laboratory workers have been reported to acquire shigellosis and salmonellosis from working in high-burden laboratories. Even the most minor breaches of protocol can result in infection as a very small infectious dose may be required. The likelihood of recovery of such organisms is highest for technologists culturing stool specimens. If routine biosafety principles are followed, contamination of bench surfaces should not occur with such organisms.

Objective
We evaluated routine bench cleaning practices in at a busy clinical microbiology laboratory to determine the risk to laboratory workers

Methods
The study was carried out at the clinical microbiology laboratory of the Aga Khan University in Karachi. The laboratory processes over 100 stool specimens per day, and has standardized protocols pertaining to biosafety and bench cleaning procedures. In an effort to establish effectiveness of cleaning procedures in the laboratory we observed bench contamination by monitoring the total microbial counts (Heterotrophic plate counts – HPCs) and culturing for Shigella and Salmonella species.

Results
30 samples were collected over 1 week. HPCs were higher at the end of the work day, demonstrating the waning of bleach cleaning effectiveness. Contamination with Salmonella or Shigella spp was not observed.

Conclusion
Adequate bench disinfection protocols were implemented in the study laboratory. Frequent audits of these cleaning practices instill confidence in laboratory workers that their work environment is safe.

Owing to exhaustive test menus and prolonged work hours, modern clinical microbiology laboratories are overcrowded posing severe threat of laboratory acquired infections (LAIs) to workers. Data on laboratory-acquired infections are hard to acquire because infections don’t have readily evident symptoms and incubation period and course of infection are not very well defined. The data for laboratory acquired infections have been largely voluntary as most of the laboratories may not share the report of incidents because of the fear of punishment and accountability.1,2 One of the most common routes of exposure associated with laboratory work is ingestion of microorganisms occurs through bad laboratory practices transmission of organisms to the mouth from contaminated items and accidental splashes that fall into the mouth. Specimen processing during routine laboratory procedures often contaminates containers, bench tops, equipment, and causes generation of aerosols.1

Further details are mentioned in table 1. A study conducted by Pike et al identified 4,079 laboratory acquired infections from 1932 to 1978 out of which 17% were from clinical laboratories.2 Similarly a survey by Bayer from 1979 to 2005 showed 1,141 laboratory acquired infections, 46% were acquired in diagnostic laboratory.

Laboratory-acquired infections occur due to a wide variety of organisms including bacteria, viruses, fungi, and parasites. The most common causes of LAI’s causing agents include Shigella species, Salmonella species, Brucella species, Neisseria meningitides and Mycobacterium tuberculosis.3 Different studies conducted over the period of time concluded following results in terms of most common infections among the laboratory workers table 2.1,2,4,6

Based on the rates of LAIs, Shigella dysentriae Type 1 and Salmonella Typhi are classified as pathogens belonging to Hazard Group 3 by the Advisory Committee on Dangerous Pathogens (ACDP) and must be processed in a Biosafety Level 3 (BSL 3) facility.7 However, in high-burden laboratories, these organisms are dealt with under BSL-2 conditions on a regular basis, increasing the risk of acquisitions in laboratory workers. At the Aga Khan University clinical microbiology laboratory, work is performed in a BSL-2 facility with stringent bench cleaning protocols and biosafety training of all laboratory workers.
The main objective of our study was to assess stool bench cleaning procedures by observing for bench contamination with *Salmonella* and *Shigella* species, as these organisms are highly infectious and common causes of LAIs.

### Methods

#### a. Site and biosafety procedures

The study was conducted at the clinical microbiology laboratory of Aga Khan University. The laboratory processes over 100 stool samples for microscopy and culture daily. The laboratory also has which has approved biosafety and bench cleaning procedures.

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**Table 1: Routes of exposure associated with laboratory acquired infections.**

<table>
<thead>
<tr>
<th>Route</th>
<th>Laboratory Practices</th>
</tr>
</thead>
</table>
| Ingestion and Contamination of skin and mucous membranes. | Pipetting from mouth  
Accidental splashes of hazardous material into mucous membrane (eyes, mouth and eyes) intact or non intact skin.  
Contaminated surface, equipment and articles  
Consumption of edible items in workplace |
| Inoculation                                | Injuries from sharp objects and needle stick  
Insect and animal bites and scratches       |
| Inhalation                                  | Numerous procedures that produce aerosols                                           |

*Adapted from reference 1*

**Table 2: Most common laboratory acquired infections**

<table>
<thead>
<tr>
<th>Title</th>
<th>Findings</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory-associated infections: incidence, fatalities, causes, and prevention</td>
<td>Over 64% of the laboratory acquired infections were caused by the <em>Salmonella typhi</em>, <em>Franciscella tularensis</em>, <em>Brucella</em> species and <em>Myobacterium Tuberculosis</em>.</td>
<td>Pike <em>et al</em> 1979.2</td>
</tr>
<tr>
<td>Incidence of tuberculosis, hepatitis, brucellosis, and shigellosis in British medical laboratory workers</td>
<td>The most common infections among the laboratory workers were shigellosis, tuberculosis and hepatitis.</td>
<td>Harding <em>et al</em> 1976.4</td>
</tr>
<tr>
<td>Infections acquired in clinical laboratories in Utah</td>
<td>The most frequently occurring infections in Utah laboratories were hepatitis B, shigellosis, pharyngitis and tuberculosis. The incidence of infections was three times higher in smaller laboratories.</td>
<td>Jacobson <em>et al</em> 5</td>
</tr>
<tr>
<td>Epidemiology of laboratory associated infections</td>
<td>The most reported cases were of <em>Salmonella typhi</em>, <em>Brucella melitensis</em> and chlamydia species.</td>
<td>Harding <em>et al</em> 1995.6</td>
</tr>
</tbody>
</table>

32. *Infectious Diseases Journal of Pakistan*
procedures as described in the BMBL 5th edition. Briefly, benches are decontaminated and disinfected by 1% sodium hypochlorite (freshly prepared) before start of work, during the work if there is a small spill of any potentially infectious materials, after completing procedures, and at the end of the work shifts.

b. Study protocol
The stool processing and culture bench was divided into three equal parts A, B and C (figure 1) based on the workload. Three samples of swabs were taken from each portion of the stool bench for five consecutive days at the beginning and end of the day. These swabs were inoculated on nutrient agar, Xylose

![Stool culture bench at Aga Khan University Microbiology Laboratory, Karachi, which was divided into A, B and C for sampling.](image)

![Bacterial count on nutrient agar](chart)

Fig 2. HPCs were higher at the completion of the work day, indicating the waning of bleach cleaning effectiveness. However, no Salmonella or Shigella species were isolated.
Lysine Deoxycholate (XLD) agar and Salmonella Shigella (SS) agar. Growth was determined at 48 and 72 hours to determine Heterotrophic Plate Counts (HPCs) on the nutrient agar plate, and for any growth of non fermenters on XLD and SS agars. Any growth of non-fermenting gram negative organisms was identified further by biochemical and serological tests. Media quality control was performed as recommended by the American Society for Microbiology.

Results
30 swabs were collected over 1 week at the start and end of each day from each part of the bench. Heterotrophic Plate Counts (HPCs), were high at the end of each day irrespective of the area of the bench sampled. Bacterial counts are plotted in figure 2 demonstrating the increase in counts at the end of the day. On average, counts were higher for part B of the bench. HPC for part B on day 5 at the end of the work day are lower. This represents an anomalous result and may have been obtained due to some small spill cleaning during the work process. Salmonella and Shigella spp were not isolated from any of the swabs.

Discussion
Our results show that bench cleaning protocols are being implemented and are effective for the laboratory bench studied. Results were communicated to laboratory manager and staff and were observed to increase efficiency in cleaning protocols. Therefore, results were likely to have increased confidence among staff in bench cleaning protocols employed.

The results projected the increase of HPCs at the end of the day indicating the weakening of the cleaning effect. However, results show that bench cleaning was being implemented regularly as low counts at the beginning of each work day demonstrate efficiency of disinfection at the end of each day.

A survey was conducted in approximately 22,000 medical laboratory in Great Britain showed enteric infections (salmonellosis and shigellosis) were the major cause of LAI’s in microbiology laboratories. Another survey conducted by Miller and Baron on the risk of a laboratory-acquired infection in microbiologists versus the general population of the same relative age and found that incidence of salmonellosis is 1.5 per 100000 microbiologists and incidence of shigellosis is 6.6 per 100000. We cultured bench surfaces for these organisms on a bench which commonly cultures these pathogens and performs susceptibility testing. During the study period, 04 Salmonella and 01 Shigella spp isolates were cultured on the bench from samples. Since the study period was short, pathogen positivity remained low, and the incidence of LAIs due to Salmonella and Shigella is low globally, inferences regarding rate of acquisition of these LAIs in the study laboratory cannot be made. However, the absence of these pathogenic organisms from bench surface cultures is reassuring.

In conclusion, current disinfection protocols did not place workers at higher risk for acquisition of Shigellosis or Salmonellosis. Based on our observation that these results increased workers’ appreciation of bench cleaning protocols, we recommend that such biosafety audits be carried out regularly by clinical microbiology laboratories to ensure biosafety compliance.

Acknowledgements
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References
Prevalence, Etiology and Predictors of Urinary Tract Infections in Febrile Children under the Age of Five Years

Tufail Soomro*, Shiyam Sunder Tikmani**

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** Department of community health Sciences, Aga Khan University Karachi

Abstract

Background
Urinary tract infections (UTI) represent a major burden of infections in children; early diagnosis and appropriate treatment are important to avoid long-term morbidity. In low-middle-income countries, UTI concomitantly presents with other infections. Therefore, this study was done to determine the burden of UTI among febrile children and identify common pathogens and their sensitivity pattern. We also constructed a model that predicts UTI in febrile children.

Material and Methods
This was a cross-sectional study conducted at the Pediatric unit, Civil hospital Sukkar from 1st August 2013 to 30th September 2014. Patients up to five years of age of either gender with fever for less than two weeks were enrolled after written consent. Demographic and clinical features were recorded in a proforma. Urine culture was sent within 30 minutes of its collection. Identification of isolates using in-house biochemical tests and susceptibility to commonly used antibiotics was performed using the disc diffusion method. The data was entered and analyzed using SPSS version 20.

Results
272 patients were enrolled in the study. The median age of enrolled children was 2.8 (Interquartile range 2) years. Male patients accounted for 53.3% cases with male to female ratio of 1:1.14. The majority of children presented with low-grade fever, 182 (66.9%) followed by diarrhea (19.5%), dysuria 37 (13.6%), ureteric colic 31 (11.4%) and flank pain 28 (10.3%). Out of them, 32 (11.8%) patients had UTI. E. coli was the most commonly isolated pathogen accounting for 12 cases. Most of the pathogens were sensitive to aminoglycosides, fluoroquinolones, and fosfomycin. Female gender (OR 5.7, 95% CI: 1.88-17.41), diarrhea (OR 7.7, 95% CI: 2.36-28.82) and flank pain (OR 3.67, 95% CI: 1.02-13.21) were independent predictors of urinary tract infection in febrile children.

Conclusion
UTI is common among febrile children. Gram negative organisms are common pathogens of UTI and most are still sensitive to conventional antibiotics.

Keywords
Febrile children, UTI, Pathogen, Predictors

Introduction
Urinary tract infections (UTI) can be defined by the presence of a significant bacterial count in the urine along with signs and symptoms of infections.¹ UTI is a common diagnosis in childhood, particularly in the first three years of life.² The prevalence of UTI is 3-5% in girls compare to 1-3% in boys.³ In infants, the incidence of UTI is more in males than females due to higher incidence of obstructive anomalies of the urinary tract in boys but after one year, UTI is more frequent in girls than boys because of small urethra in females and increase chances of vaginal contamination with fecal flora and beyond two years the male to female ratio with UTI is 1:10.⁴ UTI is more common in uncircumcised boys.⁵ Literature suggests that fever is a common clinical presentation of UTI in neonates, infants, and young children whereas older children present with urinary symptoms (dysuria, polyuria, increase frequency, urgency).⁶ Eighty (80%) of the infants with culture-proven UTI in a study, presented with fever.⁷ UTI in children can occur concomitantly with other infections. Clinical features are vague and non-specific in infants so the diagnosis of UTI is missed by most pediatricians. Diagnosis and management of UTI is a challenge for physicians and leads to misdiagnosis that is often followed by ill health and renal damage.⁸

In a study, 110 out of the 3625 children seen in the out-patient department had UTI accounting for prevalence of 3.0%. The majority of the patients (59, 53.6%) were less than 2 years of age with a male: female ratio of 1:1.3. Fever was the commonest presenting symptom and the commonest organisms isolated in urine were Klebsiella (27, 24.5%), and Staphylococcus aureus (24, 21.8%). The organisms were sensitive to gentamicin (50, 45.5%), ceftriaxone (49, 44.5%), and ciprofloxacin (36, 32.7%).⁹ In another study, the prevalence of UTI was 7.87% and E. coli was reported as the most common etiological agent of UTI
(65.2%), followed by Klebsiella spp. (26%), Pseudomonas aeruginosa (3.6%), and Staphylococcus coagulase positive (3.7%). Results of antimicrobial susceptibility analysis for E. coli to commonly used antibiotics were as follows: amikacin (79.7%), ofloxacin (78.3%), gentamicin (71.6%), ceftriaxone (41.8%), cefotaxime (41.4%), and cefixime (27.8%).

In another study, the most common pathogens isolated were E. coli in 297 (64.1%) samples, Klebsiella spp and Enterobacter each in 51 (11.31%) samples, Proteus in 36 (7.8%) samples, Pseudomonas in 15 (3.27%) samples and Citrobacter in 8 (1.74%) samples. Recognizing UTI is very important to prevent long-term sequelae like renal scarring, hypertension, and chronic renal failure. To ensure optimal treatment with antibiotics it is necessary to know the causative organisms and their sensitivity pattern in patients visiting Civil Hospital, Sukkar so that appropriate antibiotics can be prescribed to prevent these long-term sequelae.

The study was done to establish the magnitude of UTI among febrile children and to determine common uropathogens and their sensitivity pattern.

Material and Methods

This cross-sectional study was conducted in the Pediatric unit Civil Hospital from 1st August 2013 to 30th September 2014. Children were included in the study through non-probability convenience sampling from both the outpatient and inpatient departments. Patients’ up to five years of age of either gender with complaints of fever (defined as the axillary temperature of ≥101°F) for less than two weeks and parents willing to participate in the study were included. Patients who were very sick, chronically ill, received antibiotics within the last 48 hours, refused to give written consent or had structural anomalies of urinary tract were excluded from the study.

The study physician explained the purpose, procedure, risks and benefits of the study to the parents and written consent was taken from them. An interview along with complete physical examination was performed and a urine sample for microscopy and culture was sent for all enrolled patients. Urine was collected following standard aseptic measures and sent to the laboratory within an hour of its collection. Aseptic bags were used for urine collection. Urethra was washed before attaching urine bag. For males, the penis was placed in the bag and for females, the bag was placed over the two folds of skin on either side of the labia. The culture was considered positive if at least 50,000 colony-forming units (CFU) per mL of a uropathogen were identified from the quantitative culture. High colony count with more than one species was considered as a contaminant; however, the clinical correlation was also done. In the case of contamination & clinically febrile children, the second culture was sent. Disc diffusion method was used to determine the susceptibility of isolates. The zone diameters of each drug were interpreted using the criteria published by the Clinical and Laboratory Standards Institute (CLSI, formerly the National Committee for Clinical Laboratory Standards or NCCLS).

The sample size was calculated using WHO software for sample size calculation. Using the prevalence of urinary tract in febrile patients of 13%, confidence level of 95% and degree of precision of 5% the total sample size was 272. Data was entered and analyzed on SPSS version 20.0. The mean ±standard deviation is presented for age. Frequency and percentage are reported for categorical variables like sex, age group, circumcision, voiding habits, urinary tract infections and causative organisms. Univariable and multivariable logistic regression analyses were done to determine the independent predictors of UTI in children.

Results

A total of 272 children of less than 5 years of age were enrolled with fever. The median age of enrolled children was 2.8 (Interquartile range 2) years. Male patients accounted for 53.3% cases with male to female ratio of 1:1.14. The majority of children presented with low-grade fever, 182 (66.9%) followed by diarrhea (19.5%), dysuria 37 (13.6%), ureteric colic 31 (11.4%) and flank pain 28 (10.3%). The other symptoms like polyuria in 24 (8.8%) case, poor stream 17 (6.3%), vomiting in 16 (5.9%), refusal to feed in 24 (8.8%) cases and convulsions in 11 (4%) cases were observed. Of 272 febrile children, 32 (11.8%) had urinary tract infection confirmed by urine culture. None of the children had received any prior antibiotics.

Comparison of demographic and clinical features of patients with culture confirmed UTI and those without UTI is summarized in table 1. In the univariable analysis, the age of the child, female gender, flank pain, ureteric colic, diarrhea, vomiting and refusal to feed were found to be associated with urinary tract infection (Table 1). However, in multivariable analysis, female gender (OR 5.7, 95% CI: 1.88-17.41), diarrhea (OR 7.7, 95% CI: 2.36-28.82) and flank pain (OR 3.67, 95% CI: 1.02-13.21) were independent predictors of urinary tract infection in febrile children. The sensitivity and specificity of the model were 100% and 90% respectively (Table 2).

Among patients with positive urine culture, 30 had a single pathogen; E. coli was most commonly isolated organism (Figure 1). Antimicrobial susceptibilities of the pathogens are given in table 3. Antimicrobial susceptibilities of the pathogens are given in table 3.

Discussion

UTI is a significant problem in children. The factors responsible for the consequences of UTI are the non-specific clinical presentation in children and lack of appreciation of high morbidity and mortality associated with UTI and the spectrum of microorganisms associated with it. Significant bacteriuria has been documented in febrile infants and children even with an alternative diagnosis of fever.

In our study children less than five years age and females were...
A quarter of the subjects had dysuria on presentation, a much higher frequency (68%) has been reported in other studies. A study conducted in Yemen reported similar results. A study in Lahore by Waqar et al reported UTI was common in patients between one to five years of age. Among 272 febrile children 32 (11.8%) had UTI in this study. This finding is comparable to a study in India conducted in 2003, which concluded incidence of UTI in febrile children as 10%. A quarter of the subjects had dysuria on presentation, a much higher frequency (68%) has been reported in other studies.

### Table 1: Comparison of demographic and clinical characteristics between children with and without urinary tract infection

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Children with urinary tract infection (n=32)</th>
<th>Children without urinary tract infection (n=240)</th>
<th>Univariable analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age Median (IQR)</td>
<td>2.3 (2.2)</td>
<td>2.7 (2)</td>
<td>1.37 (1.02-1.83)</td>
</tr>
<tr>
<td>Male</td>
<td>10 (31.3%)</td>
<td>135 (56.3%)</td>
<td>1</td>
</tr>
<tr>
<td>Female</td>
<td>22 (68.8%)</td>
<td>105 (43.8%)</td>
<td>2.82 (1.28-6.23)</td>
</tr>
<tr>
<td>Low grade fever</td>
<td>17 (53.1%)</td>
<td>165 (68.8%)</td>
<td>1</td>
</tr>
<tr>
<td>High grade fever</td>
<td>15 (46.9%)</td>
<td>75 (31.3%)</td>
<td>1.94 (0.92-4.09)</td>
</tr>
<tr>
<td>Dysuria</td>
<td>4 (12.5%)</td>
<td>33 (13.8%)</td>
<td>1.12 (0.36-3.38)</td>
</tr>
<tr>
<td>Polyuria</td>
<td>4 (12.5%)</td>
<td>20 (8.3%)</td>
<td>1.57 (0.50-4.93)</td>
</tr>
<tr>
<td>Flank pain</td>
<td>10 (31.3%)</td>
<td>18 (7.5%)</td>
<td>5.61 (2.31-13.63)</td>
</tr>
<tr>
<td>Ureteric colic</td>
<td>16 (50%)</td>
<td>15 (6.3%)</td>
<td>15 (6.29-35.73)</td>
</tr>
<tr>
<td>Poor stream</td>
<td>4 (12.5%)</td>
<td>13 (5.4%)</td>
<td>2.49 (0.76-8.17)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>26 (81.3%)</td>
<td>27 (11.3%)</td>
<td>34.18 (12.91-90.52)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>7 (21.9%)</td>
<td>9 (3.8%)</td>
<td>7.87 (2.46-20.96)</td>
</tr>
<tr>
<td>Refusal to feed</td>
<td>7 (21.9%)</td>
<td>17 (7.1%)</td>
<td>3.67 (1.38-9.71)</td>
</tr>
<tr>
<td>Convulsions</td>
<td>2 (6.3%)</td>
<td>9 (3.8%)</td>
<td>1.71 (0.35-8.29)</td>
</tr>
</tbody>
</table>

*OR-Odds ratio, †CI-Confidence interval

### Table 2: Predictive model for diagnosis of children with febrile urinary tract infections

<table>
<thead>
<tr>
<th>Predictors</th>
<th>OR</th>
<th>95% CI Lower</th>
<th>95% CI Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.23</td>
<td>.846</td>
<td>1.80</td>
</tr>
<tr>
<td>Gender</td>
<td>5.72</td>
<td>1.88</td>
<td>17.41</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>7.73</td>
<td>2.36</td>
<td>28.82</td>
</tr>
<tr>
<td>Flank pain</td>
<td>3.67</td>
<td>1.02</td>
<td>13.21</td>
</tr>
</tbody>
</table>

Sensitivity 100% Specificity 90%

### Table 3: Antibiotic sensitivity pattern of causative organisms

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>E. coli (n=12)</th>
<th>Klebsiella (n=8)</th>
<th>Pseudomonas spp (n=4)</th>
<th>Proteus spp (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin/Clavulanic acid</td>
<td>8</td>
<td>6</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Cefaclor</td>
<td>10</td>
<td>3</td>
<td>NT</td>
<td>2</td>
</tr>
<tr>
<td>Cefixime</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Ciprofloxacil/Ofloxacil</td>
<td>10</td>
<td>4</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>10</td>
<td>5</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Amikacin</td>
<td>10</td>
<td>6</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Fosfomycin</td>
<td>12</td>
<td>7</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

Predominant. A study conducted in Yemen reported similar results. A study in Lahore by Waqar et al reported UTI was common in patients between one to five years of age. Among 272 febrile children 32 (11.8%) had UTI in this study. This finding is comparable to a study in India conducted in 2003, which concluded incidence of UTI in febrile children as 10%. A quarter of the subjects had dysuria on presentation, a much higher frequency (68%) has been reported in other studies.
Female preponderance has been documented worldwide in all settings.12,13 This finding was due to the short urethra of females and perineal contamination. In our study febrile children with UTI presented with diarrhea and vomiting each accounting for 33.3%. Diarrhea has been significantly associated with UTI; reported by many studies conducted in India.14,15

In our study UTI was common in uncircumcised males; similar findings have been reported by other investigators. This finding is due to colonization of bacteria in foreskin that may cause contamination of urine.12,13

In the present study factors that predicted UTI in febrile children were female gender, diarrhea, and flank pain. These factors can be used in developing countries to predict UTI in children in a place where urine culture is not routinely done. Diagnosis of UTI in children poses a significant challenge due to the fact that most of the clinical characteristics in children suspected to have UTI are not reliable, in the present study as in other studies19 dysuria, flank pain, vomiting, failure to thrive, irritability were not statistically significantly associated with UTI. There is no specific sign or symptom that can predict the presence of UTI in infants and children. Combinations of findings, including a prior history of UTI, should be taken into account when making a decision to evaluate for UTI.19-20 The limitation of this study is that other causes of fever were not investigated. In our study E. coli was most commonly isolated organism followed by Klebsiella pneumoniae. E. coli was commonly reported organism in the study at Yemen16 and a study conducted in Ayub teaching hospital reported similar results comparable to our study. However, a few studies have indicated a lower percentage of E. coli and higher infection with Proteus and Klebsiella.17 Proteus was isolated from 2 male subjects. This has been explained by the ability of the Proteus species to swarm the long urethra of males and ascend to cause infection.11

E. coli was sensitive to Co-amoxiclavulanic (66.6%), quinolones (83.3) and resistant to nalidixic acid and co-trimoxazole (83.3%). Unlike our study, in Yemen, E. coli were sensitive to nalidixic acid (70%).

In conclusion, a high prevalence of UTI is observed among febrile children in our setting and is predicted by female gender, diarrhea, and flank pain. Gram negative organisms are common pathogens of UTI and most are still sensitive to conventional antibiotics.

References


Report Antibiotics Stewardship Activities

Two Interactive sessions on “Antibiotics Stewardship in Community” were held at PC Hotels, Rawalpindi and Peshawar on 13th April and 12th May 2016 respectively. This event was organized in collaboration with SanofiPastuer Pakistan. 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 speakers included Dr. Ejaz Khan, Brig Dr Qudratullah Malik and Dr Farah Qamar. A total of 172 and 170 GPs and pediatricians attended these two events at Rawalpindi and Peshawar respectively. The audiences were very active and asked a lot of related questions. Important material related to antibiotic stewardship was also distributed. A survey form gathered showed how the participants felt that this was an important and crucial topic and wanted this activity to continue.

The following important topics were deliberated:

a. Overview of Antibiotics Stewardship
b. Neonatal Sepsis
c. Community acquired Pneumonia
d. Meningitis

Antibiotic Resistance -- act today for safer tomorrow

GSK and MMIDSP held a press briefing titled ‘Antibiotic resistance -- act today for safer tomorrow’ on April 12, 2016. Ejaz Khan, infectious diseases consultant at Shifa International Hospital highlighted how antibiotic has been accelerated by misuse and overuse of antibiotics, as well as poor infection prevention and control. Physicians, health workers, pharmacists, policy makers and agriculturists can help spread awareness about appropriate antibiotic usage. Dr. Mohammad Usman, associate consultant microbiologist at Shifa International Hospital said some resistant infections cause severe illness, require increased recovery time, tend to incur increased medical expenses, or may die from the infection if not treated properly. Dr. Khalid Naeem Khawaja, consultant microbiologist at the National Agricultural Research Centre (NARC), said that antibiotics are commonly used in animals that are used as food to prevent, control, and treat disease, and to promote the growth of food-producing animals. Dr. Shafqat Ali Hamdani, pharmacist and consultant healthcare system at Shifa Pharmacy and Laboratories, said “Public should only use antibiotics when prescribed by a certified health professional and should always take the full prescription; never using left-over antibiotics; and never sharing antibiotics with others.”
Instructions to Authors

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

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

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

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

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

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

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

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

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

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

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

Acknowledgments
Acknowledge any sources of support, in the form of grants, equipment or technical assistance. The source of funding (if any) for the study should be stated in this section. Please see below for format of References, Figures and Tables.
II. Review Articles
Authoritative and state of the art review articles on topical issues are also published, with a word limit of 2000. It should consist of critical overview of existing literature along with reference to new developments in that field. These should be comprehensive and fully referenced. Articles should contain an Abstract; Main Text divided into sections, Conclusions and References.

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

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

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

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

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

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

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

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

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

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

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

Instructions updated - April 2012.
Editor IDJ