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Acknowledgement: Dr. Viqar Zaman
Parasitic Diseases: The Forgotten Burden

Parasitic diseases cause tremendous mortality and morbidity worldwide, and of 300 million people affected at least 50% are school-age children living in developing countries. According to recent WHO estimates one in every four persons harbors parasitic worms. The commonly neglected parasitic diseases include malaria, schistosomiasis, hook worm disease, leishmaniasis, giardiasis and amebiasis. Malaria, the eighth leading contributor to global diseases burden measured in disability adjusted life years (Daly’s), poses a risk to 50% of the world’s population in 107 countries.

Intestinal parasitic infections (IPIs) are globally endemic and have been described as constituting the greatest single worldwide cause of illness and disease. They are associated with lack of sanitation, inaccessibility to safe water and improper hygiene, and therefore they are linked directly to poverty. IPIs deprive the poorest of health, whilst contributing to economic instability and social marginalization. The poor people of developing nations experience perpetual cycles where under nutrition and repeated infections lead to adverse consequences that continue from generation to generation. People of all ages are affected by prevalent parasitic infections; however, children are worst affected.

Ascaris lumbricoides, Trichuris trichiura and hookworms referred to as soil-transmitted helminths (STHs), are the most common intestinal parasites. Ascaris lumbricoides is the largest and the most common helminth parasitizing the human intestine, which currently infects about 1 billion people worldwide. Hymenolepis nana is the most common parasitic cestode prevalent globally. Giardia duodenalis/Giardia intestinalis, previously known as Giardia lamblia, is a prevalent protozoan parasite worldwide causing giardiasis, with about 200 million people currently infected. Another common intestinal protozoan Blastocystis hominis whose parasitic status is under debate is also widely distributed.

Pakistan is an endemic country for parasitic diseases owing to the sub-tropical climate, high level of illiteracy, rapid population growth, urbanization, overcrowding, poor hygiene, and lack of awareness and lack of access to basic health facilities. Malaria is a major health problem in Pakistan with estimated number of annual episodes of about 1.6 million of which about 33% are reported as falciparum malaria cases.

It is essential to formulate an indigenous medical curriculum which builds a competent graduate who can serve societal needs and is adequately prepared to deal with common diseases. Traditionally, Parasitology has been taught in medical schools as a part of Pathology/Microbiology, decreasing its significance in comparison to Virology and Bacteriology. We need more emphasis on parasitic diseases in the medical curricula and make sure that our medical graduates are not only knowledgeable but aware of the huge impact these diseases have on ordinary lives.

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Acknowledgement
This editorial draws excerpts from two articles submitted by the author and in so doing I would like to acknowledge my collaborators.
Detection of Blastocystis hominis in Humans and Poultry

Syeda Sadaf Haider, Rakhshanda Baqai
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Abstract

Background
Blastocystis hominis, previously only considered a commensal, is now also being reported as a causative agent of zoonotic infection and diarrhea in humans. The objective of this study was to determine the prevalence of B. hominis infection in poultry and humans in different age and sex groups and its seasonal pattern.

Methods
Two hundred and thirty fecal samples were collected from patients with gastrointestinal symptoms and their clinical histories were recorded. Direct microscopy and formol ether concentration method was used. Cryptosporidium spp. were detected by Kinyoun method. Bacteriological study was done by inoculating fecal samples on appropriate media. Identification was done by gram staining, standard biochemical methods and Quick test strip (QTS). Blood samples were also collected for hematological tests. One hundred and fifty poultry fecal samples were collected from Poultry Research Institute and examined by direct microscopy.

Result
Results indicate that out of 230 faecal samples from patients, parasites were detected in 161 (70 %) of cases. B. hominis was present in 31 % followed by Entamoeba histolytica (18 %), Cryptosporidium spp. (16 %), Giardia lamblia (9 %), Ascaris lumbricoides (6 %), Hymenolepis nana (1 %). B. hominis cases were found to be more in April and September and more cases were detected from Malir area. Infection with B. hominis was found to be more in females between 0 - 19 years of age and more commonly in children. B. hominis infection as a single pathogen was found in 60 %, and 40 % in combination with one or more pathogens. The most predominant symptom was abdominal pain (24 %) followed by diarrhea (22 %), constipation (21 %), fever (18 %) and vomiting (15 %). No bacterial pathogen was found in combination with B. hominis. Higher eosinophil count was observed in patients positive for blastocystosis. Out of 150 poultry faecal samples, 40 (27 %) were positive for B. hominis.

Conclusions
This study indicates that B. hominis can be a causative agent of zoonotic disease. It was found to be a causative agent of diarrheal disease in humans either alone or in combination with other parasites.

Keywords
Blastocystis hominis, Zoonotic Infection, Diarrhea

Introduction
Blastocystis hominis is a polymorphic protozoa found commonly in the intestinal tract of humans. It occurs world-wide, and infection in developing countries such as Pakistan and Bangladesh is relatively high. Its prevalence was found to be higher (25 % – 46 %) than in immunocompetent persons (0.5 % - 11.5 %).

B. hominis has been reported in many animals including invertebrates (house fly, earthworm), reptiles, birds and mammals, including humans. B. hominis consist of several different species with distinct host ranges, and different bird species are infected with morphologically distinct Blastocystis. Fecal-oral route is considered to be the main mode of transmission; zoonotic transmission of B. hominis also occurs. Human-to-human transmission of B. hominis infection between two small communities has also been reported. Moreover, people working closely with animals in zoos and abattoirs and food handlers are at higher risk of acquiring this infection.

B. hominis may act as a commensal but can be pathogenic under specific conditions, such as immunosuppression, poor nutrition or concomitant infections. Recent evidence indicates that B. hominis is potentially pathogenic if found in stool between five or more organisms per high power field and in the absence of other pathogenic organisms, and shows response to treatment with iodoquinol or metronidazole. Blastocystis infection depends upon a variety of factors, including the number of infected human and nonhuman hosts, seasonal influence and duration of infection, agricultural practices, host behavior and activity, socioeconomic and ethnic differences in human behavior, geographic distribution, sanitation, climate and hydrogeology of the area.

The most common complaint of blastocystosis is of intense abdominal discomfort accompanied by pain. Other symptoms are diarrhea, vomiting, anorexia, bloating, constipation, dehydration, sleeplessness, nausea, weight loss, fatigue, dizziness.
Blood in the stool as well as excessive mucus and leukocytes have been reported. B. hominis has been associated with many chronic conditions, including irritable bowel syndrome, chronic fatigue and arthritis.

This study was done to determine the prevalence of B. hominis as a zoonotic infection and to determine whether it is a commensal or a pathogen.

**Material and Method**

Two hundred and thirty stool samples were collected from February 2005 through December 2005 from patients with abdominal pain and acute and chronic diarrhea. Samples were collected through laboratories of Malir Hospital and Nadeem Medical Centre, Karachi, Sindh, Pakistan. Clinical histories were recorded from patients. Direct microscopy and formalin-ether concentration method was used for the detection of helminths, ova and protozoan cysts. Cryptosporidium spp was detected by Kinyoun method. One hundred and fifty faecal samples from poultry birds were collected from Sindh Poultry Vaccine Centre, Karachi, Sindh, Pakistan and examined by direct microscopy.

Direct Microscopy: A smear was prepared by mixing small amount of faecal material with 1 drop of normal saline and iodine on a glass slide. A coverslip was applied and the smear was observed under low and then high power objective.

Formol-ether concentration method: 1 gram of faeces was mixed with 3-4 ml of 10% formol water in a glass tube and then 3-4 ml of 10% formol water was added and mixed for 20 seconds. It was then sieved in a beaker and transferred to a centrifuge tube. 3-4 ml ether was added and the tube was shaken for 15 seconds and then centrifuged for 1 minute at 3000 rpm. After centrifugation faecal debris, ether and formol water were discarded. A drop of sediment was taken and after applying a coverslip it was observed under low and then high power objective.

Kinyoun method: A smear was prepared by mixing a small amount of faecal material with 1 drop of normal saline on a glass slide, it was air dried and fixed with methanol for 3 minutes. The smear was stained with 1% basic carbol fuschin for 2 minutes, washed with tap water and decolorized with 1% H2SO4 for 1 minute and counter stained with 0.5% malachite green for 30 seconds, washed with tap water, air dried and observed with oil immersion objective.

Stool specimens were inoculated on MacConkey and S.S agar for enteric bacteria (i.e., Salmonella spp, Shigella spp, Yersinia enterocolitica, Escherichia coli and Vibrio cholerae ). Incubation was done at 37°C and examined after 24 hours. Identification was done by colony morphology; gram staining and biochemical tests by standard methods and Quick test strip (QTS). Blood samples were also collected from patients for hematological study. Total leukocytes, erythrocytes and hemoglobin were estimated and differential leukocyte count was performed by Field’s stain. Eosinophilia is defined as = 250 eosinophils per µl of peripheral blood and has often been associated with parasitic diseases.

**Results**

In this study 230 fecal samples were collected from patients with gastrointestinal symptoms. All samples were observed with direct microscopy, Blastocystis hominis, Entamoeba histolytica, Giardia lamblia, Ascaris lumbricoides, Hymenolepis nana were observed. Cryptosporidium was stained with Kinyoun method. 16 out of 26 samples were positive for Cryptosporidium. Culture results of samples were not significant for detecting any bacterial pathogenic. No significant eosinophil count was observed in the blood profile.

**Fig. 1: Isolated from 230 stool samples.**

![Fig. 1](image)

Fig. 1 shows pathogens isolated from stool samples. Out of 230 faecal samples from patients, parasites were present in 161 (70%) of cases.

**Fig. 2: Age and sex distribution**

![Fig. 2](image)

Fig. 2 shows distribution of B.hominis according to age and sex. Infection was found to be more prevalent in females, and between 0 - 19 years of age.
Fig. 3: Symptoms in patients with *B. hominis* infection

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>No. of patients (n)</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal pain</td>
<td>39</td>
<td>24</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>35</td>
<td>22</td>
</tr>
<tr>
<td>Constipation</td>
<td>34</td>
<td>21</td>
</tr>
<tr>
<td>Fever</td>
<td>29</td>
<td>18</td>
</tr>
<tr>
<td>Vomiting</td>
<td>24</td>
<td>15</td>
</tr>
</tbody>
</table>

Fig. 3 shows symptoms of patients with *B. hominis* infection. The most predominant symptoms were abdominal pain (24 %) followed by diarrhea (22 %), constipation (21 %), fever (18 %) and vomiting (15 %).

Fig. 4: Seasonal pattern of *Blastocystis*

Fig. 4 shows seasonal pattern of *B. hominis*. Infection was found to be more in April and September.

Fig. 5 a: Detection of *B. hominis* and other parasites in faecal samples from patients

<table>
<thead>
<tr>
<th>Parasites</th>
<th>No. of patients (n)</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Protozoa:</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>B. hominis</em></td>
<td>50</td>
<td>31</td>
</tr>
<tr>
<td><em>E. histolytica</em></td>
<td>29</td>
<td>18</td>
</tr>
<tr>
<td>Cryptosporidium spp.</td>
<td>26</td>
<td>16</td>
</tr>
<tr>
<td><em>Giardia lamblia</em></td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td><em>Helminths:</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. lumbricoides</em></td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td><em>H. nana</em></td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Fig. 5 shows detection of *B. hominis* with other parasites. *B. hominis* was present in 31 %, followed by *Entamoeba histolytica* (18 %), *Cryptosporidium spp.* (16 %), *Giardia lamblia* (9 %), *Ascaris lumbricoides* (6 %), *Hymenolepis nana* (1 %). *B. hominis* 60 % was found as a single pathogen and 40 % in combination with other pathogens. No bacterial pathogen was found in combination with *B. hominis*.

Fig. 5 b: *Hominis in Fecal Samples from Patients* (Saline Staining 40 x)

Fig. 6: Area wise distribution of *B. hominis* in patients

<table>
<thead>
<tr>
<th>Areas</th>
<th>Number of cases (n)</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malir</td>
<td>55</td>
<td>34</td>
</tr>
<tr>
<td>North Karachi</td>
<td>41</td>
<td>25</td>
</tr>
<tr>
<td>Nazimabad</td>
<td>40</td>
<td>25</td>
</tr>
<tr>
<td>Gulshan-e-Iqbal</td>
<td>32</td>
<td>20</td>
</tr>
<tr>
<td>Landhi</td>
<td>32</td>
<td>20</td>
</tr>
<tr>
<td>Saddar</td>
<td>32</td>
<td>20</td>
</tr>
<tr>
<td>Defence</td>
<td>33</td>
<td>20</td>
</tr>
<tr>
<td>Orangi Town</td>
<td>33</td>
<td>20</td>
</tr>
<tr>
<td>Korangi</td>
<td>26</td>
<td>16</td>
</tr>
<tr>
<td>Baldia Town</td>
<td>25</td>
<td>15</td>
</tr>
<tr>
<td>F.B Area</td>
<td>19</td>
<td>12</td>
</tr>
<tr>
<td>Linez Area</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td>Gulistan-e- Jauhar</td>
<td>14</td>
<td>8</td>
</tr>
</tbody>
</table>

Fig. 6 shows the area wise distribution: *B. hominis* was found mostly in Malir area.

Fig. 7 a: *Blastocystis hominis in faecal samples from poultry*

**Discussion**

Asymptomatic and symptomatic *B. hominis* infections in humans have been reported worldwide. Infection is associated with variable gastrointestinal and generalized symptoms in humans.
Transmission of B. hominis can occur via animal-to-animal, human-to-animal and animal-to-human. Zoonotic transmission of B. hominis from animal handlers and normal healthy individuals was reported in 41/105 animal handlers, and 17/163 healthy individuals who were positive for B. hominis. People who work closely with animals are at risk of acquiring this infection. Many species of animals harbor Blastocystis and a potentially widespread transmission occurs between animals and humans. B. hominis infections may be of zoonotic origin but due to non cooperation of poultry workers we were unable to collect faecal samples from them.

**Conclusion**

In this study we detected B. hominis in 70% of stool samples from humans and 27% from faecal samples from poultry. B. hominis was present in both symptomatic as well as asymptomatic patients. 31% of symptomatic patients had abdominal pain, constipation, diarrhea, vomiting and fatigue. It was detected alone or in combination with other intestinal pathogens. No significant eosinophil count was observed. It has a seasonal preference for summer months with peaks in April and September; it affects all ages but is more likely to affect children 0-19 ages and females are more affected. B. hominis infection was more prevalent in suburban areas where animal contact is more common than in townships. This study also indicates that B. hominis can be a causative agent of zoonotic disease, and as such animal handlers would be at a higher risk of acquiring B. hominis infection.

**References**

13 Cheesbrough, Medical laboratory manual for tropical countries. 1 (2): 185-186
22 Rajah Salim H, Suresh Kumar G, Khairul Anuar A, Ini I. Blastocystis hominis in animal handlers. Parasitol Res. 1999; 85 (12); 1032-33
23 Smith HV. Detection of parasites in the environment. Parasitology. 1999; 117: 113-141
Frequency of Extended-Spectrum -Lactamases (ESBLs) and Blood Stream Infections

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**Assistant Professor Microbiology, Foundation University Medical College, Rawalpindi.
***Professor of Microbiology, Department of Pathology, Rawalpindi Medical College,
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Abstract

Objective
To determine the frequency and distribution of ESBLs -producing Enteric Gram- negative rods (EGNRs) in blood stream infections.

Materials and Methods
This descriptive study was conducted during 04/2004-03/2006 at the Microbiology department of Fauji Foundation Hospital, Rawalpindi. ESBL production was studied in Enteric Gram-negative rods isolated from blood samples by the help of double disc diffusion technique.

Results
Out of 46 EGNRs 15 each were Enterobacter coli and Pseudomonas aeruginosa (32.6%), 8 were Klebsiella pneumoniae (17.3%), 4 were Aeromonas spp (8.69%), 3 were Enterobacter spp (6.52%) and only one was Citrobacter spp (2.17%). Prevalence of ESBLs-production was 32.6% (15 out of 46). The most prevalent ESBLs-positive EGNR was E. coli n=8 (53.3%), followed by K. pneumoniae, P. aeruginosa, Aeromonas spp n=2 each (13.3%) & Enterobacter spp n=01 (6.66%). Escherichia coli showed the highest frequency of positivity (53.3%), followed by Aeromonas (50%) & Enterobacter spp (33.3%), Klebsiella pneumoniae (25%) and Pseudomonas aeruginosa (13%).

Conclusions
It is important to screen for ESBL production routinely by all the laboratories, as administration of 3rd generation cephalosporins and aztreonam in an infection with ESBL-producer would not only be ineffective but also further promote ESBL production, leading to increasing resistance.

Key Words
Extended- Spectrum Beta-lactamases, Enterobacteriaceae, Enteric Gram -Negative rods, Escherichia coli, Klebsiella pneumoniae.

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A susceptibility disk containing amoxicillin-clavulanate (20 \( \text{g amoxicillin/10 g clavulanic acid} \)) was placed in the center of the inoculated plate. Disks of cefotaxime, ceftazidime, ceftriaxone and aztreonam were placed 30 mm (center to center) from the amoxicillin-clavulanate disk. Enhancement of the zone of inhibition of the oxyimino-\( \beta \)-lactam due to synergy of the clavulanate in the amoxicillin-clavulanate disk was considered as evidence of ESBL-production (Fig 1). *Escherichia coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used as control strains.

**Fig 1. Detection of ESBL Double Disc Diffusion Technique Results**

Out of 46 Enteric gram-negative rods (EGNRs) isolated from the blood cultures, *Escherichia coli* and *Pseudomonas aeruginosa* were 15 each (32.6%), followed by *Klebsiella pneumoniae* \( n=8 \) (17.3%), *Aeromonas spp* \( n=4 \) (8.69%), *Enterobacter spp* \( n=3 \) (6.52%) and *Citrobacter spp* was only one (2.17%) (Table 1). Prevalence of ESBLs-production was 32.6% (15 out of 46) (Fig 2).

**Table 1. Frequency of Enteric Gram-negative rods in bloodstream infections**

<table>
<thead>
<tr>
<th>Enteric Gram–Negative Rods</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>15</td>
<td>32.6</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>15</td>
<td>32.6</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>8</td>
<td>17.3</td>
</tr>
<tr>
<td><em>Aeromonas spp</em></td>
<td>4</td>
<td>8.69</td>
</tr>
<tr>
<td><em>Enterobacter spp</em></td>
<td>3</td>
<td>6.52</td>
</tr>
<tr>
<td><em>Citrobacter spp</em></td>
<td>1</td>
<td>2.17</td>
</tr>
<tr>
<td>Total</td>
<td>46</td>
<td>100</td>
</tr>
</tbody>
</table>

Out of 15 ESBLs-positive EGNR, *Escherichia coli* \( n=8 \) (53.3%) was most prevalent, followed by *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Aeromonas spp* \( n=2 \) each (13.3%) . *Enterobacter species* showed the lowest positivity rate \( n=1 \) (6.66%) (Table 2). Highest frequency of positivity was noted with *Escherichia coli* 53.3% (8 out of 15), followed by *Aeromonas* 50% (2 out of 4), *Enterobacter spp* 33.3% (1 out of 03) *Klebsiella pneumoniae*, 25% (2 out of 8), *Pseudomonas aeruginosa* 13.3% (2 out of 15) (Table 3).

**Table 2. Prevalent Organism causing ESBLs in 46 blood isolates**

<table>
<thead>
<tr>
<th>Type of Enteric Gram-negative Rods</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>8</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>2</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>2</td>
</tr>
<tr>
<td><em>Aeromonas spp</em></td>
<td>2</td>
</tr>
<tr>
<td><em>Enterobacter spp</em></td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
</tr>
</tbody>
</table>

**Discussion**
The SENTRY Program has previously shown that extended-\( \beta \)-lactamases are prevalent in many countries in the Asia pacific region and South Africa having a common monitoring in 1998. Over time, the frequency of ESBLs is increasing. The prevalence of ESBLs-production in this study was 32.6%. Variable results were noted in other studies, 4.3-89%. Prevalence of ESBL-producing strains in various species of
Enterobacteriaceae differs in different countries & in different hospitals. Usually one of the three species (Klebsiella pneumoniae, Escherichia coli, Enterobacter) predominates.  

Table 3. Frequency of Prevalent Organisms among ESBLs -Positive isolates

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Total</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(No)</td>
<td>No</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td>Aeromonas spp</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Enterobacter spp</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td>Citrobacter spp</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>46</td>
<td>15</td>
</tr>
</tbody>
</table>

The most prevalent ESBLs-producing EGNR in this study was Escherichia coli (53.3%), followed by Klebsiella pneumoniae, Pseudomonas aeruginosa and Aeromonas spp (13.3% each). Enterobacter species showed the lowest positivity rate (2.17%). Escherichia coli (48%) was the most prevalent organism reported by Shah et al (2004), from Islamabad, Pakistan. The prevalence of ESBL-producing Escherichia coli ranges from 7%–28.5% in various studies. During a five-year surveillance study in France, the prevalence of ESBL-production was 11.4% in Klebsiella species and 47.7% in Enterobacter aerogenes. According to & , (2006) from Saudi Arabia, 15.8% of the isolates from blood cultures were ESBL-producers.

There is a marked increase in the incidence of infections due to ESBL-producing E. coli, especially in the community. ESBLs were present in high proportions of Escherichia coli (25%) and Klebsiella pneumoniae isolates (17%) causing septicemia at a tertiary hospital in Tanzania. Klebsiella spp (80%) was the commonest ESBLs-producer, reported from Armed Forces Institute of Pathology, Rawalpindi, and all India institute of Medical Sciences, New Delhi. A study carried out by Shah et al (2004) in Quaid-i-Azam University Islamabad, revealed a high frequency of ESBLs (70%) in Klebsiella pneumoniae. According to & (2006) among the ESBLs-producer in blood cultures, 48.4% were Klebsiella pneumoniae, followed by 15.8% of Escherichia coli and Enterobacter cloacae.

Secondary β-lactamases have been reported widely in Pseudomonas aeruginosa but much more rarely than in Enterobacteriaceae. According to et al (2006) 3.5% of P. aeruginosa were ESBLs-producers. Incidence of 13% from France, 7% from Spain and 2.5% from England has been reported. In the present study, the incidence of ESBLs in Pseudomonas spp was 14.2%.

Conclusions

32.6% of the EGNRs were ESBL-producers, E. coli being the most prevalent pathogen. The most frequent ESBL-producer was E. coli followed by K. pneumoniae. Administration of third generation cephalosporins and aztreonam in an infection with confirmed ESBL-producers would be disastrous, because this would not only be ineffective thus increasing mortality but simultaneously promoting ESBLs-production and leading to increased resistance. Therefore, it is very important to screen for ESBLs-production routinely by all the laboratories.

References:


50. Infectious Diseases Journal of Pakistan


Childhood Tuberculosis: a Review of Epidemiology, Diagnosis and Management

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Introduction
It is estimated that about one third of the world’s population is infected with *Mycobacterium tuberculosis*¹. Not all people infected have the disease, and most of them are adults. Tuberculosis (TB), however, remains an under-diagnosed and neglected entity in children. A total of 8.3 million new cases of TB were reported worldwide in 2000, of which an estimated 11% cases were children and the reported percentage of all TB cases occurring in children varied from 3% to more than 25% in different countries²,³. Most Asian countries have a lower proportion of children with TB compared to countries in sub-Saharan Africa. This discrepancy may be due to differences in the population structure of TB patients, or to differences in case detection or reporting of TB in children⁴. The World Health Organization currently reports only smear-positive cases by age. This underestimates the magnitude of TB in children because young children cannot produce sputum and approximately 95% of cases in children <12 years of age are smear-negative⁶. Rates of smear-positive disease among children <15 years of age range from 0 to 3 per 100,000 population. However, there is wide variation among individual countries, with Bolivia and Djibouti reporting the highest smear-positive rate (12/100,000) in 2001⁷. Two-thirds of the countries with case rates of more than 5/100,000 among children are located in sub-Saharan Africa.

According to the WHO there were 450,000 deaths due to TB in children under 15 years of age in 1989⁸. Seventy-five per cent of all childhood TB cases occur annually in 22 high-burden countries, mostly in sub-Saharan Africa². In developing countries, TB is responsible for 10% or more of childhood hospital admissions, and 10% or more of hospital deaths⁹. Recently, the global burden of TB in children and its impact on child health are being increasingly recognized, in part because of the re-emergence of TB as a major public health problem in developing countries¹⁰.

Epidemiology and risk factors
Most children acquire the infection from adults in their surroundings. That is why the epidemiology of childhood TB follows that in adults¹¹. Understanding the risk factors and disease outcome in children is fraught with challenges. These include absence of a gold standard of diagnosis, difficulty in establishing a definitive diagnosis, the increased prevalence of extra-pulmonary disease and a lower public health priority given to childhood TB compared to adult disease. The latter is due to the fact that children do not contribute significantly to the spread of the disease. Not all children develop the disease once infected. The factors that determine a child’s risk of developing disease include younger age, malnutrition, recently acquired infection, and immune suppression, particularly due to measles or HIV infection². Children less than 5 years old and infected with TB are at higher risk of developing disease probably due to immature cellular immunity¹²,¹³. Exposure to TB is determined by the degree and nature of contacts with source cases as well as duration of infectiousness of the source cases. Factors such as family size, living space, population density, and the age of TB patients in a given setting determine the number of infections per infectious case. High incidence of TB in children aged 0–5 years in an area of South Africa correlated with a lower level of parental education, low annual household income, and household crowding¹⁴. A study in Turkey found that TB was associated with malnutrition, measles, and pertussis¹⁵. Studies from low- and middle-income countries confirm associations with poverty, crowding, and malnutrition as risk factors for TB in children²,¹⁶. In industrialized countries, childhood TB constitutes about 2–7% of all TB cases and many of these cases were detected through contact tracing, and had low death and high treatment completion rates. Whereas in low and middle-income countries, childhood TB constitutes about 15–40% of all TB, with higher TB death rate and low treatment success rates. A number of studies in North America and Europe showed that immigrant children had a higher risk of disease than local children¹⁷–²¹.

Pathology
Children acquire TB usually from a droplet infection. The TB bacteria, *Mycobacterium tuberculosis*, spreads from aerosol formation of sputum expectorated by infected adults²².

Pulmonary tuberculosis
Exposure to TB leads to the development of a primary

The latter is due to the fact that children do not contribute significantly to the spread of the disease. Not all children develop the disease once infected. The factors that determine a child’s risk of developing disease include younger age, malnutrition, recently acquired infection, and immune suppression, particularly due to measles or HIV infection². Children less than 5 years old and infected with TB are at higher risk of developing disease probably due to immature cellular immunity¹²,¹³. Exposure to TB is determined by the degree and nature of contacts with source cases as well as duration of infectiousness of the source cases. Factors such as family size, living space, population density, and the age of TB patients in a given setting determine the number of infections per infectious case. High incidence of TB in children aged 0–5 years in an area of South Africa correlated with a lower level of parental education, low annual household income, and household crowding¹⁴. A study in Turkey found that TB was associated with malnutrition, measles, and pertussis¹⁵. Studies from low- and middle-income countries confirm associations with poverty, crowding, and malnutrition as risk factors for TB in children²,¹⁶.
parenchymal lesion (Ghon focus) in the lung with spread to the regional lymph node(s). In the majority of cases, the resultant cell-mediated immunity contains the disease process at this stage. Progression of disease occurs by 1) extension of the primary focus with or without cavitary lesions; 2) the pathological processes caused by the enlarging lymph nodes, or by 3) spread through lymphatic and/or hematogenous spread. The typical lesion in lymph nodes and in lung tissue is caseation necrosis, found more often in adults than in children.

**Extrapulmonary tuberculosis**

Extrapulmonary tuberculosis (EPTB) refers to TB of organs other than the lungs. Diagnosis should be based on one culture-positive specimen, or histological or strong clinical evidence consistent with EPTB, followed by a decision to treat with a full course of anti-TB chemotherapy. EPTB is common among children and the most common forms include TB lymphadenopathy, TB meningitis, TB effusions (pleural, pericardial and peritoneal) and spinal TB. According to WHO, the ratio of pulmonary and EPTB in children is usually around 1:3. However, a retrospective study in Brazil found that among under-15 children, pulmonary TB was most frequent (57.8%), EPTB occurred in 24.4% of the cases, while both forms occurred together in 17.8%. A nationwide case finding study in Malawi showed that most childhood EPTB was TB lymphadenopathy (41% of all EPTB cases), pleural effusion was found in 12% cases, spinal disease in 10% cases, pericardial disease in 7% cases, and ascites in 5% cases. Miliary TB accounted for 4% of EPTB cases, TB meningitis another 4% cases, bone TB other than spinal in 1% cases, while in 16% the type of EPTB could not be ascertained. The highest morbidity and mortality occur in young children who are at increased risk of developing the severe forms of TB i.e. miliary TB and TB meningitis.

**Diagnosis**

The diagnosis of childhood TB is difficult largely due to the fact that the disease in children produces non-specific clinical manifestations. Unlike adults, infants and young children usually do not develop the cavitary form of lung TB that can be more readily detected by a chest X-ray. Moreover, young children less than 10 years old cannot produce sputum and this makes the detection of *M. tuberculosis* and diagnosis of TB difficult. Bacteriological confirmation, however, by culture of *M. tuberculosis*, the ‘gold standard’ of diagnosis, rarely exceeds 30–40%. Gastric aspirate, an alternative to sputum in young children, is positive on smear or on culture in less than 20% and 50% respectively in children with pulmonary TB. This procedure is also rarely performed outside urban centers, as is the case with mycobacterial cultures. Most often, no effort is made to confirm a diagnosis of TB bacteriologically in a child because of cost, limited resources, training, and lack of appropriate laboratory facilities. Widespread childhood malnutrition in developing countries not only predisposes children to TB infection and increases the severity of the disease, but also renders diagnosis of TB difficult because the tuberculin skin test for TB is often negative in such a population.

To overcome the problem of diagnosis of TB in children, a combination of clinical features, history of exposure to adult patients with TB, result of tuberculin test and radiological findings has been used. Various scoring systems and diagnostic classifications have been developed based on these variables. Emphasis is given to laboratory tests i.e., detection of acid-fast bacilli (AFB), tubercles in biopsy, suggestive radiology and tuberculin test giving an induration of >10 mm. As many as 16 diagnostic approaches have been mentioned in a review by Hesseling et al. Investigators have modified them to make them more relevant to specific settings such as in a pediatric population with high prevalence of malnutrition. The basis of a scoring system is the careful collection of diagnostic information. The scoring system is actually a screening test that helps clinical judgment.

Edwards et al., compared different scoring systems in a resource-limited setting with a high prevalence of TB. They found that the various scoring systems for the diagnosis of childhood TB correlated poorly with each other. There was also considerable disagreement on when to initiate TB treatment. Findings were similar for HIV-infected and non-infected children. Hesseling et al., concluded that comparison of different systems was difficult because the definitions of symptoms and other diagnostic characteristics were not standardized. A few diagnostic approaches were developed using the gold standard of diagnosis, bacteriological confirmation, and a few studies looked at validation of these diagnostic approaches. It is felt that the newly developed diagnostic approaches should be assessed objectively and an independent reference standard should be used for diagnosis to avoid bias. It is also recommended that these scoring systems be validated in individual countries before being used for screening children for TB.

The Dhaka Hospital of ICDDR,B conducts a program for the diagnosis, treatment and follow-up of children with TB. Since 1994 around 700 patients have been treated, of whom more than 600 were children. Over 75% of the children were diagnosed to have TB while undergoing nutritional rehabilitation for severe malnutrition. Anecdotal experience suggests that childhood TB is under-diagnosed at the hospital as elsewhere. Therefore, the actual number of children with TB reporting to the hospital could be much higher. At ICDDR,B the modified version of the Kennet-Jones score (KJS) is used to diagnose TB. The median age of 398 children diagnosed in the last five years on the basis of the modified KJS was 15 months with a male:female ratio of 1.6:1, 95% of children were severely malnourished with a weight-for-age of 46±8%, (mean±SD) of the National Centre for Health Statistics (NCHS) median reference. Most of these children came from families having a monthly income of approximately US$ 50 and poor parental schooling.
According to this scoring system, 7 or more points indicate unquestionable TB; 5-6 points indicate probable TB, therapy may be justified; 3-4 points indicate that further investigations are needed.

A score of 7 or more indicates a high likelihood of TB, treatment is justified.

A history of contact with a TB patient could be obtained in 40% cases, the Mantoux test (MT) was positive in 33%, and radiological evidence of parahilar or paratracheal lymphadenopathy was observed in 58% of children. The median KJS in this group of children was 5 with an interquartile range of 4-6 (data unpublished). In contrast to the modified KJS, the WHO-recommended TB score (TBS) does not require the use of chest x-ray to diagnose childhood TB, (table 2). A comparison of the different diagnostic approaches is shown in

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### Table 1. Modified Kenneth-Jones' criteria for the diagnosis of TB in children

<table>
<thead>
<tr>
<th>Score +3</th>
<th>Score +2</th>
<th>Score +1</th>
<th>Score -1</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Recovery of AFB from sputum, gastric aspirate, laryngeal swab, etc.</td>
<td>1. X-ray chest suggestive of para-hilar lymphadenopathy with or without parenchymal lesions</td>
<td>1. Non-specific chest X-ray changes</td>
<td>BCG vaccination in the last 2 years</td>
</tr>
<tr>
<td>2. Tuberculous granuloma, granulomatous lesions in lymph node biopsy or choroidal tubercles on fundoscopy</td>
<td>2. Suggestive physical findings: pleurisy, skin lesion, osteomyelitis, Pott's spine etc.</td>
<td>2. Compatible physical findings: erythema nodosum, phlyctenular conjunctivitis, meningitis, cervical lymphadenitis, arthritis, hemoptysis, etc.</td>
<td></td>
</tr>
<tr>
<td>3. Positive Mantoux test (MT) induration exceeding 10 mm</td>
<td>3. Doubtful MT (5-9 mm)</td>
<td>3. History of contact with a patient suffering from TB</td>
<td></td>
</tr>
<tr>
<td>4. Recent MT conversion from negative to positive</td>
<td>4. Non-specific granuloma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Contact with sputum smear-positive patient</td>
<td>5. Age below 2 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Non-response to therapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. 3rd degree protein-energy malnutrition</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 2. WHO TB score for use in the diagnosis of TB in children

<table>
<thead>
<tr>
<th>Feature</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>GENERAL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of illness (weeks)</td>
<td>&lt;2</td>
<td>2-4</td>
<td>&gt;4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nutrition (% weight-for-age)</td>
<td>&gt;80</td>
<td>60-80</td>
<td>&lt;60</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TB in family (past or present)</td>
<td>Nil</td>
<td>Reported by family</td>
<td>Proved sputum smear positive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tuberculin test</td>
<td></td>
<td></td>
<td>Positive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malnutrition</td>
<td></td>
<td></td>
<td>Not improving after 4 weeks of treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unexplained fever and night sweats</td>
<td>No response to malaria treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOCAL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large, painless lymph nodes, sinus in neck, axilla, groin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Joint or bone swelling</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdominal mass or ascites</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNS signs (change in temperament, lethargy, fits, coma, and abnormal CSF findings)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angle deformity of spine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A score of 7 or more indicates high likelihood of TB.
Table 3. Review of different approaches for the diagnosis of childhood TB

<table>
<thead>
<tr>
<th>Diagnostic approach</th>
<th>Characteristics of the different approaches</th>
<th>Setting</th>
<th>Statistical parameters</th>
<th>Remarks</th>
</tr>
</thead>
</table>
| Kenneth-Jones criteria              | • Bacteriological confirmation  
• Tuberculous granuloma  
• Mantoux test  
• Chest X-ray  
• Physical findings relating to TB  
• History of contact with TB patient  
• Points for age below 2 years  
• Non-response to therapy  
• Nutritional status  
• BCG during last 2 years, etc. | India (38)  
(no HIV)  
Brazil (39)  
(no HIV) | Specificity 73%  
Sensitivity 56%  
Specificity 94% | Not validated against bacteriological confirmation. |
| WHO TB Score Chart /Keith Edwards system | • Duration of illness  
• Nutritional status  
• History of TB in family  
• Mantoux test  
• Malnutrition not improved after 1 month  
• Enlarged painless neck gland  
• Night sweating or prolonged fever  
• Deformity of spine  
• Swelling of joint  
• Ascites  
• Coma >48 h or slowly developing neurological sign | Papua New guinea (40)  
(low HIV prevalence)  
Zambia (37)  
(high HIV prevalence)  
India (41)  
(no HIV)  
Brazil (39)  
(no HIV) | Sensitivity 62%  
Specificity 95%  
Sensitivity 88%  
Specificity 25%  
PPV 55%  
NPV 67%  
Sensitivity 91%  
Specificity 88%  
Sensitivity 84%  
Specificity 97% | Not validated against bacteriological confirmation. |
| WHO scoring system (diagnostic classification) | • Contact with TB patient  
• Not regaining health after measles and whooping cough  
• Loss of weight  
• Cough and wheeze not responding to antibiotic therapy  
• Painless swelling of superficial nodes  
• Tuberculin test  
• X-ray chest  
• Histology/Response to anti-TB  
• Bacteriological confirmation | South Africa (26, 42)  
(low HIV prevalence area) | PPV 63%, false positive rate 24%. | A useful screening tool in areas where TB is prevalent. |
| IUATLD (point scoring system)       | • Contact with TB patient  
• Mantoux test  
• Persistent cough  
• Low weight for age  
• Unexplained prolonged fever | International pooled data (43)  
(no HIV) | Sensitivity <70%  
Specificity <70%  
PPV 60-77% | A useful screening tool for further investigation |

IUATLD, International Union Against Tuberculosis and Lung Disease; PPV, positive predictive value; NPV, negative predictive value
Among different scoring systems, the Keith-Edwards scale is the most widely used, appears in clinical texts and was recommended in the first edition of the World Health Organization TB/HIV clinical manual in 1996. A study conducted to assess the Keith-Edwards scoring system demonstrated sensitivity of 62% and specificity of 95%. A recently published study of the same diagnostic approach, however, concluded that this scoring system should not be used in its present form as a diagnostic tool in HIV-endemic areas due to its low specificity in such a population (sensitivity 88% and specificity 25%). In HIV-endemic settings, it can lead to over diagnosis and thus potentially unnecessary treatment.

Community-based trials have shown that child TB can be diagnosed using simple symptom-based approach. A community-based study in South Africa has shown that a combination of three symptoms (a persistent non-remitting cough of >2 weeks duration, documented failure to thrive (in the preceding 3 months), and fatigue may provide reasonable diagnostic accuracy in TB detection (sensitivity 62.6%, specificity 89.8%, positive predictive value 83.6%) 44.

Diagnosis by culture, PCR and serology Though the conventional culture technique for M. tuberculosis is simple, an incubation period of 7-10 weeks is necessary for isolation of the organisms 45. This long period required for isolation led to the development of other techniques for culture such as the BACTEC radiometric assay, SeptiCheck AFB system, and mycobacterial growth indicator tube system (MGIT). The average time for detection is 18 days for the BACTEC system, 26 days for SeptiCheck AFB system and 14 days for MGIT. The capability of performing rapid mycobacterial drug sensitivity is an additional advantage of the BACTEC system and MGIT 46.

The polymerase chain reaction (PCR) is now a commonly used technique for early diagnosis of TB. The PCR can be used to (i) diagnose TB rapidly by identifying DNA from M. tuberculosis in clinical samples that are negative by microscopic examination; (ii) determine rapidly whether acid-fast organisms identified by microscopic examination in clinical specimens are M. tuberculosis or atypical mycobacteria; (iii) identify the presence of genetic modifications known to be associated with resistance to some antimycobacterial agents and, (iv) DNA typing to differentiate between reactivation of disease and exogenous reinfection, and to track transmission and internal laboratory contamination (10). The sensitivity of PCR ranges from 4%-80% and the specificity 80%-100% 46-49.

Recently, a new test QuantiFERON®-TB test (QFT) was approved by the Food and Drug Administration of USA for use in TB patients and their contacts to detect latent M. tuberculosis infection. It measures the cell mediated immune responses to peptide antigens (early secretory antigenic target-6 (ESAT-6) and culture filtrate protein-10 (CFP-10)) by production of interferon gamma. These two antigens are highly specific for M. tuberculosis complex species (50). Commercial serological antibody-based tests are available but they are expensive and can not replace sputum smear microscopy for pulmonary TB in adults (51). These tests have little or no role in case detection in children.

WHO recommended treatment regimen for childhood TB

The treatment of TB in children is divided into two phases: an intensive phase for 2 months and a continuation phase for at least 4 months. The intensive phase uses a greater number of drugs with the goal of rapidly eliminating the majority of organisms and to prevent the emergence of drug resistance. Fewer drugs are generally used in the continuation phase because the risk of acquiring drug resistance is low, and most of the organisms have already been eliminated during the intensive phase. In either phase, treatment can be given daily or three times weekly. Table 4 shows the first-line anti-TB drugs and their recommended doses (3).

<table>
<thead>
<tr>
<th>Essential drug</th>
<th>Recommended dose in mg/kg body weight (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid</td>
<td>Daily 5 (4.6) maximum 300mg daily</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>10 (8–12) maximum 600mg daily</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>25 (20–30) maximum 600mg daily</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>20 (15–25) maximum 600mg daily</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>15 (12–18) maximum 600mg daily</td>
</tr>
</tbody>
</table>

There are concerns about the toxicity of ethambutol, particularly for optic neuritis, in young children. However, a literature review indicates that it is safe in children at a dose of 20 mg/kg (range 15–25 mg/kg) daily. Streptomycin should be avoided when possible in children because the injections are painful and irreversible auditory nerve damage may occur. It is mainly reserved for the first 2 months of treatment of TB meningitis.

The recommended treatment regimens for different TB categories are generally the same for children as for adults and are shown in table 5 3.

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Table 5. Recommended treatment regimens for different categories of TB

<table>
<thead>
<tr>
<th>Category</th>
<th>TB cases</th>
<th>Intensive phase (daily)</th>
<th>Continuation phase (3 times weekly)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Smear-positive pulmonary TB Smear-negative pulmonary TB with extensive parenchymal involvement Severe forms of EPTB (other than TB meningitis) Severe concomitant HIV disease</td>
<td>2HRZE</td>
<td>4HR or 6HE‡</td>
</tr>
<tr>
<td>II</td>
<td>Previously treated smear-positive pulmonary TB: —relapse —treatment after interruption —treatment failure</td>
<td>2HRZES followed by 1HRZE</td>
<td>5HRE</td>
</tr>
<tr>
<td>III</td>
<td>Smear-negative pulmonary TB Less severe forms of EPTB eg TB lymphadenopathy</td>
<td>2HRZ</td>
<td>4HR or 6HE</td>
</tr>
<tr>
<td>IV</td>
<td>Chronic and MDR-TB</td>
<td>Specially designed standardized or individualized regimens</td>
<td></td>
</tr>
</tbody>
</table>

‡ This regimen (2HRZE/6HE) may be associated with a higher rate of treatment failure and relapse compared with the 6-month regimen with R in the continuation phase.

TB = tuberculosis; H = isoniazid; R = rifampicin; Z = pyrazinamide; E = ethambutol; EPTB = extra-pulmonary TB; HIV= human immunodeficiency virus; S = streptomycin; MDR-TB= multidrug resistant TB.

Adjunctive treatment with corticosteroids

Corticosteroids may be used for the management of complicated forms of TB, e.g., tuberculous meningitis (TBM), the complications of airway obstruction by TB lymph glands, and TB pericarditis. In advanced TBM cases, corticosteroids have been shown to improve survival and reduce morbidity. Prednisone is used in a dosage of 2 mg/kg/day (maximum dosage 60 mg/day) for 4 weeks. The dose should then be slowly reduced (tapered off) over 1–2 weeks before stopping. Miliary or hematogenously disseminated TB should be managed similar to TB meningitis. All children with miliary TB (or suspected of having military TB) should undergo lumbar puncture to evaluate the presence of meningitis.

Drug resistant TB in children

The treatment of TB is complicated when the organism is resistant to anti-TB drugs. Acquired resistance is defined as resistance to one or more anti-TB drugs, which arises during the course of treatment. It is usually due to non-adherence to the recommended regimen or due to incorrect prescription and intake of anti-TB drug. Primary resistance is defined as the presence of resistant strains of *M. tuberculosis* in patients who have been infected with the resistant bacilli by another patient and subsequently develop the disease. Multidrug resistant TB (MDR-TB) describes strains of TB that are resistant to at least the two main first-line TB drugs - isoniazid and rifampicin. Extensive Drug Resistant TB or XDR-TB (also referred to as Extreme Drug Resistance) is MDR-TB that is resistant to three or more of the second-line drugs (52). Mortality rate of MDR-TB among adults is as high as 43–93% (52, 53). Costs of drug therapy of MDR-TB cases have been reported to be as much as 100-fold higher than those of treating drug-susceptible cases (54).

Information about MDR-TB in children is scarce. Resistance patterns in children have generally been found to be similar to those of adults from the same areas and similar backgrounds (53, 54). A prospective study in the Western Cape province of South Africa evaluated 149 child contacts of 80 adult MDR pulmonary TB cases (55). This study revealed that most of the child contacts of adults with MDR-TB are likely to be infected by these MDR source cases. In a report of 306 under-five children, the incidence of INH resistance was 5.6% and MDR 1%. Clinical features were similar in children with drug-susceptible and drug-resistant TB (56). In India, the prevalence of resistance to any drug was 32.4%, while that of multi-drug resistance was 13.3% during 1994-1997 (57). Drug-resistance surveillance in childhood TB in South Africa between 2003 and 2005 had shown a similar rate of MDR-TB but a significant increase in isoniazid resistance, from 6.9% to 12.8% which reflected the ongoing transmission of drug-resistant TB (58).

**Treatment of multidrug resistant TB (MDR-TB)**

Children with multidrug resistant TB (MDR-TB) should be treated with the first-line drugs to which they (or their source case) are susceptible. Ethambutol is bactericidal at higher doses, so doses of up to 25 mg/kg/day should be used in children with MDR-TB. Table 6 summarizes the reserve (or second line) anti-TB drugs for treatment of MDR-TB in children.

Impact of HIV on childhood TB

HIV infection is probably one of the most important factors for the resurgence of TB in adults as well as in children (11, 59). Globally 13% of adults with newly diagnosed TB are co-infected with HIV, with 34% in the African continent to 1.4% in the Western Pacific region. The rates of HIV infection in patients with TB have so far remained below 1% in Bangladesh, China,
Table 6. Reserve or second-line anti-tuberculosis drugs

<table>
<thead>
<tr>
<th>Reserve (second-line) drug</th>
<th>Recommended daily dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dose (mg/kg)</td>
</tr>
<tr>
<td>Ethionamide or prothionamide</td>
<td>15–20</td>
</tr>
<tr>
<td><strong>Fluoroquinolones</strong></td>
<td></td>
</tr>
<tr>
<td>Ofloxacin</td>
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<td>Levofloxacin</td>
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<td>Moxifloxacin</td>
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<td>Gatifloxacin</td>
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<tr>
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<td>Kanamycin</td>
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<tr>
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<tr>
<td>Cycloserine or terizidone</td>
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<tr>
<td>Para-aminosalicylic acid (PAS)</td>
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Indonesia and Pakistan\(^60\). In a cohort study in Addis Ababa, HIV-positive children with TB were younger, more underweight and had a 6-fold higher mortality than HIV-negative children with TB\(^61\). The tuberculin skin test was found to be less sensitive and chest radiography less specific in HIV-infected patients. The cure rate was 58 per cent for HIV-positive and 89 per cent for HIV-negative pediatric TB patients. Both diagnostic error and delay in diagnosis of TB are common in case of HIV-positive children. Clinical manifestations are more severe and progression to death more rapid in HIV-positive children\(^11, 61\). Among 118 culture-proven TB patients in South Africa, 48% children were found to be HIV-1 infected and 37% non-HIV-1-infected \(^62\). Hospital-related mortality was higher (17.5%) in HIV-1-infected children than in non-infected group (11.4%). Reported co-infection of HIV and TB in various Indian studies ranged from 16%-68% \(^63-65\). Experience from the Tuberculosis Research Centre in Chennai also suggests that TB manifestations are more severe in HIV-positive children, with lower cure rates and higher mortality\(^11, 57\).

TB prevalence in the first 4 years of life in children born to HIV-infected mothers is 10 times higher than in non-HIV-infected mothers. It is also 30 times higher in HIV-infected than uninfected children (66). A prospective study has shown that children with a CD4 percentage below 15% had four times higher risk of TB than the children with normal CD4 counts \(^67\).

**The role of BCG vaccination**

The efficacy of BCG vaccination in the prevention of TB has varied from 0% to 80\(^%\). Its overall protective effect is 50%-68\(^\%\), the effect being greatest in preventing TB meningitis and miliary or disseminated TB. Therefore, even if it cannot reduce the disease burden in general, the vaccine can reduce the severity of disease. Among infants who were vaccinated, BCG had a 64\(^\%\) protective effect against TB meningitis and a 78% protective effect against disseminated TB \(^2, 68\). For this reason, the WHO continues to recommend BCG vaccination of infants. The efficacy of BCG vaccination may be reduced in HIV-infected individuals. The conversion to a positive tuberculin skin test after BCG vaccination is less frequent in HIV-infected individuals\(^11\). Although there have been reports of a higher risk of disseminated BCG disease in HIV-infected children, BCG appears to be safe in the vast majority of cases \(^17, 69\). A recently published study assessed the cost-effectiveness of BCG vaccination against childhood TB meningitis and miliary TB worldwide\(^70\). The number of TB meningitis and miliary TB cases prevented in children born in 2002 up to age 5 years worldwide was 29,729 and 11,486 respectively. Most of the cases were prevented in Southeast Asia (46%), Africa (27%) and the Western Pacific (15%). Incremental cost-effectiveness and cost-utility ratios were calculated to combine the costs and benefits of vaccination versus no vaccination. In case of TB meningitis, the incremental cost per case or death prevented was $8,592 while the incremental cost per disability adjusted life year (DALY) gained was $285. In case of miliary TB, the incremental cost per case or death prevented was $23,294 and incremental cost per DALY gained was $771 \(^70\).

**DOTS and childhood TB**

There is little information on how well Directly Observed Therapy-short course strategy (DOTS) programs have addressed childhood TB \(^2, 14\). An observational trial evaluated directly observed therapy 6-month regimen for pulmonary, pleural and lymph node tuberculosis in children. The trial used isoniazid, rifampicin and pyrazinamide therapy daily for only 2 weeks; followed by 6 weeks of twice weekly isoniazid, rifampicin and pyrazinamide therapy; and 16 weeks of twice weekly isoniazid and rifampicin. Only 37% of patients had complete resolution of disease at the end of treatment, but all continued to improve after therapy was stopped \(^71\). Since DOTS depends on demonstration of AFB in sputum smear, the major problem in inclusion of children in DOTS is difficulty in demonstration of AFB and classification of different clinical manifestations according to categories described for adults \(^11\).

**Preventive therapy for childhood TB**

Young children living in close contact with a source case of smear-positive pulmonary TB are at particular risk of TB infection and disease. The risk of infection is greatest if the contact is close and prolonged such as the contact of an infant with the mother. Isoniazid preventive therapy for young children with infection who have not yet developed disease will greatly reduce the likelihood of developing TB during childhood. Recommended treatment for a healthy contact aged less than 5 years is isoniazid 5 mg/kg daily for 6 months. Follow-up should be carried out at least every 2 months until treatment is complete\(^1\). The other important measure to control TB in children is BCG vaccination. It provides protection against dissemination.
of illness. It is recommended that all household contacts be screened for symptoms of the disease and isoniazid preventive therapy should be provided to children aged less than 5 years and to all HIV-infected children. Close contacts of MDR-TB patients should receive careful clinical follow-up for a period of at least 2 years. If active disease develops, prompt initiation of treatment with a regimen designed to treat MDR-TB is recommended. However, chemoprophylaxis with second-line drugs for MDR-TB is not recommended.

Conclusion
Tuberculosis is among the top ten causes of global mortality. This is compounded by the dual TB–HIV epidemic. Diagnosis of TB is difficult in children, particularly in developing countries. Children have non-specific clinical signs, variable chest X-ray features, infection is paucibacillary in nature with low rates of bacteriological confirmation. Diagnosis of TB in children, therefore, has relied mainly on clinical case definitions, tuberculin skin-testing and chest radiography. Child DOTS needs to be implemented in earnest by national TB control programs. In every aspect of childhood TB, from epidemiology to risk factors to laboratory and community based diagnosis, TB/HIV coinfection, drug resistance in children, and preventive therapy, there is need for additional research.

References
46. Comparative study of different methods of identification of Mycobacterium tuberculosis in gastric aspirate of children suffering from pulmonary tuberculosis. MD thesis. All India Institute of Medical Sciences, New Delhi; 1997.
49. Comparative study of different methods of identification of Mycobacterium tuberculosis in gastric aspirate of children suffering from pulmonary tuberculosis. MD thesis. All India Institute of Medical Sciences, New Delhi; 1997.
Molecular Epidemiology of Mycobacterium Tuberculosis

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Abstract

Despite BCG vaccination and effective antituberculous therapy (ATT), TB causes 3 million deaths worldwide. Multidrug resistant (MDR) TB specially is on rise globally. The global distribution of TB cases is skewed heavily towards developing countries. Pakistan together with other Asian countries; such as China, India, Bangladesh, and Indonesia, makes up over 50 percent of the global burden of the TB cases. Mycobacterium tuberculosis (MTB) is the causative agent of tuberculosis. Comparative genome sequencing of MTB strains revealed that MTB complex with highly conserved genome has polymorphic genomic regions. This polymorphism in MTB genome has led towards the development of several molecular typing methods for MTB such as IS6110 based restriction fragment polymorphism (RFLP), spoligotyping, Polymorphic GC-rich sequence genotyping (PGRS) and variable number of tandem repeat-Mycobacterial interspersed Repetitive Unit (VNTR-MIRU) typing methods. Utility of these genetic tools to study the epidemiology of infectious diseases has introduced the concept of molecular epidemiology. IS6110-RFLP, spoligotyping and VNTR-MIRU typing methods have been used for genotyping of MTB strains from all over Pakistan which revealed Central Asian Strain and Beijing strain as predominant strains types amongst our isolates.

This review describes the utility of these molecular typing methods for epidemiological studies for strain differentiation and disease transmission of MTB.

Tuberculosis in Pakistan

Pakistan, together with other Asian countries; such as China, India, Bangladesh, and Indonesia, makes up over 50 percent of the global burden of the tuberculosis (TB) cases. Pakistan ranks sixth amongst the 22 high burden TB disease countries, with an estimated incidence rate of 181/100,000 population. In addition about 80 percent of new TB cases occur in this 22 high disease burden countries annually. Moreover, this estimated TB burden in Pakistan is an underestimated figure as many cases in the country go unreported due to lack of access to health care facility, over crowding, poverty and other social constraints. Majority of TB cases occur predominantly in the economically most productive, 15 to 54-year age group which further hinders socioeconomic development especially in low income countries.

Genetic organization of Mycobacterium tuberculosis

Complete genome sequencing of Mycobacterium tuberculosis H37Rv led TB research community to enter in genomic era. Genome sequencing of MTB H37Rv revealed that it consists of 4.4 mega base pairs with densely packed coding regions. It is estimated to comprise 4000 protein coding regions and has a very high guanine and cytosine content. Since then MTB clinical strain CDC1551 and six related mycobacteria i.e. M. bovis, M. leprae, M. ulcerans, M. avium, M. avium paratuberculosis and M. smegmatis have been fully sequenced. Comparative genome sequencing of MTB strains revealed that Mycobacterium tuberculosis (MTB), the main causative agent of TB, while display diverse phenotypic characteristics and host ranges, has an overall genomic similarity of 99.9% throughout the world. However studies have shown that MTB complex with highly conserved genome has polymorphic genomic regions. Like eukaryotic genomes, those of MTB are separated by periodic repeat of monomeric sequences, called repeat sequences. There are two types of repeat sequences, interspersed repeats and tandem repeats.

Interspersed repeats

It includes insertion sequence (IS) and direct repeat (DR). IS are small mobile genetic elements which are widely distributed throughout the MTB genome. Over 14 different kinds of IS have been identified in the MTB genome which are usually less than 2.5 kb in size. The most widely utilized IS in epidemiological studies is IS6110, which is present in the range of 0-26 copies in MTB strains. In 1993, van Embden and colleagues proposed a standardized Southern blot hybridization method based on the frequency of IS6110 in the MTB genome which allows strain differentiation.

In the chromosomal region MTB strains also contain multiple 36-bp DRs, interspersed by unique 35 to 41 bp spacer DNA sequences. Thus this information led to another most widely used rapid strain typing method known as Spoligotyping. This method is based on the detection of presence or absence of 43 interspersed spacer sequences in the genomic DR region of MTB using Polymerase Chain Reaction (PCR).
Tandem repeats
Tandem repeats (TRs) or variable number tandem repeats (VNTRs) are array of consecutive base pair repeats in non-coding region of MTB DNA. Polymorphism i.e. difference in number of VNTRs are used to identify various types in a MTB population using PCR based method. Frothingham and Meeker O’Connel reported 11 VNTR loci in the MTB complex strains, five (A to E) were reported as major polymorphic tandem repeats (MPTR) and six (A to F) as exact tandem repeats (ETR) ranging in size from 53 to 79 bp . Since then additional VNTRs have been reported. Supply et al reported 41 VNTR of mycobacterial interspersed repetitive units (MIRU) of 40 to 100 bp size scattered around the genome H37Rv and other MTB complex strains . Twelve of the 41 polymorphic MIRU loci have been for genotyping of clinical MTB strains. High-throughput automation of MIRU-VNTR genotyping analysis has made the process more interesting and less laborious. Discriminatory power of MIRU-VNTR analysis increases when more than 12 MIRU loci are used. In the result of concerted effort to select a better combination of VNTR for MTB genotyping, presently fifteen out of twenty-nine MIRU-VNTR has been selected which need to be evaluated in different settings .

Thus a number of DNA fingerprinting methods are based on analysis of the degree of similarity and distribution of these variable elements between isolates, which are used as genetic markers.

Molecular epidemiology of tuberculosis and its significance
Molecular epidemiology has emerged from integration of molecular biology and epidemiology. Specifically in infectious diseases it aims to investigate whether a particular clinical strain differs in infectiousness, severity of disease or drug susceptibility locally as well as globally. Thus molecular epidemiologic methods in TB have facilitated studies that address some of the key areas. These include the current rates of active transmission by differentiating recent or previous infection; the determination of whether all M. tuberculosis strains exert similar epidemiologic characteristics in populations and understanding of transmission dynamics. However, with availability of a range of genotyping methods, genetic diversity and polymorphism in MTB are the key aspects to determine for the application of molecular epidemiology in a population.

Genotyping Methods for Mycobacterium tuberculosis
There is a wide variety of genotyping markers for Mycobacterium tuberculosis as discussed earlier. However a few of these exhibit enough discrimination and reproducibility for wide scale adaptation. Some of such genotyping methods have been discussed here.

**IS6110 Restriction Fragment Length Polymorphism (IS6110-RFLP)**
In 1993, this technique was adopted as the standard method for routine MTB typing (van Embden, 1993). This technique based on the occurrence of number and location of Insertion Sequence 6110 (IS6110), which is unique to MTB complex and is found in multiple copies in most strains. In this method, chromosomal DNA is digested with restriction enzyme *Pvu*II. The digested DNA is separated on an agarose gel, blotted on a nitrocellulose membrane and hybridized with peroxidase labeled IS6110 probe. Thus IS6110- containing digested fragments are detected using enhanced chemiluminescence (ECL). IS6110- RFLP typing has gained recognition as the gold standard in the molecular epidemiology of TB. This typing method is highly discriminatory and reproducible (van Soolingen et al, 1995). However there are certain limitations to the application of this technique. Firstly a proportion of MTB isolates contains few or no copies of IS6110 element and the method is unreliable for typing such strains. Secondly it needs large amount of DNA and several weeks are required to culture enough viable organisms. Thirdly the method is labor intensive, technically demanding and expensive.

**Spoligotyping**
Another typing method, spoligotyping (spacer-oligonucleotyping), has extensively been used as a secondary typing method and as a marker to study the MTB phylogeny (Kwara et al, 2003 & Filliol et al, 2002, 2003). As mentioned earlier this method is based on the polymorphism in the direct-repeat (DR) region of MTB strains. The 36 bp size DRs are interspersed by unique spacer DNA sequences of 35–41 bp. Spoligotyping identifies the presence or absence of 43 spacer DNA sequences between the variable direct repeats using PCR.

Although spoligotyping is simple, rapid and highly reproducible, it cannot totally replace IS6110-RFLP typing because of lower discriminatory power, except for strains with low copy numbers of IS6110 (van Soolingen et al, 2001). In addition, spoligotyping has less discriminatory power as it targets less than 0.1% MTB genomic area as compared to IS6110 based typing which examines the entire genome.

Polymorphic GC-rich sequence genotyping (PGRS) PGRS is a short sequence which is repeated multiple times in MTB genome. This is a Southern blot hybridization technique that use a PGRS- specific probe (3.4 kb fragment of PGRS) cloned in pTBN12 plasmid. Studies have shown that MTB strains with fewer copies (1-5) could be further distinguished by PGRS-RFLP analysis. However unlike IS6110-RFLP system, the hybridization patterns generated by PGRS typing are too complex to computerize for analysis .

**Variable Number of Tandem Repeat-Mycobacterial interspersed Repetitive Unit (VNTR-MIRU) typing:**

Mycobacterial interspersed repetitive units variable number
tandem repeat (MIRU-VNTR) is another PCR based genotyping method which has higher discriminatory power than spoligotyping. Spoligotyping while instrumental in identifying MTB genogroups is unable to help discriminate amongst them. MIRU-VNTR is based on detection of independent mini satellite like loci scattered throughout the MTB genome and has been shown to be a reliable and reproducible typing method with high discriminatory power for studying the MTB population structure in different countries. The typed strains are expressed by a 12-digit numerical code, corresponding to the number of repeats at each locus. This numerical code is easy to compare and exchange at inter-, and intra-laboratory level. The discriminatory power of MIRU-VNTR analysis is proportional to the number of loci evaluated. In general, the discriminatory power of standard twelve loci based typing only slightly lower than that of the IS6110 based restriction fragment length polymorphism (RFLP), which is currently the gold standard for MTB genotyping. However MIRU-VNTR has several advantages over gold standard IS6110 based Restriction Fragment Polymorphism (RFLP). Such as it requires little culture growth or DNA and provides comparable, numerical data by using standard gel electrophoresis or alternatively could be developed as a high throughput system. MIRU typing is also a method of choice for MTB strains with zero to five copies of IS6110 element, which have been reported from south Asian countries.

Single nucleotide polymorphism (SNP)
Extensive comparative genomic analysis of MTB has also revealed nucleotide polymorphism which provided markers to differentiate clinical isolates as well their phylogenetic relatedness. There are two types of single-nucleotide polymorphism (SNP), nonsynonymous single-nucleotide polymorphism (nsSNP) and synonymous single-nucleotide polymorphism (sSNP). nsSNP create amino acid change which might lead to change of phenotypic character. Such as MTB resistance to anti tuberculosis agents usually correlate with genetic alteration i.e. nsSNP, in drug resistance genes.

In contrast, sSNP are considered functionally neutral and do not alter the amino acid profile. However these neutral alterations in the structural or house keeping genes can provide basis for evolutionary studies. Sreevatsan et al divided MTB complex strains into three principle genetic groups (PGGs), PGG1, PGG2 and PGG3, using two functionally neutral polymorphism in codons 463 and 95 of katG and gyrA genes respectively. Other researchers have similarly used sSNP analysis to explore the phylogenetic structures of MTB strains and have reported consistent findings.

Thus SNP may provide significant information in the area of phylogenetic analysis, drug resistance and virulence of MTB strains.

Large sequence polymorphism (LSP) or Deletion analysis Comparative genome analysis of H37Rv and CDC1551 strains has revealed large sequence polymorphisms (LSP) in addition to SNPs. LSP mainly occur due to genomic deletion and rearrangements without horizontal gene transfer. Therefore it has been proposed for genotyping as well as for phylogenetic analysis. Up to 43 genomic regions have been utilized for large scale deletion analysis or deligotyping. Using deleted segment as genetic markers, this analysis can be performed by conventional PCR based method or by automated GeneChip techniques.

![Figure 1. Dendrogram of Pakistani isolates (Pearson correlation). All isolates were spoligotyped, and data were analyzed with the Bionumerics software program. A dendrogram was calculated on the basis of the Jaccard index for pairwise analysis of strains by the unweighted [reproduced with permission]](image)
Study described MIRU loci 26, 31, 16, 10, 27, 39 and 40 as most discriminatory for differentiation of MTB isolates from Pakistan (Figure 2). This information could be instrumental in the identification of predominant CAS1 from our MTB isolates. In addition study also reports prevalence of high copy IS6110 isolates using IS6110-RFLP method (Figure 2).

**Figure 2**: IS6110-RFLP typing of *Mycobacterium tuberculosis*. The figure illustrates a composite analysis of IS6110-RFLP and MIRU-VNTR of 78 MTB strains using Bionumerics software (Applied Maths). The strains included 29 CAS1 and 49 ‘unique’ strains. *denotes CAS1 strains exhibiting heterogeneous IS6110-RFLP profiles [reproduced with permission]

**Conclusions**

Genetic variation analysis is used to track different MTB strains in order to obtain information regarding patterns of spread, infectivity and pathogenicity which has enabled better understanding of *Mycobacterium tuberculosis* and its interaction with the host possible. This in turn could greatly assist in the control of tuberculosis.

**References**

Primary Amebic Meningoencephalitis

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Abstract

Free-living amoebae constitute an important, though uncommon cause of human disease. Human infections due to free living ameba involving brain, skin, lung and eyes have increased significantly during the last few years. Much more so than Naegleria, Acanthamoeba is ubiquitous in the environment, with amebae being widely disseminated in soil and water. Unlike the healthy individuals acquiring Naegleria infections, persons contracting Acanthamoeba infections of the CNS are compromised hosts, suffering from concurrent diseases such as AIDS or other conditions such as alcoholism that predispose them to opportunistic infections. Balamuthia infections have also been reported in individuals with compromised health status, with AIDS patients among those diagnosed, as well as in immunocompetent individuals. While this ameba undoubtedly occurs in nature, it has been isolated only from CNS tissue of individuals or animals that have died from the infection. Naegleria is associated with primary amebic meningoencephalitis (PAM), a fulminating, rapidly fatal infection of the central nervous system. PAM can present as acute pyogenic meningitis in a previously healthy individual and the organism is acquired through swimming in infested waters. Diagnosis and treatment are difficult and the condition is associated with high mortality. We report the first confirmed case of PAM from Karachi, Pakistan.

Case History

A 22 year old male presented to the Aga Khan University Hospital, Karachi, Pakistan, in the month of July, 2005, with complaints of dry cough for 15 days, fever for 4 days and generalized tonic, clonic seizures for 1 day. He had no known chronic illnesses. CT scan of head on admission showed cerebral edema. On examination he was febrile with maximum temperature of 39°C, obtunded and had neck stiffness. A clinical diagnosis of severe sepsis secondary to meningitis and left-sided pneumonia was made. He had to be intubated in the Emergency Room because of severe respiratory distress and was admitted to the intensive care unit where lumbar puncture was done.

Cerebrospinal fluid examination detailed report revealed glucose of 59 mg/dl, protein 1374 mg/dl (Ref range 25-45 mg/dl), total leucocyte count 1520/cmm, with 95% polymorphs, 05% lymphocytes and 6730/cumm RBCs with xanthochromia. Latex particle agglutination (LPA) test for detection of capsular antigens of Streptococcus pneumoniae, Haemophilus influenzae and Neisseria meningitidis was negative. Microscopy of gram stained smear of CSF revealed numerous pus cells but no bacteria. In view of negative LPA and gram stain results a wet preparation of CSF was examined which showed numerous actively motile trophozoites throwing out pseudopodia.

Further history from the family revealed that the patient had gone for a picnic with his friends to “Sakran”, a lake slightly further than Hub Choki, 3 weeks earlier and had been swimming in the lake waters. Another lake which he frequented regularly was Northern Bypass pool, close to Sohrab Goth. The patient died of septic shock within 12 hours of admission before treatment could be initiated.

Based on the morphology of trophozoites in the CSF, a diagnosis
of Naegleria fowleri meningoencephalitis was made. This was later confirmed on culture of Naegleria fowleri on agar medium containing low concentrations of nutrients (peptone 0.05%, yeast extract 0.05%, glucose 0.1%) in the presence of live Escherichia coli in our laboratory.

Discussion

This young man displayed characteristic clinical features of PAM including hyperacute clinical course, unremitting signs and symptoms of meningitis and encephalitis, the latter confirmed by CT imaging, high peripheral leukocyte count, mainly polymorphonuclear leukocytes, pleocytosis, hyperproteinosis, low normal glucose level, and absence of bacteria and fungi, and rapid worsening of disease eventually leading to death. Autopsy of the patient could not be carried out.

Free-living amoebae are known to be capable of causing human disease for over 50 years. They were previously regarded as harmless soil organisms or commensals of mammals1. Pathogenic ameba include Naegleria fowleri, Acanthamoeba spp. and Balamuthia mandrillaris. Sappinia diploidea, a soil ameba, has recently been added to the list of amebae pathogenic for humans2.

These pathogenic protozoa are distinct from other free living amebae. These organisms have no known insect vectors, there are no human carriers, and there is little relationship of poor sanitation to spread of infection as evidenced by antibody titers in surveyed human populations3. Cosmopolitan distribution in soil and water provides multiple opportunities for contacts with humans.

Named after Malcolm Fowler who described the initial case of PAM in 1965, the predominant pathogenic spp is Naegleria fowleri (also called N. aerobia and N. invadens) N. australiensis and N. italica. N. fowleri is distributed worldwide and infections have been reported in countries in every continent1. Its presence in fresh water is related to water temperature2. Pathogenic species proliferate at higher temperature with growth occurring at up to temperatures of 40 to 45°C. An increase in surface temperature create ideal niches for the thermophilic N. fowleri. Persons who bathe, swim, or dive in pools or freshwater natural basins increase their chances of coming into contact with N. fowleri and contracting PAM1. Infection can occur rarely after washing and bathing if household water supply is contaminated6. Not everyone exposed to contaminated water contracts the disease7. This was also evident in this case, where only the patient developed PAM while his friends, who swam with him in the contaminated waters, remained healthy and asymptomatic.

Around 200 cases of PAM have been reported worldwide to date. Victims are healthy, young individuals with a history of recent water-related sport activities. Clinical features are characterized by severe headache and other meningeal signs, fever, vomiting, and focal neurologic deficits which progress rapidly (<10 days) and frequently to coma and death.

The life cycle of N. fowleri consists of three stages: cysts, trophozoites, and flagellated forms. Trophozoites can turn into temporary flagellated forms which usually revert back to the trophozoite stage. Infection occurs when trophozoites cross the cribriform plate to enter the olfactory neuroepithelium and subsequently reach the brain. N. fowleri trophozoites are found in CSF, olfactory nerves and in adventitia and perivascular spaces of small to midsize arteries and arterioles, while flagellated forms are found in CSF. The pathologic changes include acute hemorrhagic necrotizing meningoencephalitis with modest purulent exudate, mainly at the base of the brain, brain-stem and cerebellum. Cortical hemorrhages and edema are seen with uncal or cerebellar herniation; olfactory bulbs are hemorrhagic and necrotic7. The tissue necrosis in meningoencephalitis is partly mediated by a secreted cysteine protease, pore forming proteins, low molecular mass thiol compounds and calcium-mediated complement resistance8. Additionally, pseudopodia-specific Nfa1 protein may also be involved in the pathogenicity of N. fowleri9.

In the laboratory amebae are easily seen and recognized by their slug-like movement on wet preparation examination. The CSF should not be centrifuged or refrigerated. The organism can be easily cultured on nutrient medium in the laboratory. The most common finding on CT is that of cerebral edema, as seen in this patient.

The results of treatment have been disappointing. Only eight reports of cured cases have appeared in the medical literature to date10. The only drug that appears to be promising against N. fowleri in vivo is amphotericin B at a dose of 1 mg/kg/day administered intravenously as a slow infusion or combined with 0.7 mg of amphotericin B intrathecally on alternate days. Other drugs which may be useful in combination with amphotericin are fluconazole and rifampicin10.

It is important to identify all contaminated sites within and outside the city of Karachi and caution the public against bathing and swimming in Naegleria infested waters. Raising the level of awareness is important in preventing the infection. In individual cases, suspicion of PAM should be aroused when the CSF is purulent and no bacteria identified. Diagnosis rests on history of exposure which should be actively sought and demonstration of trophozoites in the CSF. Early institution of amphotericin B in high doses appears to be absolutely essential for chances of successful outcome.

References:


Infective Endocarditis by *Burkholderia cepacia*

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Abstract

*Burkholderia cepacia* has been known for its association with life threatening respiratory infections in patients of cystic fibrosis. We report a laboratory confirmed case of infective endocarditis caused by this environmental bacterium; yielded from paired blood samples and surgically removed cardiac vegetation. Infective endocarditis caused by *Burkholderia cepacia* has not been reported earlier.

Introduction

*Burkholderia cepacia*, commonly known as environmental bacteria, is associated with life threatening respiratory infections especially in cystic fibrosis. Sub-acute infective endocarditis is a microbial infection of the heart valves with variable course depending upon the pathogen, nature of the valve (native or prosthetic), immune status of the patient and comorbid conditions like previous valvular damage, septal defects, IV drug abuse etc. Nevertheless, this condition remains fatal, if untreated. Common etiological agents include *Staphylococci*, *Streptococci* and culture negative causes like HACEK group, *Brucella* spp, *Chlamydia* spp, *Coxiella burnetti* etc. Pseudomonads (a group of aerobic Gram negative bacilli that includes genus *Pseudomonas*) have also been implicated as potential cause. *Burkholderia cepacia*, another member of Pseudomonads family, so far has not been reported as the causative agent for infective endocarditis. We report isolation of *Burkholderia cepacia* from a complicated case of infective endocarditis.

Key words

Case Report

A 42-yearold male with known mitral valve disease underwent percutaneous transvenous mitral commissurotomy (PTMC) and three weeks later developed intermittent fever with generalized body aches and chest discomfort. On admission to the hospital his pulse was 96/ minute, blood pressure 110/70 mm of Hg, respiratory rate 20/minute and temperature 101° F. There was no history of myocardial ischaemia, hypertension or any chronic respiratory illness. Electrocardiography did not reveal any signs of ischaemia. Physical examination revealed a systolic murmur of moderate severity while rest of the systemic examination was unremarkable. His blood complete picture showed a normochromic normocytic peripheral film, slightly raised leucocytes (12000/µl), with neutrophilic predominance (78%). Hepatic and renal functions were within normal limits. On transesophageal echocardiography, large mobile vegetation (14 mm) was detected on the atrial aspect of the posterior mitral leaflet (Figure1). Grade I mitral regurgitation and grade II aortic regurgitation were also noticed. Repeated echocardiographic studies showed no further change in the size of the vegetation. Due to high grade fever, empirical therapy was initiated, comprising of intravenous Inj Vancomycin 1g 12 hourly and Inj Ceftriaxone 1g OD. The patient, meanwhile, started having cardiac discomfort and heaviness in the chest. Mild ischaemic echocardiographic changes led to the decision of carrying out coronary angiogram which demonstrated patent coronary vasculature. After 8 days of the empirical therapy, the urea level started rising and in the following 3 days reached 74 mmol/L. Renal ultrasonography showed Grade I parenchymal disease with bilateral enlarged kidneys. Keeping in view drug-induced nephropathy, antimicrobial therapy was stopped for a while. The case was discussed with Microbiology Department, Armed Forces Institute of Pathology; paired samples for blood culture were advised 8 hourly apart for two consecutive days. Out of

![Figure 1. Transesophageal echocardiograph of patient showing vegetation on mitral valve.](image-url)
these, growth yielded from the last two blood culture samples. Late oxidase positive, non-lactose fermenting rounded colonies appeared on MacConkey agar, showing Gram negative rods on staining. Biochemical profile with api 20NE (bioMérieux, France) identified the isolate as *Burkholderia cepacia*.

Sensitivity was performed by Kirby-Bauer disc diffusion method, according to Clinical and Laboratory Standard Institute (CLSI) recommendations. The isolate was sensitive to meropenem (10µg) with zone diameter 21mm and resistant to ceftazidime (30µg), minocycline (30µg) and trimethoprim-sulfamethoxazole (1.25/23.75µg). Meropenem was immediately recommended while minimum inhibitory concentrations (MICs) were done (Meropenem MIC 0.25µg/mL; Ceftazidime MIC > 32µg/mL) in accordance with CLSI standards. Surgical removal of the vegetation and repair of the regurgitation defect was planned due to the deteriorating condition of the patient especially chest discomfort. During operation, the vegetation was detected on the anterior mitral leaflet and posteromedial commissure, while aortic valve had fusion of commissures and calcifications causing grade II regurgitation murmur. Both the valves were replaced with prosthetic valves. The excised mitral valve with the vegetation (Figure 2) also yielded growth of *Burkholderia cepacia* with similar antibiogram, supplementing the clinical significance of the isolate in this case. Post operatively fever started settling down but the renal status rapidly deteriorated. Peritoneal dialysis was carried out but the patient expired due to renal failure.

![Figure 2. Excised mitral valve showing vegetation (encircled) on gross examination.](image)

**Discussion**

*Burkholderia cepacia* has been isolated from the environmental sources like soil, water, and plants as well as from the hospital settings like distilled water, nebulizers, medical equipment etc. The organism has the potential to infect and shorten the survival of patients suffering from respiratory illnesses like cystic fibrosis. In this case although the patient had no previous history of respiratory illness, nevertheless, developed a highly invasive infection, reflecting the pathogenic potential of this organism beyond the respiratory system. However, our patient had undergone an invasive procedure (PTMC) 3 weeks earlier, before he reported back to the hospital with the intra cardiac vegetation. The definitive diagnostic criteria for infective endocarditis (Modified Duke criteria) was met, with positive evidence of valvular involvement on transesophageal echocardiography, mitral valve disease, fever >100.4° F and paired positive blood cultures. Surgical removal of the vegetation was warranted for two reasons: first, the fever was not responding to empirical therapy for more than a week, and secondly, the size of the vegetation was large enough, posing a possible threat of dislodgement. Although the empirical therapy of blood culture negative native valve endocarditis was started immediately, an unexpected exaggerated nephrotoxic effect (Vancomycin) was observed in this case. The empirical therapy was modified following culture and sensitivity of the isolate but by that time enough renal damage had already occurred, resulting in fatal outcome.

The isolation of *Burkholderia cepacia*, not only from the blood but also the vegetation from the patient without any history of respiratory illness, convincingly supports that bacteria can cause invasive disease in a person without previously known predisposing conditions. Since there is no previous evidence of isolation of *Burkholderia cepacia* from patients of infective endocarditis, it is difficult to decide the best empirical antimicrobial therapy, alone or in combination. Nevertheless, meropenem appears to be an option against *Burkholderia cepacia*. Timely paired blood sampling, aseptic collection, early transport and close liaison with the laboratory are the crucial features for successful isolation of the known as well as previously unknown pathogens for infective endocarditis, thus saving precious lives.

**References:**

7. Timely paired blood sampling, aseptic collection, early transport and close liaison with the laboratory are the crucial features for successful isolation of the known as well as previously unknown pathogens for infective endocarditis, thus saving precious lives.


Gastrointestinal Obstruction due to Ascariasis – Management Issues

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Abstract

Ascariasis is one of the common parasitic infections worldwide especially in the tropical and subtropical developing countries where sanitation and hygiene are poor. Children are vulnerable because their high risk behavior. Complications such as GI obstruction, acute appendicitis with perforation, ileal perforation, volvulus, biliary tract obstruction and intussusception may occur. Non-operative and conservative management followed later by an anthelmintic agent will help relieve the symptoms in most cases. We present here a case of ascariasis in a 5 year old boy complicated by GI obstruction and treated conservatively and review recent literature.

Key words
Ascariasis, gastrointestinal obstruction, child

Introduction

Ascariasis is the most common type of roundworm infestation in humans, infecting approximately 25 percent of the world's population or nearly 807 million cases. The global prevalence of severe ascariasis results in approximately 60,000 deaths annually; primarily as a consequence of intestinal obstruction. Risk factors include poor personal hygiene, poor sanitation and places where human feces are used as fertilizer, warm climate and humidity. The highest rates of infection occur in developing countries and in tropical and subtropical areas where sanitation and hygiene are poor. Complications due to ascariasis include gastrointestinal (GI) obstruction and migration to other organs such as biliary tract. In Pakistan ascariasis has been well described in children. We present a child with GI obstruction due to *Ascaris lumbricoides* and review some of the management issues.

Case Report

A 5-year-old male child presented to the clinic with the complaints of abdominal pain for 4 days. The pain was colicky, griping, intermittent, and mostly central and had increased over last 48 hours. He also had vomiting, decreased oral intake, and obstipation with distension of abdomen. In addition, he also complained of cough and low grade fever. He was given albendazole 2 days back after which his pain worsened. The parents reported passage of multiple large worms through mouth and nose. Laboratory tests done prior to admission showed WBC count 8300/μL with neutrophils 78% and eosinophils 8%; erythrocyte sedimentation rate 15 mm/hr and ALT 95 mg/dl. An ultrasound of the abdomen showed dilated gas filled bowel loops. The child had been previously healthy with no major illnesses.

On general physical examination the child was pale and coughing. Vital signs were normal. Chest examination showed decreased breath sounds on right and mild wheezing. Abdomen was soft but distended. Rectal examination revealed tenderness and a questionable mass or fecal matter. The rest of the systemic examination was unremarkable.

Laboratory investigations at admission revealed an ALT 50 mg/dl with normal electrolytes and urine analysis. A chest x-ray (Fig 1) showed bilateral minimal alveolar infiltrates seen in the para-cardiac region. Supine and erect abdominal x-rays (Fig 2) revealed large bowel obstruction with fecal impaction in recto-sigmoid colon. Areas of linear tubular soft tissue densities were seen in the gas filled transverse and descending colon, suggestive of worms (ascaris). A diagnosis of massive ascariasis with partial gastrointestinal (GI) obstruction and associated Loeffler syndrome was made.

![Fig 1. Chest X-Ray showing bilateral minimal alveolar infiltrates seen in the para-cardiac region and right lower lobe.](image)

The patient was given intravenous fluids and given plain “Kleen” enema and oral laxative. The next day the child passed a large bolus of entangled worms with relief in pain and swelling. He was discharged with instructions to continue oral laxatives for few days and repeat the plain “Kleen” enema followed by repeat albendazole after a week. At follow-up after 2 weeks he was doing well with no complaints.
Discussion

Ascariasis is an infection caused by a parasitic roundworm, *Ascaris lumbricoides*. Children are infected more often than adults. It is found in association with poor personal hygiene, poor sanitation, and in places where human feces are used as fertilizer. Intake of food or drink contaminated with roundworm eggs may also cause infection. Life cycle is shown in figure 3.

During movement through the lungs the larvae may produce an uncommon form of pneumonia called eosinophilic pneumonia or Loeffler syndrome. Once back in the intestines, they mature into adult roundworms. Adult worms live in the intestine where they lay eggs that are passed in feces. Sometimes a large number of adult worms may cause GI obstruction which may be fatal. De Silva particularly looked at ascaris-induced GI obstruction from 9 different studies. GI obstruction was the single most common complication (38-87%, weighted mean 72%). The proportion and the number of cases of ascaris-induced GI obstruction per year per 1000 population were significantly related to the local prevalence of ascariasis. It is estimated that the incidence of such an event is 2 per 1000 ascaris-infected children per year. The case fatality rates range from 0 to 8.6% (weighted mean 5.7%).

The infection is often asymptomatic. Children may, however, present with symptoms indicative of larval migration like pneumonitis, urticarial rash or have some vague abdominal discomfort. Other symptoms may include a low grade fever, fatigue, weight loss, cough, bloody sputum, wheezing, shortness of breath and vomiting when worms pass through the lungs. Sometimes a worm may leave the body through vomitus or stool. Common GI complications include partial and complete intestinal obstruction, acute appendicitis with perforation, ileal perforation, Meckel's diverticulitis, disruption of post operative intestinal anastomosis, volvulus, biliary tract obstruction and intussusception.

Diagnosis of uncomplicated ascariasis is by history of passage of large worms through various body orifices and by microscopic identification of ova in the stool (Fig 4).

“Deworming” ascariasis with mebendazole or albendazole is curative for uncomplicated infestation. In GI obstruction, nasogastric suctioning controls vomiting. For GI obstruction due to a large number of worms, a paralyzing vermifuge (such as pyrantel pamoate or piperazine) can make the worms relax and pass through the intestine to relieve obstruction. Many advocate a conservative approach to these cases, favoring non-surgical management.
management for GI obstruction due to ascariasis.

Soomro et al has advocated non-operative management for GI obstruction using hypertonic saline enema in 45 children from Karachi. Villamizar et al reported 87 children (mean age 4.6 yrs, 55% girls) with bowel obstruction caused by ascaris. Sixty four children (75%) presented with a subacute clinical course and 24 children (25%) had acute presentation. Plain abdominal x-rays showed a “whirlpool or tubular” pattern of intraluminal worms with or without air fluid levels. Most cases responded to conservative management consisting of oral administration of racine oil and piperazine. Gangopadhyay et al described 19 of 22 children who had GI obstruction due to ascariasis being conservatively managed using oral piperazine and glycerine with liquid paraffin emulsion enemas. Tondon et al from Jabalpur, India also described highly successful conservative management by use of hypertonic saline enema which is given similar to an ordinary soap water enema. The enema works by irritatating and disintegrating the worm bolus commonly situated in the terminal ileum as well as helps to increase the intestinal motility and passage of worms into the colon. The success rate in their series of 250 children with GI ascariasis using this conservative technique was 95.6%. In our patient with partial GI obstruction we used simple Kleen enema and oral laxative which proved to relieve the worms and the symptoms. If obstruction is not relieved or other complications occur such as perforation, bile duct obstruction or appendicitis, in which case laparotomy is indicated. Up to 24% children may require operative management including external “milking” of the obstructing bolus of worms from the gut, intestinal resection, appendectomy for obstructing worm or an enterotomy to manually extract the worms.

Numerous anthelmintics are used for uncomplicated ascariasis. The most common are mebendazol, albendazole and pyrantel pamoate which act by killing the adult worms as well as larvae and eggs to prevent reinfection. However anthelmintics are not recommended during GI obstruction and should only be administered after the passage of nearly all adult worms. Studies have also shown that prior anthelmintics treatment, particularly with mebendazol, and certain paralyzing agents, especially those causing spastic paralysis, should be avoided because of the risk of causing complete obstruction by ascaris in children.

This seems to be the case in our patient whose symptoms worsened and mimicked obstruction after being given albendazole. Good hygiene, travel precaution, proper cooking of food, pet care and keeping children from putting things in mouth are some of the preventive measures.

In summary ascariasis may present with GI complications including obstruction. Non-operative and conservative management (nil by mouth, intravenous fluids, a paralyzing agent, an enema followed later by an anthelmintic agent) will help relieve the symptoms in most cases.

Reference
Events and Programs organized by the Infectious Diseases Society of Pakistan (IDSP)

February 2, 2008
Infection Control Workshop at Children Cancer Hospital, Karachi. Facilitators were: Dr. Altaf Ahmed (President, IDSP), Dr. Seema Irfan (Assistant Professor, Microbiology, AKUH), Dr. Kausar Jabeen (Assistant Professor, Microbiology, AKUH), Dr. Mohammad Zeeshan (AKUH) and Dr. Shamvil Ashraf. Over forty doctors, nurses, and paramedics attended the workshop.

March 13, 2008
Workshop on “Antibiotic Use in General Practice” in Lahore. IDSP Lahore Chapter held a Seminar on “Antibiotics Use in General Practice” by Dr. Hammad Nazeer, Consultant in Infectious Diseases, Fatima Memorial Medical College and Hospital, Lahore, talked about “Rational use of Antibiotics”. The talk focused on when to start antibiotics, fundamentals of appropriate selection, dose, duration, etc. Dr. Mateen Izhar, Professor of Microbiology, Shaikh Zayed Postgraduate Medical Institute, Lahore, gave a presentation on “Laboratory diagnosis and treatment of common community acquired infections”. The presentation concentrated on Respiratory tract infections, Urinary tract infections, Soft tissue infections, and Enteric fever.

The seminar was attended by approximately 30 leading GPs of Lahore. There was active participation by the attendees during the question and answer discussion sessions. The IDSP’s effort seemed to be well appreciated.

March 15, 2008
Seminar on “ESBL: Emerging Threat to Clinical Therapeutics” in Karachi. Speakers were: Dr. Naseem Salahuddin, Dr. Altaf Ahmed, Dr. Afia Zafa, and Dr. Bushra Jamil. Gram-negative ESBL producing microorganisms are now becoming a serious problem among hospitalized patients in most hospitals across Pakistan, resulting in high morbidity and mortality. Coupled with the fact that new antibiotics are not in the pipeline, extreme measures must be taken to implement infection control.

IDSP has partnered with Center for Injection Safety and others on propagation of “Epidemiology of Unsafe Injections in Pakistan.”

Corrigendum
The Guest Editorial appearing in IDJP Vol. 17 #1 reading: “alarming statistics of 31% MDR are provided by Mahmood and Ahmad in this issue” should read “alarming statistics of 31% MDR rate in laboratory based samples are provided by Aamer Ikram, Sakhawat Ali, Wajid Ali, M Amin Wiqar in this issue”. The error is regretted.

IDSP is affiliated with the following organizations and is already working closely with them.

The International Federation of Infection Control (IFIC) is an umbrella organization of societies and associations of healthcare professionals in infection control and related fields worldwide. The goal of IFIC is to minimize the risk of infection within the healthcare setting worldwide through the development of a network of infection control organizations for communication, consensus building, education, and sharing expertise.

Founding of IFIC
For many years, the annual meetings of the Infection Prevention Society of the UK (IPS) and the Association for Professionals in Infection Control and Epidemiology (APIC) in the US have attracted infection control professionals from around the world. These meetings were found to be valuable for making international contacts and ultimately interest developed for formalizing such contacts. As a result of networking between infection control professionals from the UK, the US, Canada, Denmark, Sweden, and the Netherlands, an international conference entitled The Role of the Infection Control Nurse in the Surveillance, Prevention and Control of Hospital Associated Infections was held in 1978 at the European office of the World Health Organization in Copenhagen, Denmark. At the close of the conference, consensus was reached for establishing a multidisciplinary, international association for the control of hospital-associated infections. This was the major first step toward the establishment of the International Federation of Infection Control. IFIC was officially inaugurated as a multidisciplinary organization in 1987.

Membership
Membership in IFIC is extended to societies of healthcare professionals in infection control and related fields in countries throughout the world. The organizations that join IFIC are then called member societies. Currently IFIC has 66 members from 51 countries. Individuals in countries without infection control societies or associations that are not formally constituted may become associate members of IFIC at a reduced membership fee. IFIC also has patron members: persons, firms, or companies which, whilst they have an interest in or concern about infection control, are commercial organizations. The patron members may on agreement contribute to IFIC activities such as conferences and publications but are not entitled to vote.
International Nosocomial Infection Control Consortium

The International Nosocomial Infection consortium (INICC) was established in the 90’s when selected hospitals in Latin America began routinely collecting health care associated infection surveillance data to be included in an international database.

Hospitals invited to participate in the consortium, provide general medical and surgical inpatient services to adults and children requiring acute care.

The INICC, founded and lead by Dr. Victor D. Rosenthal, has focused initially on determining the incidence of device associated health care associated infections and surgical site infections in medical centers.

Data from the participating hospitals is collected using standardized health care associated infections definitions of NNIS- CDC and specific protocols developed by INICC.

At the present , INICC counts with the contribution of about 200 researchers worldwide , from several medical centers at more than 60 cities from 15 countries of four continents: 1- Argentina, 2- Brazil, 3- Colombia, 4- Equator, 5- Guatemala, 6- Mexico, 7- Panama, 8- Peru, 9- Dominican Republic, 10- Italy, 11- Turkey, 12- India, 13- Philippines, 14- Malaysia, and 15- Morocco.

Through participation in the study Health Care Centers and their professional team will acquire new effective tools to improve the measurement of health care associated infection rates, extra length of stay, attributable cost, mortality, risk factors analysis, and other related data.

Information gathered by professionals at each Health Care Center is part of a pool of worldwide collected data which allows accurate risk factors analysis and evaluation of useful and cost effective interventions to be made.

By applying the infection control guidelines, performance feedback, and other measures, behavior of the health care workers at r medical centers improves significantly. By joining INICCs initiatives most relevant goals can be achieved: reduction of the mortality, rates, costs and bacterial resistance.

The Central Office team draft monthly full reports, showing charts and tables of the global rates per 100 patients, and per 1000 bed days, device-associated health care associated infections per 1000 device days, microbiological profile, attributable mortality by type of device associated infection, extra length of stay, hand hygiene compliance, and CVC and urinary catheter compliance and others to be sent to each participating hospital. As participants of an International Research Institute, professionals of each Health Care Center are co-author of many multi-center studies presented at international scientific meetings and also published at international peer review journals.

Pakistan Antimicrobial Resistance Network (PARN)
High levels of antimicrobial resistance (AMR) and frequency of health care associated infections (HAI) in Pakistan are a significant cause of concern. Limited awareness about these issues suggests a need for wider discussions.

Pakistan Antimicrobial Resistance Network (PARN) set up in March 2007 in association with the Infectious Diseases Society of Pakistan is a coalition of individuals, organisations and public health agencies concerned about antibiotic resistance and health care associated infections with the specific aim of creating awareness through sharing of information and development of a support group to help address these issues. The coalition partners include National Institute of Health Pakistan and Pakistan Medical Research Council.

Alliance for the Prudent Use of Antibiotics (APUA):
The Alliance for the Prudent Use of Antibiotics (APUA) was founded as a non-profit global organization in 1981 to contain antibiotic resistance and improve antibiotic effectiveness. APUA’s mission is to strengthen society’s defenses against infectious disease by promoting appropriate antimicrobial access and use and controlling antimicrobial resistance on a worldwide basis. With affiliated chapters in over 60 countries, many in the developing world, APUA stands as the world’s leading organization conducting antimicrobial resistance research, education, capacity building and advocacy at the global and grassroots levels.

Antimicrobials are uniquely societal drugs because each individual patient use can propagate resistant organisms affecting entire health facilities, the environment and the community. Wide-scale antimicrobial misuse and related drug resistance is challenging infectious disease treatment and healthcare budgets worldwide.

APUA’s goal is to improve antimicrobial policy and clinical practice worldwide so as to preserve the power of these lifesaving agents. Our programs seek to reduce drug resistance and improve the effectiveness of treatment for acute bacterial diseases, tuberculosis, AIDS and malaria in the industrial and developing world.

IDSP has partnered with Center for Injection Safety and others on propagation of “Epidemiology of Unsafe Injections in Pakistan”
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<th>Event Description</th>
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<td>1. 6th International Symposium on Pneumococci &amp; Pneumococcal Diseases, ISPPD-6</td>
<td>June 08, 2008 - June 12, 2008</td>
<td>Reykjavik</td>
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<td>2. 1st Internat. Congress on Infection in Transplantation &amp; Cancer</td>
<td>10-12 June 2008</td>
<td>Tehran, Iran</td>
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<td>3. Infectious Disease Update</td>
<td>June 16 - 27 2008</td>
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<td>5. 5th Annual Infectious Disease and Internal Medicine ACP Recertification Seminar</td>
<td>June 23, 2008 - June 27, 2008</td>
<td>Myrtle Beach, United States</td>
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<td>6. 29th Advances in the Management of Infectious Diseases</td>
<td>July 11, 2008 - July 13, 2008</td>
<td>Mackinac Island, United States</td>
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<td>7. Emerging Infectious Diseases Conference</td>
<td>23-26 July 2008</td>
<td>Big Island of Hawaii</td>
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<td>8. 26th Annual Pediatric Infectious Disease Conference</td>
<td>July 27, 2008 - July 31, 2008</td>
<td>Vail, United States</td>
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<td>9. Cardiology &amp; Infectious Disease</td>
<td>July 28, 2008 - August 09, 2008</td>
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<td>14. 60th Annual Meeting of the German Association for Hygiene and Microbiology (DGHM)</td>
<td>21-24 Sept 2008</td>
<td>Dresden, Germany</td>
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<td>17. International Symposium on Infections in Immunocompromised Host</td>
<td>18-20 Nov 2008</td>
<td>Riyadh, S. Arabia</td>
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<td>19. 164th SGM Meeting (Society for General Microbiology)</td>
<td>30 March-3 April, 2009</td>
<td>Yorkshire, UK</td>
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<td>21. 48th Annual ICAAC/46th IDSA meeting</td>
<td>25-28 October 2009</td>
<td>Washington DC</td>
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Instructions for Authors

Scope
The Infectious Diseases Society of Pakistan sponsors the Infectious Disease Journal of Pakistan (IDJP). 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; with laboratory, clinical, or epidemiological aspects.

Criteria for publication
All articles are peer reviewed by the IDSP panel of reviewers. The Editors review Correspondence. Authors may also submit the names and contact informations of two 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 . Please submit one complete copy of the manuscript and all enclosures to The Editor, Infectious Diseases Journal of Pakistan, Department of Pathology and Microbiology, The Aga Khan University, Stadium Road, P.O. Box 3500, Karachi 74800, Pakistan. An electronic copy of the manuscript must also be sent to (masim.beg@aku.edu) . 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’.

Manuscript Categories

I. Original Articles
Articles should report original work in the fields of microbiology and infection.

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.

Abstract
Abstract should not exceed 200 words and must be structured in to separate sections headed Background, 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. The section should end with a very brief statement of what is being reported in 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.

Results
Present results in logical sequences in the text, tables and illustrations. Articles can have a maximum of five illustrations (in a combination of figures and tables) per article.

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 their limitations. 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.

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. 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, 2 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, 2 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.


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.

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 (eg graphs): 600 dpi
- Black & white halftone illustrations (eg photographs): 300 dpi
- Colour illustrations: 400dpi (note that colour images should be split CMYK not RGB)

Important Notice
Financial support: All authors must disclose any financial support they have received during the course of the study or investigation.

Conflict of Interest: If there is a conflict of interest then this must be disclosed by the author or authors at the end of the article to be submitted.

Ethical Guidelines
All clinical research articles/studies involving human subjects submitted to the IDJP must adhere to ethical guidelines of their institutions and have informed consent from their patients. The Editors may require this statement.

All scientific research that uses animals in their study protocols must include a statement on the ethical treatment of animals during the study.

Revised 2006
MEMBERSHIP APPLICATION FORM

INFECTION DISEASES SOCIETY OF PAKISTAN

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Signature General Secretary: ___________________

FULL MEMBERSHIP:
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PRIVILEGES OF MEMBERSHIP:

FULL MEMBER:
All the members shall have the right to:
1. Participate in all activities of the society.
2. Receive all publication including quarterly ID Journal free of cost.
3. Vote according to constitution of the society.

ASSOCIATE MEMBERS:
All the members shall have the right to:
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2. Receive all publication including quarterly ID Journal free of cost.

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

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