An Overview of
Department of Microbiology

Dhaka Shishu Hospital

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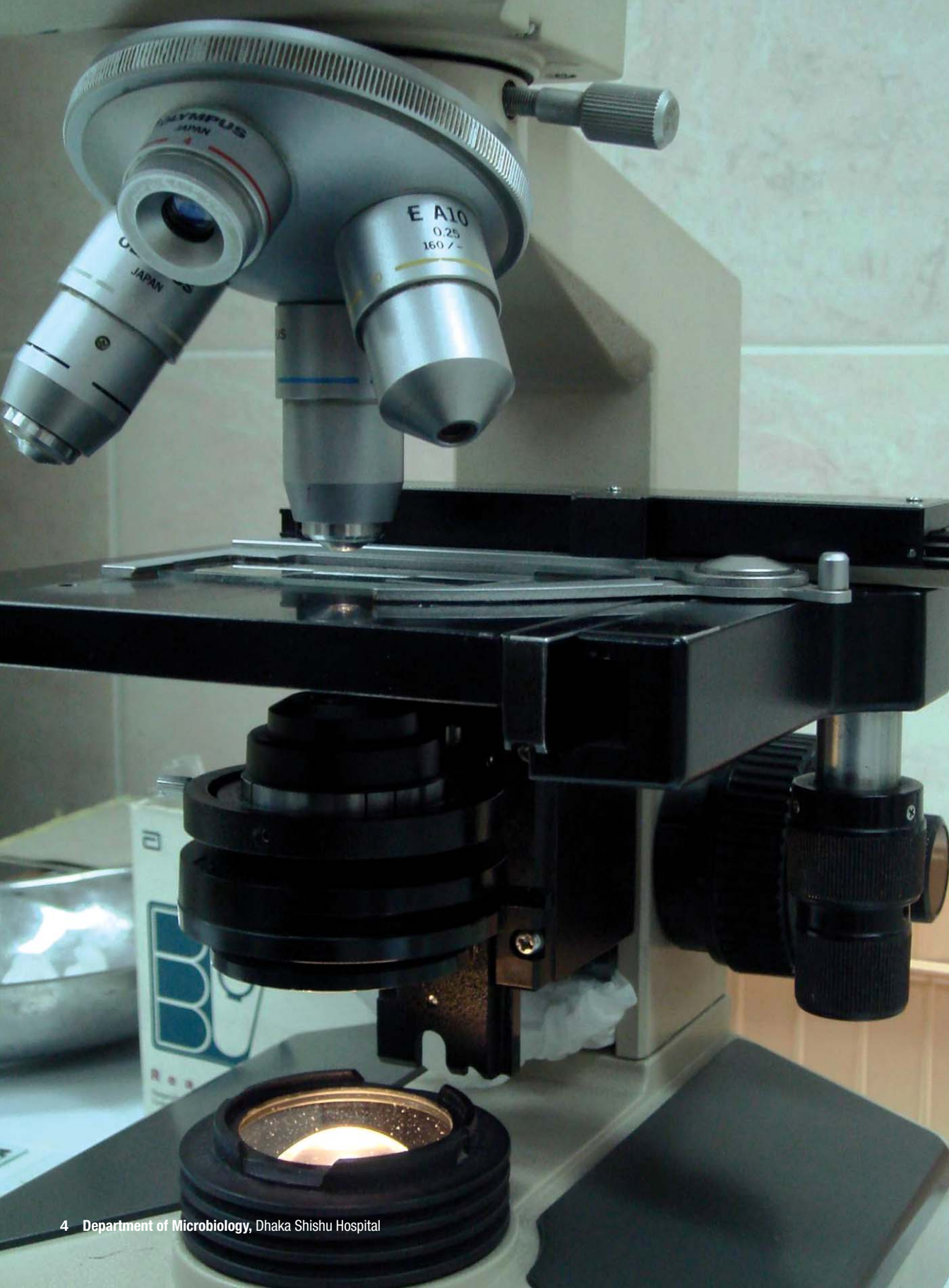
Table of Contents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Preface

Department of Microbiology of Dhaka Shishu Hospital began its journey in 1983 with only a pressure cooker and a few petri plates on a small table, at the corridor of the Department of Pathology. Since its inception, the department took initiatives to build its diagnostic capacity and do much more. As dreamt by the founders of the hospital, the Microbiology department had always been wholeheartedly interested in research, besides providing diagnostic services to the patients of the hospital.

From the very beginning, the department has been playing a proactive role in establishing strong relationship with the clinical colleagues to customize the work of the department according to the need of the clinicians and offer better services for the patients. Schemes and ideas of the Department of Microbiology did the amazing job of bringing the diverse groups of colleagues of the administration, the pediatric departments, the laboratory personnel and the collaborators close together and forming a 'zone of performance'. This is inevitably helping the department to perform better in both the sectors --- patient service and research.

By gradual expansion and capacity building, the department has emerged itself as a "state of the art" facility of international reputation, in only around a couple of decades. The research findings of this laboratory are published in various reputed international journals. The department now proudly participates in several global policy matters in the field of treatment and prevention of pneumonia, meningitis and typhoid. It must be mentioned that the British Broadcasting Corporation (BBC) made and televised a documentary based on this laboratory's work in the field of Hib-meningitis.

The department has mentored several graduates. Most of them are now working in different parts of the world for their doctoral or postdoctoral degrees. Some of them are already back here at the department, after specialised training in renowned institutions across the world. In addition, the laboratory is enthusiastically contributing in the capacity building of other microbiology laboratories of the leading medical colleges and hospitals of Bangladesh, and working as the reference centre for the surveillance on invasive pneumococcal diseases.

Most of the resources of the Department of Microbiology have been pulled together by itself. In addition to that, the department also intents to gather resources for the hospital, and thus facilitates the use of the hospital's limited resources for other priorities like serving the poor children.

However, this massive development from a small table to a huge laboratory with the latest technologies at finger tips would not be possible without the generous support from the hospital authority, friends in the society and research collaborators at home and abroad. Collaborators of this department include Centre for Disease Control (CDC), GlaxoSmithKline, Hib Initiative, ICDDR,B, Johns Hopkins University, Nagasaki University, Oxford University, PneumoADIP, Wellcome Trust, World Health Organization, etc.

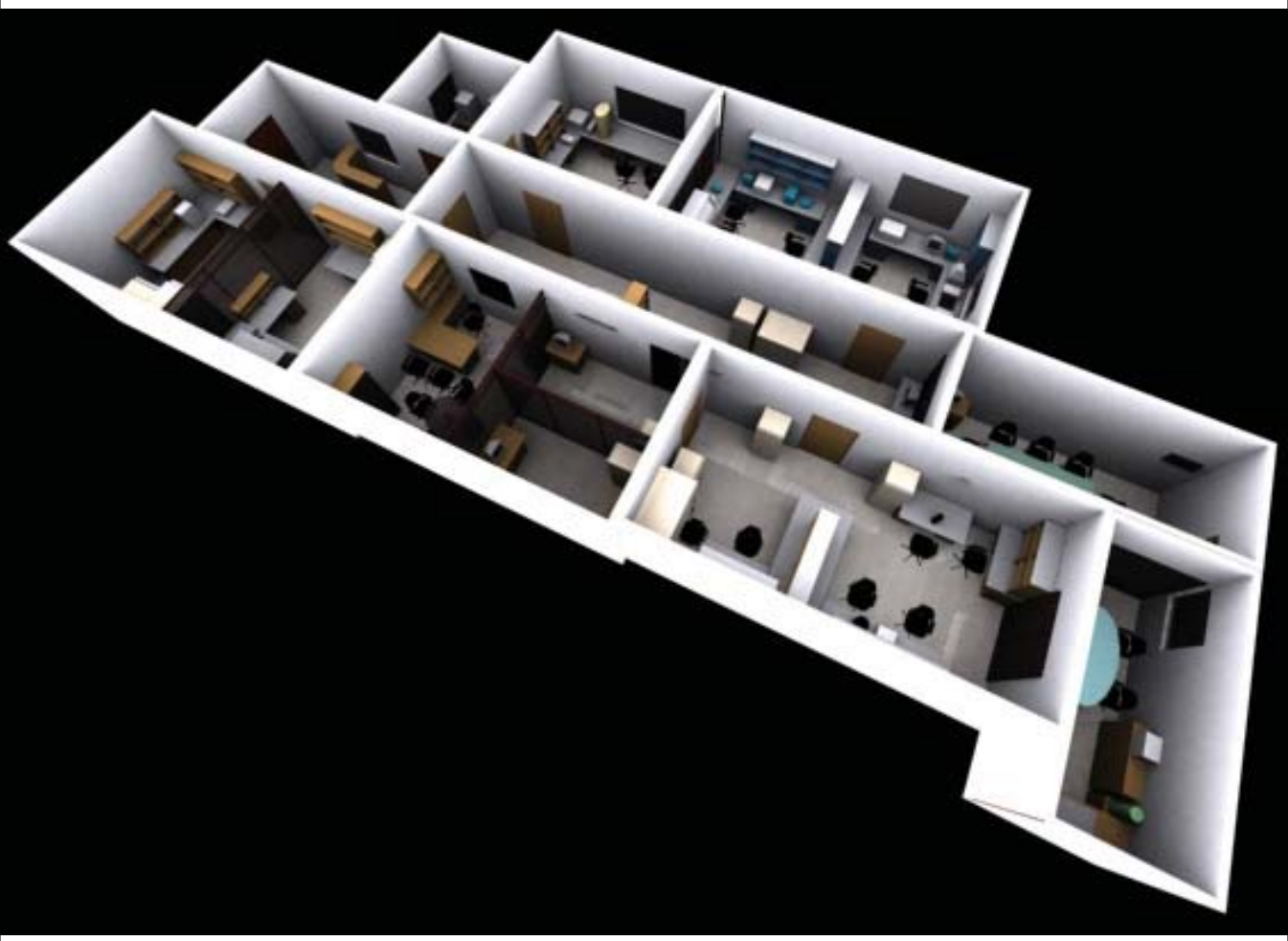
The department has the potential to take the leadership in the field of pediatric infectious diseases, if the "zone of performance" remains intact, as it is now.

Samir K Saha, Ph.D.

OVERVIEW

Laboratory Portrait

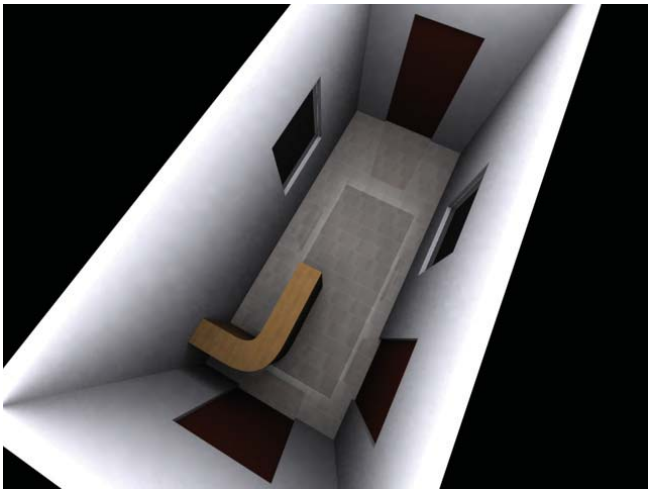
As mentioned before, the department has walked forward from the corridor of another laboratory to have its own space through several steps of extension and renovation. Now, the department has eight functional rooms like reception corner for the patients, room for preparation of media and their quality control, data management section, meeting room and so on. The biggest area is devoted for routine bacteriological and serological work, while there are also laboratory areas for molecular biology and immunology related works.



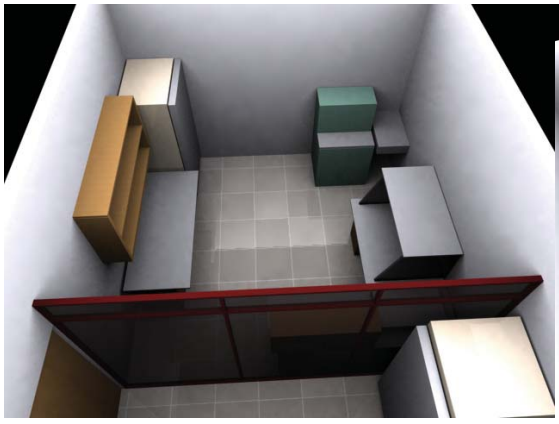
Functioning Area

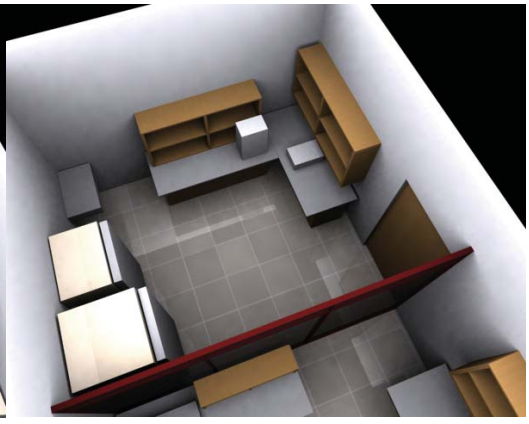
Reception
The reception is at the entrance of the department where specimens are received and reports are delivered.

Media preparation room
All media are aseptically prepared in this room. Entrance into this room is restricted. This area is occupied by a laminar flow, a distillation plant and refrigerators.



Reception





Media preparation room

Data management section
The corridor of the department is customized to be used as the computer sections. Few other equipment are also kept here.

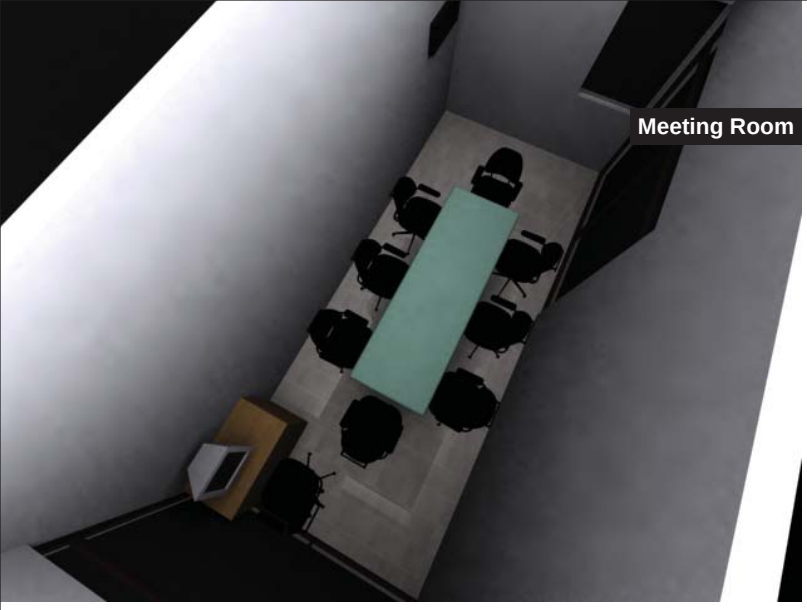
Corner for the Head of the Department

Routine Laboratory 1

This section is the core region for most of the experiments and diagnosis. All hospital diagnosis and routine works like isolation, identification, serology and typing of the organisms are done in this section. There are biosafety hood, refrigerators, horizontal shaker, pH meter, electric bright field microscope etc. available in this room.

Routine Laboratory 2
This section is well equipped with ELISA microplate reader, lyophilizer, electric bright field microscope, pH meter and refrigerator.

Molecular Biology Section
This laboratory is equipped with sophisticated machines like PCR machine, PFGE, PCR work station, high speed centrifuge etc.


Meeting Room


Tea Room


Autoclave Room

Laboratory Tests

SEROLOGY
Widal test
Febrile Antigen
ASO titre
RA test
CRP
VDRL
HbsAg
ICT for Kalazar
ICT for Malaria
ICT for Filaria
ICT for Dengue
Others

MICROSCOPY
CSF Cytology
Skin Scraping for fungus
Gram's stain
AFB stain
KLB Stain

LATEX
CSF

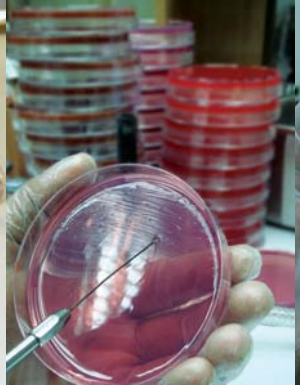
CULTURE & SENSITIVITY
Blood
Urine
CSF
Stool
Throat Swab
Pus
Wound Swab
Eye Swab
Umbilical Swab
Catheter tip
Catheter Swab
Acitic Fluid
Pleural Fluid
Ear Swab
Sputum
Others


ACTIVITIES
eppendorf
Research

Department of Microbiology of Dhaka Shishu Hospital (DSH) started its journey in 1983 with very limited resources in the corridor of the Department of Pathology. However, lack of space and limitation of resources were taken as a challenge, and that was the driving force to move forward.

As a whole, DSH is a resource poor organization. However, commitments are enormous and priorities are many. There is staggering number of issues to be addressed and so it is not easy to decide on the priorities to be addressed. The priorities of DSH start from providing free beds to 'no cost' investigation facilities, through free food for the mothers and the children of more than 50% of in-patients. Considering this fact, the department, since its commencement, aimed to collect its major supplies, capital equipments, etc. from external sources, so the hospital can use its limited resources for other competing priorities.

Although the department primarily started with the responsibility to give services to the hospital by building its diagnostic capacity, research came in a passive way as an intrinsic component of basic science. Department of Microbiology, therefore, aimed to do the best in both --- diagnostics and research. These two components are complementary to each other as we always prioritize those research topics which have implications in improving the diagnostic microbiology and influencing the child health policy matters. This small brochure will describe different components of the department including its capacity, activities, achievements, contributions and so on.


Diagnostic Microbiology

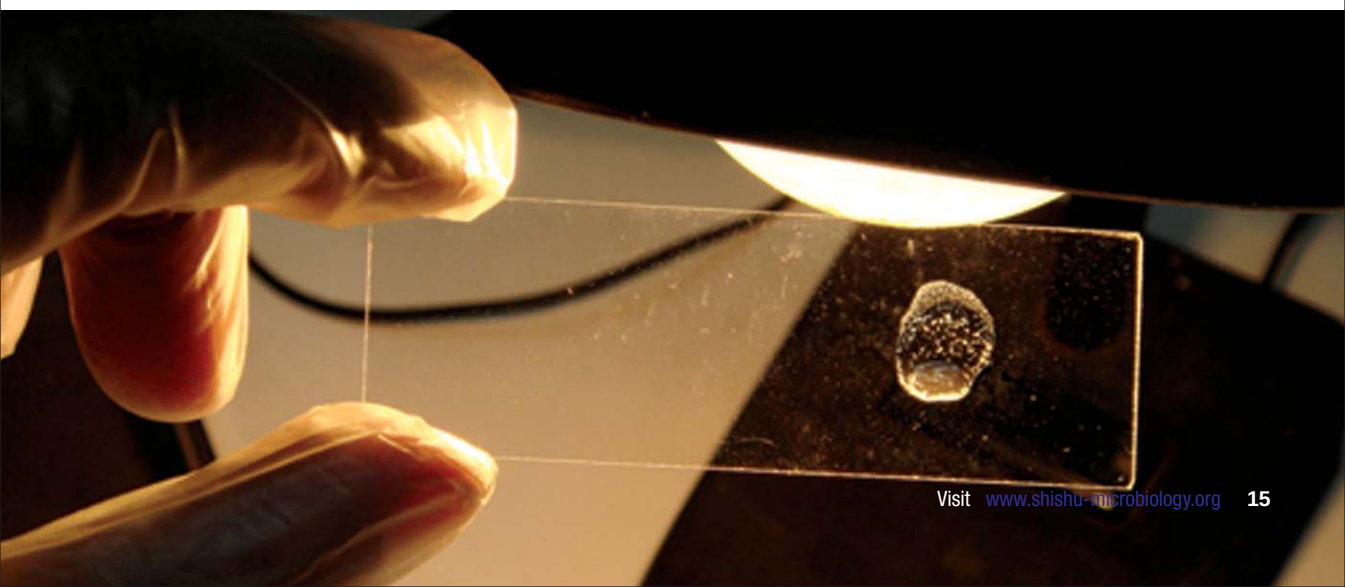
Arrival of Specimens

The laboratory receives clinical specimens from the wards at the reception area or in the laboratory. Processing of the specimens starts at real time, specifically for cerebrospinal fluid (CSF) and blood culture. Other specimens, if not processed instantly, are kept in the cool box or refrigerator to minimize the impact of time gap.

In this department, improvement of laboratory procedures and techniques is a continuous process. The advancements in processing the specimens and improvement of diagnostic capacity are coming from the experiments and findings of this laboratory, and published literature from the other laboratories.

Once the specimens arrive in the Department of Microbiology, they are handled with care following standard operation procedure (SOP), depending on type of specimens.

Specimens like cerebrospinal fluid (CSF) are handled with high priority. cytology, Gram stain and antigen detection tests are done and reported at real time.



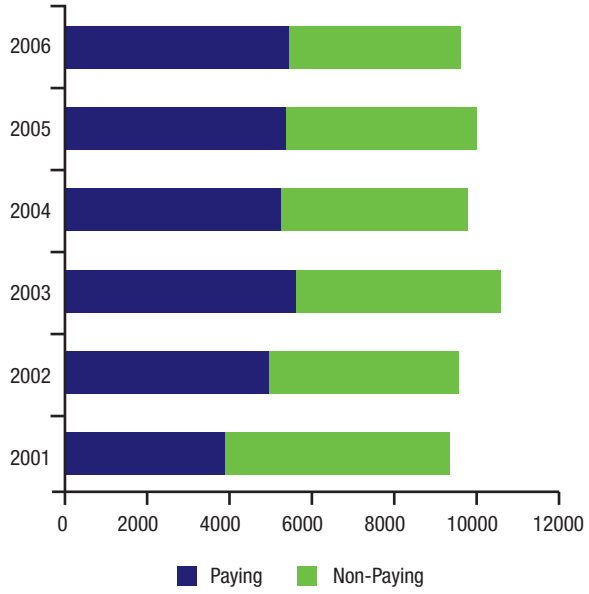


Figure 1: **Number of specimens from paying and non-paying patients (2001 - 2006)**

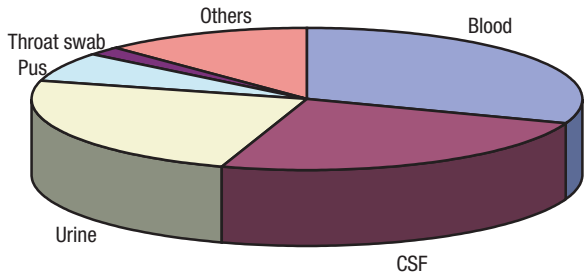


Figure 2: **Predominant type of specimens received for culture during the period 2001 - 2006**

Bacteriological culture is the main service of the whole department. The specimens are processed in aerobic and micro-aerophilic conditions. Since 2001 to April 2007, 58,900 specimens have been cultured and 52% of them were from paying patients (Figure 1).

Among the cultures, blood (31%), CSF (24%) and urine (24%) were the most predominant specimens followed by pus (7%) and throat swab (2%). (Figure 2).

In the recent years, the department introduced enriched blood culture media with the support from GAVI's PneumoADIP, and that has lead to progressive increase in blood culture (Figure 3) and higher rate of isolation. All the blood culture media, irrespective to the enrolment of the cases in the study, are provided by PneumoADIP of Johns Hopkins University.

Over all Hib is the predominant cause of pyogenic meningitis followed by *S. pneumoniae* (Figure 4). However, most of the CSF specimens are culture negative due to prior antibiotic use. Etiology of these cases is detected by detection of antigen either by latex agglutination test and / or by Immuno-chromatographic test. Both the tests, for antigen detection, are available in the microbiology laboratory, through PneumoADIP of Johns Hopkins University.

Salmonella typhi is the most common organism among the culture positive cases. Predominance of this organism is more conspicuous in the 2-59 months age group. Other organisms like Klebsiella pneumoniae, Acinetobacter and Serratia are mostly isolated from neonates (Figure 5).

This department is one of the few laboratories in the country and the region that does cultures routinely and isolates and characterizes the fastidious organisms like *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Neisseria meningitidis*. This capacity

is known to the whole world, through the publications and presentations, and thus the department is functioning as the reference laboratory of seven hospitals of Bangladesh and ICDDR,B for surveillance of invasive Pneumococcal diseases in children.

Serological tests

In addition to bacteriological culture, serological tests, mostly relevant to the paediatric age group, are also done in this department. As with the culture, increase in trend is noted here too. On an average, based on last 5 year-data, the department deals with 7479, 4733, 1652, 400, 320 specimens for CRP, Widal, febrile antigen respectively.

Communication between ward and Laboratory: Microbiology Department maintains a constant communication with the wards for collation and interpretation of the results.

This helps the department to provide better service to the patients and to improve the knowledge. Communication helps us to i) inform about the contaminated specimen and request for a fresh one; ii) get additional information about the patient and/or iii) inform about any urgent report like KLB or any growth from CSF, etc.

Capacity to Identify the Organisms: In the last several years, the capacity of the laboratory has substantially increased to precisely identify the most of the bacterial isolates upto the species level. Identification is done based on the standard procedures. Primarily the organisms identified based on growth requirements, biochemical tests, analytical profile index (API) and agglutination with specific antisera. Specific antisera are available to confirm the identification and typing of the organisms, like Salmonella, Haemophilus, Pneumococcus and Meningococcus.

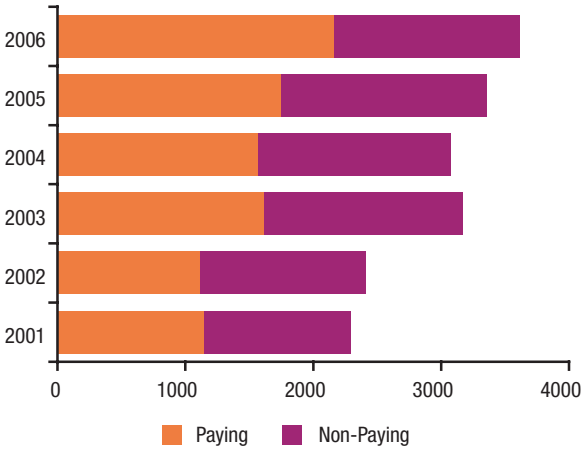


Figure 3: **Trend of blood cultures by year, from paying and non-paying patients**

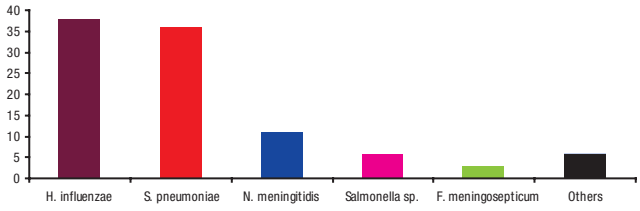


Figure 4: **% of organisms among culture positive meningitis cases (2001 -2006)**

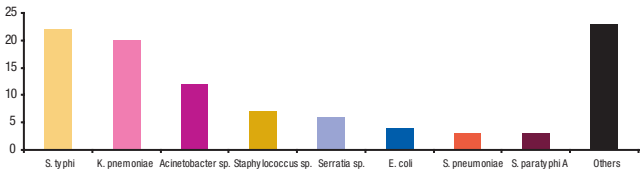
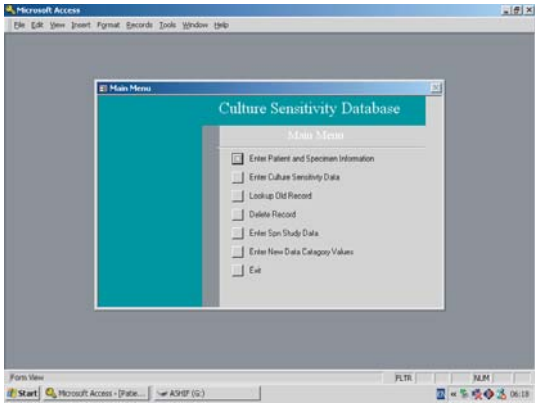
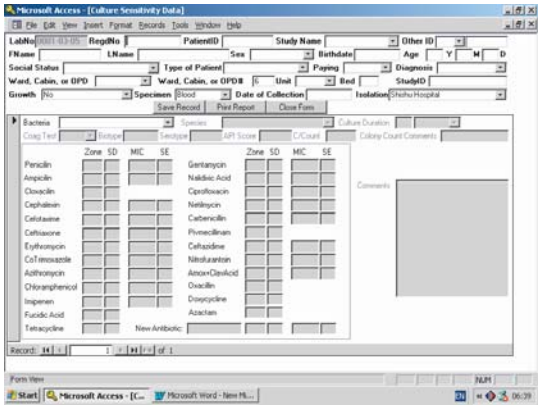


Figure 5: **% organisms isolated from blood of all age group, 2001-2006**

Preparation of Reports

The department uses software, developed by a graduate of Johns Hopkins University, which automatically interprets the antibiotic susceptibility results from the measured zone of inhibition, based on the Clinical Laboratory Standard Institute (CLSI) guidelines. Computer generated reports are then delivered to the wards.





Availability of Data

The test results of the department of microbiology are stored in the computer for any future reference. These data can be analysed to find the prevalence and the trend of the infection causing organisms, and their susceptibility pattern. Data are accessible to our clinical colleagues, if they want to look at the trend of infections among their own patients. These are useful for the treatment policy in the wards and the hospital as a whole.

Delivery of Report

The Department of Microbiology volunteer to deliver the reports to the respective wards. This facilitates the availability of test results within a short time. The results are also communicated to the doctor of the respective unit over telephone. However, this mostly occurs when the duty doctor or the consultant calls the department and enquires about the test results.

Specimens from other hospitals/clinicians: In addition to in house specimens, microbiology laboratory also receives specimens from other hospitals and private practitioners. These specimens are processed in the same way, and sometime with higher care, to attract more specimens from outside.

Waste disposal

With the continuous improvement in the diagnostic capacity, the staffs are also committed to conserve the environment by following the safe waste disposal strategies. Although complete safe waste disposal depends on national program, the department puts its effort to discard the sharps and infectious materials in a safe way, so that environment does not get affected.

Media preparation and Quality Control

The department has separate sections for media preparation which is well equipped with laminar hood, autoclave, distilled water plant and dispensers. Media are prepared following the standard procedures and labelled with date and batch numbers. All these media are then checked for quality, using reference strains from American Type Collection Centre (ATCC), by a person who is not involved in media preparation. Specimens are processed on these media only if they are approved by the quality control (QC) person. Failure of any batch to pass through QC is reviewed and discussed about to find the cause, and appropriate measures are taken to avoid the future mistakes.

Safe disposal of sharps

Research

Typhoid

For more than 10 years the laboratory is conducting the surveillance on prevalence and drug resistance of *Salmonella typhi*. The work, published in the British Antimicrobial Society Journal, showed the emergence of multidrug (Amoxicillin, Cotrimoxazole and Chloramphenicol) resistant *S. typhi*. Continued monitoring on trend of drug resistance revealed a decrease in drug resistance, and a marked difference between the strains isolated from hospital and community (Figure 6).

All these research outcome have paramount implications in typhoid treatment policy in Bangladesh and beyond. The department is still continuing the surveillance to monitor the trend of drug resistance of *S. typhi*.

Recently, the department found ciprofloxacin resistant strains of *S. typhi* and this was reported in the American Society of Microbiology (ASM) Journal. It was the first report ever, with molecular aspects of resistance. Figure 8 showing the evidence of double mutation by PCR-RFLP done in our department.

In addition to that molecular finger printing of these strains is also done which revealed that all of them are identical, indicating that the same clone is circulating in the country (Figure 11).

References:

- Saha SK *et al,* Journal of Antimicrobiol Chemotherapy. 1997; 39: 554-556
- Saha SK *et al,* Pediatric Infectious Diseases Journal. 1999; 18(3): 387
- Saha SK et al, Journal Antimicrobiol Chemother. 33:190-191
- Saha SK *et al,* J Clin Microbiol, 2006; 44:3811-3833
- Hermans PWM, Saha SK *et al,* Journal of Clinical Microbiology. 1996;34:1373-

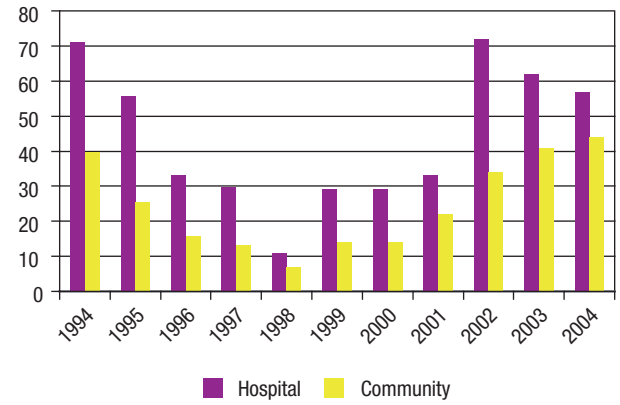


Figure 6: **Trend of drug resistance of *S. typhi* isolated from hospital and community patients**

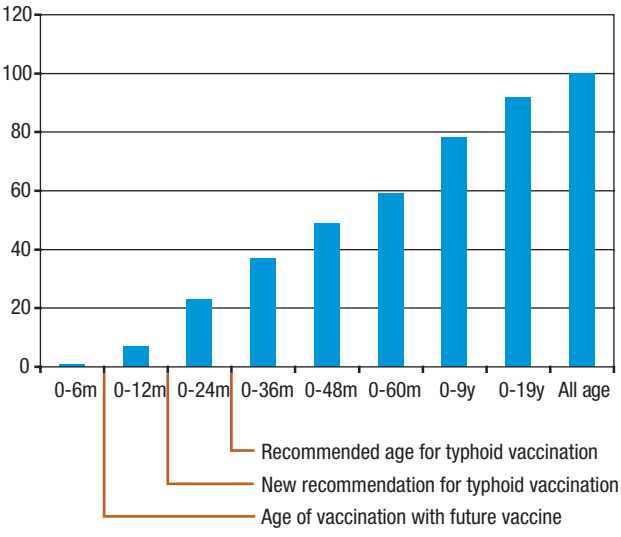


Figure 7: **Age group distribution of typhoid fever: Implication in vaccination policy**

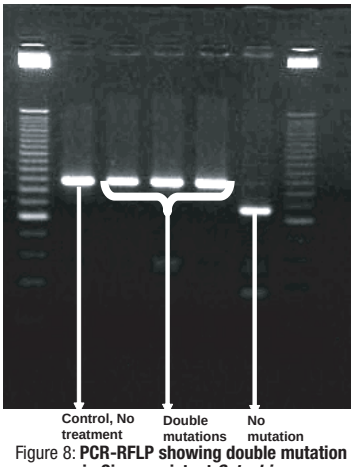


Figure 8: **PCR-RFLP showing double mutation in Cipro-resistant *S. typhi***

The department did the pioneering work to determine the value of widal test in diagnosing typhoid fever. This finding on cut-off value in this report is still used as the only reference for sero-diagnosis of typhoid in children of Bangladesh.

Reference: Saha SK ***et al,*** Annals of Tropical Paediatrics. 1996: 16:75-78

In addition to drug resistance, the Department of Microbiology has also been monitoring the prevalence of typhoid fever in different age groups for last several years. This part of work revealed the fact that the present concept of typhoid fever is misleading for immunization policy and that the existing typhoid vaccines are not appropriate to protect the most vulnerable children (Figure 7). This finding from our department was published in the Pediatric Infectious Disease Journal of USA. Publication of this report from our group has contributed to change the typhoid vaccination policy. Further, few multinational research companies are now working to produce an appropriate vaccine for young infants, which is suitable to be incorporated in the national immunization program.

Reference: Saha SK, Pediatric Infectious Disease Journal 2001;20(5):521-4.

Pneumonia and Meningitis

Streptococcus pneumoniae and *Haemophilus influenzae* are the most common cause of pneumonia and meningitis, and are globally responsible for more than 1.5 million deaths of children every year. Department of Microbiology of Dhaka Shishu Hospital is working on these organisms, isolated form pneumonia and meningitis cases, since 1990.

Streptococcus pneumoniae

This laboratory did a pioneering and ground breaking work for diagnosis of culture negative pneumococcal meningitis cases. The immuno-chromatographic test (ICT) developed for testing the pneumococcal antigen in urine was successfully used to detect the antigen in CSF with 100% sensitivity and specificity. This result was well taken by the pneumococcal research group, and now it is in use in a multicountry study (figure 9). This department is also monitoring the drug resistance and serotype distribution of this organism.

Report form the department showed that most of the S. pneumoniae strains are sensitive to penicillin and other drugs, except cotrimoxazole. About 70% of the strains are resistant to cotrimoxazole (Figure 10), which is recommended by WHO as the drug of choice at community level. The reports are further strengthened by the similar findings from other countries in the region. These reports were enough influential to convince WHO to consider the modification of recommendation from contrimoxazole to amoxicillin.

S. pneumoniae has 90 different serotypes and the types vary from population to population and from country to country. Information about serotype distribution is very important for appropriate vaccine formulation. However, there are only few laboratories in the world that has the expertise to do serotyping of Pneumococcus. These studies on prevalence of pneumococcal serotype revealed that the serotypes of Bangladesh are different from those of other countries, and the existing 7 valent vaccine (Pravner), which are in use in the USA, will cover only 23% and 22% of pneumococcal pneumonia and meningitis cases respectively. On the other hand the coverage with the upcoming vaccine can be as high as 59% for the pneumococcal pneumonia cases of Bangladesh (Figure 12).

These studies and capacities in the field of pneumococcal diseases are being documented through publications of results in reputed international journals. In the recent years, the department have established a surveillance for invasive pneumococcal diseases that includes 7 hospitals and ICDDR,B. It can be mentioned that the contribution in the field pneumococcal diseases has helped to establish the department as the reference laboratory for 7 hospitals and also ICDDR,B. All these work have placed the laboratory among the leading laboratories in the field of pneumococcus. Findings on distribution of pneumococcal serotype have influenced the initiation of research groups of the multinational

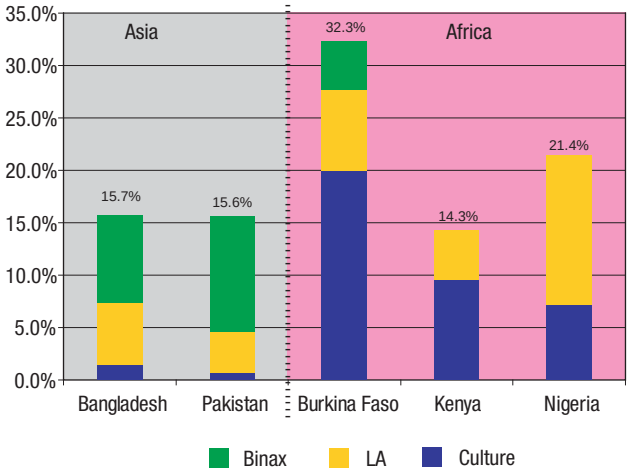


Figure 9: **Proportion of CSF specimen positive for S. pneumonae, culture, lates or Binax**

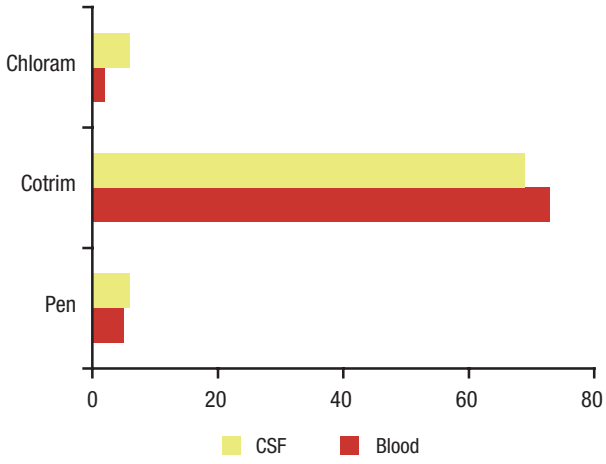


Figure 10: **Susceptibility pattern of *S. pneumoane***

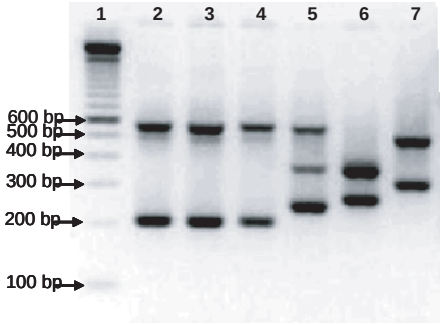


Figure 11: **VNTR Pattern of Ciprofloxacin Resistant S. Typhi compared to Sensitive Strains**

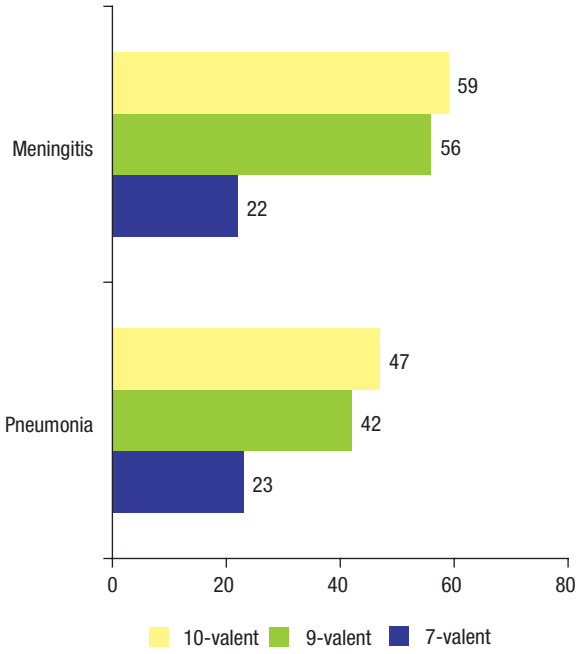


Figure 12: **Serotype specific coverage by the existing and upcoming pneumococcal vaccine**

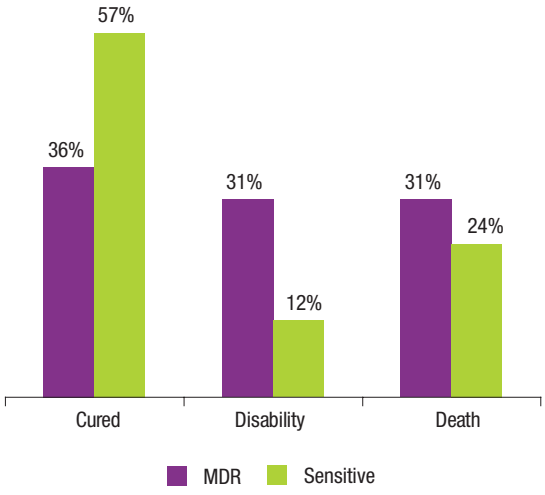


Figure 13: **Outcome of Hib meningitis cases in relation to drug resistance**

vaccine companies to work on new formulation of pneumococcal vaccine(s), and the new generation of vaccines will contain the predominant serotypes of our country.

Reference:
Saha SK *et al*, Pediatric Infectious Disease 2005. 24(12):1093-1098
Saha SK *et al*, Journal of Clinical Microbiology. 1999; 37:798-800.
Saha SK *et al*, Journal of Clinical Microbiology. 1997; 35:785-787
Saha SK *et al*, Lancet. 1991 337: 734 735

Haemophilus inlfuenzae

This is another important cause of meningitis and pneumonia. The department has reported the prevalence of this organism in causing meningitis with gradual increase, in 1997.

Reference: Saha SK, Annals of Tropical Paediatrics. 1997;17:5-8

Since then we are continuing the work with this organism specifically on prevalence and drug resistance. Age distribution of Hib cases in Bangladesh was found to be different from those of the developed part of the world in pre-vaccine era (Figure 17). This has a significant implication in the vaccination policy for the population of Bangladesh.

In the recent years, a progressive increase of multidrug resistance in *Haemophilus influenzae* is observed. It is also found that the cases with multidrug resistance have a significant relation with disability and death (Figure 13). All these data have implications on treatment policy for meningitis, and for the argument in favour of introducing Hib vaccine in national immunization program.

References:
- Saha SK, *et al,* Ann Trop Dis. 2006; 26: 329-336
- Saha SK, *et al,* Journal of Pediatrics. 2005; 146:227-233

The department is now working on direct detection of MDR gene of Hib in the specimens, which will facilitate the detection of drug resistance in the cases where bacteria will not grow due to prior antibiotic therapy.

Preliminary data showed that the MDR gene of Hib can be detected from culture negetive CSF specimen (Figure 16).

Vericella-zoster virus (VZV)

Infections with VZV, commonly known as chicken pox, are usually self limiting. However, the infection may be severe if it occurs in older age group or neonates. Our sero-prevalence study showed the trend of sero-protection in different age groups. About 80% of the adults have seroprotection and this antibody is successfully transmited from mother to newborns. Hoewever, the protection declines to almost baseline by the 2nd year of life. So, over all there is an "U" shaped trend of sero-protection (Figure 14). Until now this is the only available data on sero-prevalence of VZV.

Reference: Saha SK , Annals of Tropical Paediatrics (2002) 22, 341-345

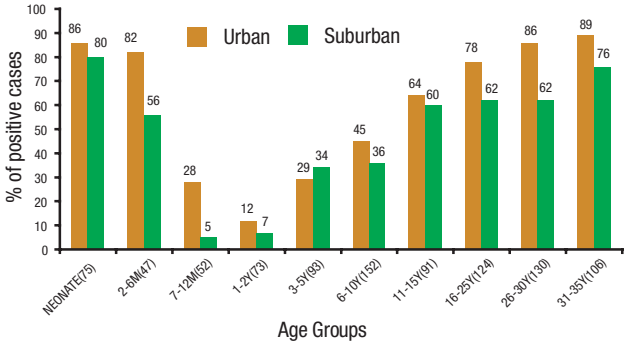


Figure 14: **Seroprevalence against chicken pox in different age group**

Hepatitis A virus (HAV)

HAV infection in childhood mostly remains asymptomatic, however, severity of infection increases with age. Improvement of hygiene contributes to a shifting in age pattern of HAV infection. We did a sero-prevalence study in different age groups of different socio-economic status to see the sero-protection in different groups.

The study revealed that population of higher socioeconomic group, are lacking protective antibody. This has an implication in vaccination policy to protect the susceptible group of children (Figure 15).

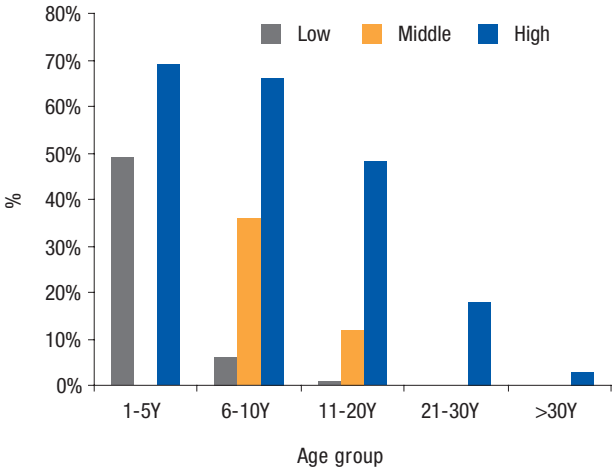
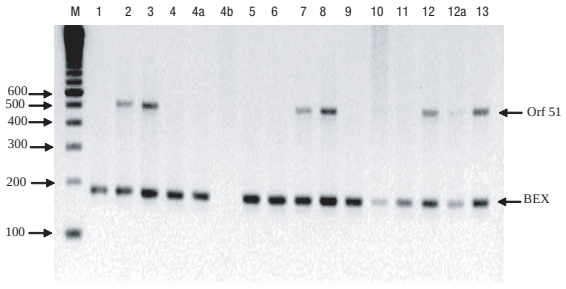


Figure 15: **Seroprotection against Hepatitis A in different age and socio-economic group**

Clinical Trials

In addition to doing the surveillances and experiments in the laboratory, the department is also involved in doing clinical trials for the improvement of treatment policies, which lead to rational use of drugs and thus prevents the emergence of drug resistance.

Most of the clinical trials are multicentred, randomized, placebo controlled double blind studies, in collaboration with World Health Organization (WHO), Geneva, Johns Hopkins University, Save the Children, USA, and Boston University of USA. In all the clinical trials the



M=Marker, lane 4, 4a & 4b serial CSF on day 0, 4 and 16, Lane 12 &12a serial CSF on day 3 and 8. Other lanes are from day 0 CSF

Figure 16: **Detection of MDR gene from culture negetive CSF of Hib meningitis**

patients are supported with free medicines and investigations, done either in the hospital or outside. The important completed and ongoing studies are as follows:

Topical emollient therapy for prevention of infections in preterm infants

In this study emollient sunflower oil or aquaphore was used as the physical barrier to prevent the entry of bacterial pathogens through the premature skins of pre term infants. This was a randomized double blind study conducted in Bangladesh. The study showed that the application of sunflower oil significantly reduces the incidence of nosocomial infection of newborns. The study was published in The Lancet and was a lead news in the BBC world.

Duration: 1998 - 2002

Principal Investigator: Gary L Darmstadt and Samir K Saha

Other investigators MAK Azad Chawdhury

Reference: Darmstadt GL & Saha SK Lancet 2005; 365:1039-45

Multicentre randomized clinical trial to compare the efficacy of chloramphenicol with that of amplicllin plus gentamicin in children aged 2 to 59 months with very severe pneumonia

This was a multicentre and randomized trial in collaboration with WHO, Boston University and Johns Hopkins University. The trial compared the efficacy of chloramphenicol Vs amplicllin plus gentamicin for the treatment of very severe pneumonia. The results were analyzed and submitted for publication in The Lancet.

Duration: 2000 to 2002

Principal Investigator: Samir K Saha and Mathuram Santhosam

Other investigators: Ruhul Amin, M Hanif

Efficacy of short course oral cotrimoxazole in treatment of non-severe pneumonia and its relationship with antimicrobial resistance: a clinical trial in the community of Dhaka

It was a placebo controlled 3 days Vs 5 days trial for treatment of non-severe pneumonia with cotrimoxazole in collaboration with WHO, Geneva. The patients were enrolled at the out patient department of DSH and given the treatment for 3 or 5 days. All the cases were followed up on day 3, 5 and 15 to assess the treatment failure or relapses.

The study showed no significant differences between 3 days and 5 days of treatment. However, it was found that emergence of resistance in nasopharyngeal *S. pneumoniae* and *H. influenzae* were lower in the 3 days group.

The study was presented in the WHO steering committee meeting in Geneva.

Duration: 2001 to 2002

Principal Investigator: Samir K Saha

Other investigators: Ruhul Amin, M Hanif

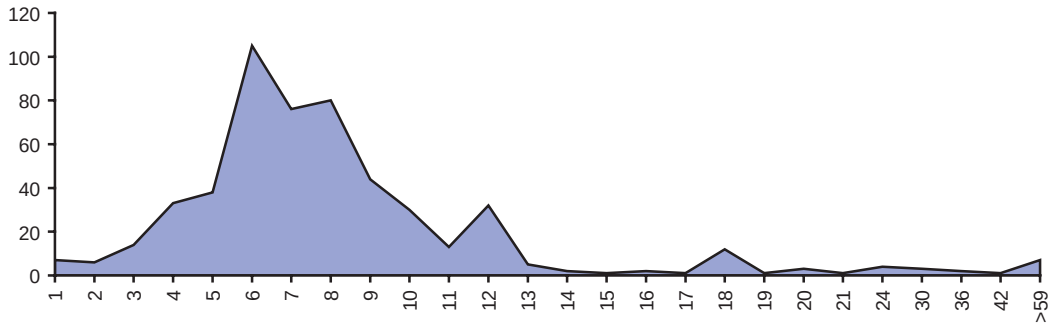


Figure 17: **Age group distribution of Hib meningitis cases**

Follow-up of Pneumococcal meningitis cases to determine its long term impact

Pneumococcal meningitis cases were comprehensively assessed by a multidisciplinary a group for developmental and neurological status of the survived children at 30-40 days and 180-190 days of discharge.

The study showed the importance of neurodevelopmental assessment in meningitis follow up to fully unearth the burden of adverse consequences of the disease, which provides compelling economic argument for introduction of pneumococcal vaccine.

Duration: 2005-2006

Principal Investigator: Samir K Saha and Naila Z Khan

Co-investigator: Ruhul Amin and M Hanif

Comparison of 5 vs 10 days of ceftriaxone therapy for bacterial meningitis in children

This was a multicentre placebo controlled double blind study in collaboration with WHO, Geneva.

In this study, bacterial meningitis cases were given either 5 or 10 days treatment with ceftriaxone. The patients were monitored every day while in the hospital. A comprehensive follow up was given to all these cases upto 190 days, after discharge from the hospital.

The data will be analyzed in July 2007.

Duration: 2002 to 2006

Principal Investigator: Samir K Saha

Other investigators: Ruhul Amin, M Hanif, Sirajul Islam, C M Haider Ali, Manzoor Hussain

Multicentre study of clinical signs predicting illness in young infant

The study was in collaboration with Save New Born Lives of Save the Children, USA, and WHO. The project aimed to validate the clinical signs in the children aged 0 to 2 months, and make an algorithm for community health workers. The children were assessed by paramedics and trained paediatricians, who were blinded to each others findings. The charts were reviewed and validated by a panel of senior paediatricians.

The data were analyzed to find the value of clinical signs, in single and in combination. The data are analyzed and the manuscript is prepared to submit in The Lancet.

Duration: 2002 to 2003

Principal Investigator: Samir K Saha and MAK Azad Chowdhury

Other Investigators: M. Monir Hossain ASM Nawshad Uddin Ahmed

Multicenter Observational Study to Assess the Safety of Outpatient Treatment of Severe Pneumonia with Oral Amoxicillin in Children aged 3 to 59 months: A pilot Study

This is an ongoing study in collaboration with WHO, Geneva. The trial is comparing the safety of out patient based oral amoxicillin therapy for severe pneumonia cases. The patients fulfilling the criteria are given oral amoxicillin and followed up at home by the trained community health workers. Any case of treatment failure are brought to the hospital and given appropriate treatment.

Duration: 2006 – 2007

Principal Investigator: Samir K Saha

Other Investigators: Salim Shakur, Ruhul Amin, M Hanif, Manzoor Hussain

ACHIEVEMENTS

Expansion of the Department

Department of Microbiology of Dhaka Shishu Hospital began its journey in 1983 in a corner of the corridor of the department of Pathology. There was lack of equipment, and constrain of space, both of which are essential to establish a good laboratory. The performance of the department in the collaborative work, achieved high appreciation from all the partners. This appreciation helped the laboratory get the necessary support to expand the department, at different stages, and ultimately bring it to the present form.

Capacity Building

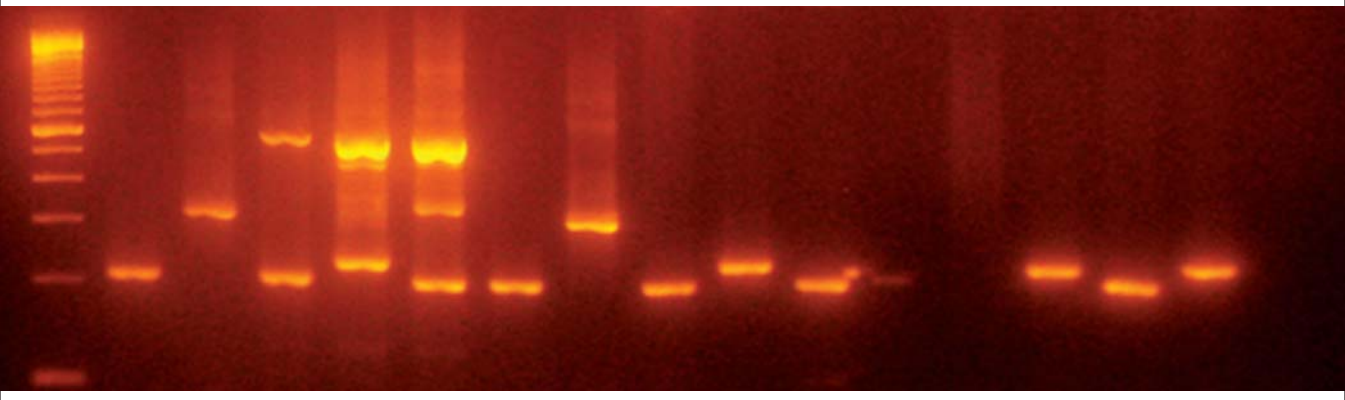
By and large there is always a huge disparity between the availability of laboratory capacity and the burden of infectious diseases. Department of Microbiology has successfully improved its capacity and applied those to explore the work on infectious diseases. The facility of the laboratory now ranges from culturing the routine specimens to molecular finger printing through PCR based identification and serotyping of the organisms. All these are achieved through collaborations with international universities and agencies and active cooperation from the hospital authority. In addition to building the capacity in respect of equipments and appliances, the laboratory has also developed the competence to maintain good laboratory practice.

Capacity and competence helped the department to achieve the recognition as the reference laboratory for *S. pneumoniae* and *H. influenzae* for several hospitals in Bangladesh and beyond.

Animal House

Sheep blood is very important to run the laboratories. Recently, Department of Microbiology closely worked with the hospital authority and arranged to build a dedicated animal house with the help of Prof. T Nagatake of Nagasaki University, Japan and Mr. Biraj Khajanchi of Asia Foundation and Construction Limited, Bangladesh.

Molecular finger printing is an important epidemiological tool to know the source of the organisms, and to design the prevention of the disease. The department has recently introduced the Variable Number Tandem Repeat (VNTR) for finger printing of different organisms. The figure shows the discriminating capacity of this method for *Salmonella typhi*.



Selected Publications

The department has more than 40 publications in peer reviewed international journals. In most of these publications, this department took the lead and was named itself as the place of correspondence. A list of important publications are given below:

1. Abdullah H Baqui, Shams El Arifeen, Samir K Saha, LarsAke Persson, K Zaman, Bradford D. Gessner, Lawrence H. Moulton, Robert E Black and Mathuram Santosham Effectiveness of Haemophilus influenzae type b conjugate vaccine on prevention of pneumonia and meningitis in Bangladeshi children. Pediatric Infectious Disease Journal 2007 Vol 26 (7)
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3. Saha SK, Darmstadt GL, Baqui AH, Crook DW, Islam N, Islam M, Hossain M, Arifeen SE, Santosham M, E Black RE. Highly Ciprofloxacin-resistant Salmonella enterica serovar Typhi in Bangladesh: molecular basis of resistance. J Clin Microbiol, 2006 ; 44:3811-3833.
4. Hoque M, Ahmed ASMU, Chowdhury MAKA1, Darmstadt GL, and Saha SK. Septicemic neonates without lumber puncture: what are we missing? Journal of Tropical Pediatrics. 2006 52(1):63-65
5. Saha SK, Darmstadt GL, Yamanaka N, Billal DS, Nasreen T, Islam M, Hamer DH. Rapid diagnosis of pneumococcal meningitis: implications for treatment and measuring disease burden. Pediatr Infect Dis 2005. 24(12):1093-1098
6. Darmstadt GL, Saha SK, Ahmed ASMU, Chowdhury MAKA, Law PA, Ahmed S, Alam MA, Black RE and Santosham M. Topical therapy with skin barrier-enhancing emollients prevents nosocomial infections in preterm infants in Bangladesh: a randomized, controlled clinical trial. Lancet 2005; 365:1039-45
7. Saha SK, Baqui AH, Darmstadt GL, Ruhul A, Hanif M, Arifeen EL, Oishi K, Santosham M, Nagatake T and Black RE. Invasive Haemophilus influenzae type b diseases in Bangladesh, with increased resistance to antibiotics. Journal of Pediatrics. 2005; 146:227-233.
8. Saha SK, Baqui AH, Darmstadt GL, RuhulAmin M, Hanif M, Arifeen SE, Santosham M, Oishi K, Nagatake T, Black RE. Comparison of Antibiotic Resistance and Serotype Composition of Carriage and Invasive Pneumococci among Bangladeshi Children: Implications for treatment policy and vaccine formulation. J Clin Microbiology. 2003; 41: 5582-7.
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12. Saha SK, Darmstadt GL, Baqui AH, Hanif M, Ruhulamin M, Santosham M, Nagatake T, Black RE. "Rapid Identification and Antibiotic Susceptibility Testing of Salmonella typhi Isolated from Blood: Implications for therapy". Journal of Clinical Microbiology 2001, 39(10):3583-5.
13. Saha SK, Baqui AH, Hanif M, Gary DL, Ruhulamin M, Nagatake T, Santosham, M,Black, RE. "Typhoid fever in Bangladesh: Implication for vaccination policy. Pediatric Infectious Disease Journal 2001;20(5):521-4.
14. Saha SK, Talukder SY, Islam M, Saha, S. A highly cefriaxone resistant Salmonella typhi in Bangladesh. Pediatric Infectious Diseases Journal. 1999; 18(3): 387.
15. Saha SK, Sack RB, Matsumoto K, Nagatake T. Determination of penicillin resistance in Streptococcus penumoniae and use of co-trimoxazole in treatment of pneumococcal pneumonia. Journal of Clinical Microbiology. 1999; 37:2743-2744
16. Saha SK, Rikitomi N, Ruhulamin M, Masaki H, Hanif M, Islam M, Watanabe K et al. Antimicrobial resistance and serotype distribution of Streptococcus pneumoniae strains causing childhood infections in Bangladesh, 1993-1997. Journal of Clinical Microbiology. 1999; 37:798-800.

17. Kalter HD, Hossain M, Burnham G, Khan NZ, Saha SK, Ali MA, Black RE. Validation of caregiver interviews to diagnose common causes of severe neonatal illness. Paediatric Perinatal Epidemiology 1999; 13(1): 99-113.
18. Utsunomiya Y, Ahmed K, Rikitomi N, Ruhulamin M, Hanif M, Masaki H, Kawakami, Watanabe K, Saha SK, Nagatake T. Isolation of pathogenic bacteria from induced sputum from hospitalized children with pneumonia in Bangladesh. Journal of Tropical Pediatrics. 1998; 44:338-342.
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22. Kalter HD, Schillinger JA, Hossain M, Burnham G, Saha SK, Wit V, Khan NZ, Schwartz B, Black RE. Identifying sick children requiring referral to hospital in Bangladesh. Bulletin of World Health Organization, 1997, 75 (Supplement 1) 65-75.
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25. Saha SK, Ruhulamin M. Hanif, Islam M. Khan WA, 1996. Interpretation of the widal test in the diagnosis of typhoid fever Bangladeshi children. Annals of Tropical Paediatrics. 1996: 16:75-78.
26. Khan NZ, Kalter H, Hanif M, Schillinger J, Saha SK, Black R. Meningitis treatment without follow-up: what we are missing? Lancet 346: 706
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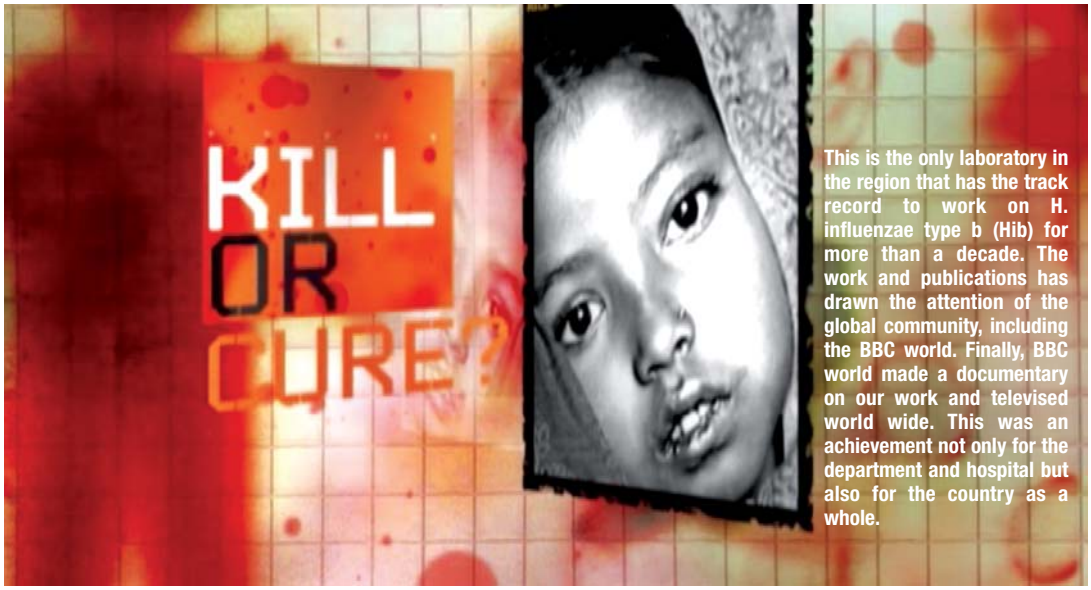
31. Saha SK, Saha S, Sanyal SC. 1991. Recovery of injured Campylobacter jejuni cells after animal passage. Applied Environmental.Microbiology. 57(11): 3388 3389.
32. Saha SK, Khan WA, Hoq MS, et. al. 1991. Penicillin resistant pneumococci in Bangladeshi children. Lancet. 337: 734 735.
33. Saha SK, Sanyal SC, 1991. Better preservation of Campylobacter jejuni/C. coli in a defined medium. Indian Journal of Medical Research. 93 26 28.
34. Saha SK, Sanyal SC, 1990. Production and characterisation of Campylobacter enterotoxin in a synthetic medium and its assay in rat ileal loops. FEMS Microbiol. Lett. 67: 333 338.
35. Saha SK, Sanyal SC. 1989. Better growth of Campylobacter jejuni using simple fortner's principle and candle extinction jar. Indian Journal of Medical Research 89: 24 27.
36. Saha SK, Sing NP, Sanyal SC. 1988. Enterotoxicity of chicken isolates of Campylobacter jejuni in ligated ileal loops of rats. Journal of Medical Microbiology. 26(2): 87 91.

Ground-breaking BBC World Documentary



This is the only laboratory in the region that has the track record to work on H. influenzae type b (Hib) for more than a decade. The work and publications has drawn the attention of the global community, including the BBC world. Finally, BBC world made a documentary on our work and televised world wide. This was an achievement not only for the department and hospital but also for the country as a whole.

Contribution to Global Health

All the publications from this department are related to public health and this created an impact in the global child health policies. The outcome of the research of this department has generated data to influence treatment and immunization policy for pneumonia, meningitis and typhoid. All in all, research findings from the laboratory, combined with others, have a significant impact on global health, specifically in children.

Recently, the department participated in the global policy making meeting on prevention of diseases caused by S. pneumoniae and H. influenzae type b, held in Bangkok. Experts from more than 20 countries from Asia, Africa, Latin America, USA, Switzerland etc. attended the meeting. The meeting was followed by a press conference, organized by GAVI initiatives, WHO and Ministry of Public Health of Thailand. The press briefing was done by (from left) Dr. Supamit Chunsuttiwat, Ministry Public Health of Thailand, Dr. Rana Hajjah, Director of GAVI's Hib Initiative; Dr. Orin Lavine, Executive Director of GAVI's pneumoADIP, Prof. Samir K Saha, Senior Consultant of Dhaka Shishu Hospital, Thomas Cherian, Coordinator EPI, Department of Immunization, Vaccines and Biologicals, WHO.

Contribution to National Health Policy

While publishing in international journals, the Department of Microbiology has always aimed to work with the common causes of child morbidity and mortality in Bangladesh. Data generated from this laboratory has direct implication to help the policy makers of this country to take evidence based decisions on introduction of HIb vaccine. Hopefully, contribution of this department through publications and the BBC documentary on Hib, will facilitate the country to get GAVI funding and introduce the Hib vaccine by 2008. It is also expected that the ongoing work of this laboratory on *S. pneumoniae* will help the development of generation of new Pneumococcal vaccine which will be appropriate for the children of Bangladesh. The department expects that the continuous input from its work will make Bangladesh eligible to get the new generation vaccine at real time, through GAVI funding.

Establishment of Collaboration

In 1990, Nagasaki University of Japan was encouraged to collaborate with this department, seeing the publications of this department. Successful collaboration with this University, created a good track record. This helped the laboratory to subsequent establishment of collaboration with many other organizations and universities of the world. All the collaborations of this department have been successful, as the department proved its capability to make significant contributions. These collaborations are further strengthened and expanded by constant communication, transparency and accountability maintained by the department.

List of the Collaborators:

CDC Centers for Disease Control and Prevention

University of Oxford

gsk GlaxoSmithkline

icddr,b

JICA Japan International Corporation Agency

Johns Hopkins Bloomberg School of Public Health

pneumoADIP

Save the children

Thrasher Research Fund

USAID From the American People

Wakayama Medical University

wellcome trust

World Health Organization

* Name of collaborators are arranged in alphabetic order

CONTRIBUTION

Self-generated Resources

Though, Dhaka Shishu Hospital was founded with strong and high ambitions to serve the poor children of the country, and to do research in the field of pediatrics, limited resources have always been a hold back to fulfill these goals. Keeping this in mind, the department of microbiology has constantly tried its best to gather most of its resources by itself. In this process, most of the capital equipments and expensive supplies of the laboratory have been pulled together from external sources.

In the last one decade, the department has collected many capital equipments, these are in use for routine diagnostic tests and researches. The capital equipments are assets of the department and thus of the hospital too.

In addition to equipments, few new and expensive diagnostic kits, like bacterial antigen detection kit, specific antisera for identification, etc. are being available through the departmental research projects. These kits are also in use for the routine diagnostic purposes. If these tests come from paying beds, they are considered as payable tests. This is another way, by which the department is contributing in generation of income of the hospital. It can be mentioned here that, since April 2004, all the blood culture bottles used in this hospital are provided by PneumoADIP of Johns Hopkins University.

It must also be pointed out that the department has reached its present form of robust infrastructure through several expansions and renovations in last several years. All these were done by the help of the research collaborators and friends in the society, without any financial implications to the hospital.

Contributions beyond Microbiology

In addition to building capacity for itself, the department also tried to contribute to the hospital as a whole, with help from several collaborators and friends of the department.

Central Oxygen Supply: Central Oxygen Supply system of the entire hospital was mainly initiated and facilitated through one of our research collaborators, Prof. K. Matsumoto of Nagasaki University. The facility was installed by Bangladesh Oxygen Corporation (BOC), with the resources from Japan International Corporation Agencies (JICA) with active cooperation of the hospital director.

BICH Auditorium: Microbiology department, along with the director of the hospital, played an important role in the initial renovation of the BICH auditorium. This was done with the resources from Dr. Mostafizur Rahaman of Popular Diagnostic Centre.

Furthermore, as part of the work with the clinical colleagues, the department arranged to build cabinets for the nursing stations of ward 1 and ward 5 of DSH. A pulse oximeter, a sucker machine and an electronic weighing machine were also given to the wards.

Exploring Child Health in Microbiology

Diagnostic departments mostly remains less conspicuous in the over all complicated dynamics of any hospital. Department of Microbiology of DSH has given a new dimension to this subject by working with pediatricians, and by contributing to the field of child health. The department is no more confined to mere laboratory experiments; it is now contributing in policy making issues of child health at home and abroad. This is attracting more and more graduates to come and work in the field of child health microbiology.

Social Welfare

By working together with the clinical colleagues, the department is also trying to contribute to 'patient care'. As part of the research projects, the department receives medicines from WHO, and all these are given to the patients of the hospital who are enrolled in the study. In addition, the department usually gets more medicines than needed for the project. These surplus medicines are given to the hospital medical store to make it available for all the patients. All these are done with proper documentation and communication with the hospital administration.

In the recent years, the department arranged Hib vaccination program for 2,315 children of a sub urban area of Dhaka. The vaccines were donated by the Rotary club of Japan. The program was jointly conducted through the Thana Health Complex, a non government organization and Rotary Club of Dhaka with the leadership of the Department of Microbiology, Dhaka Shishu Hospital.

As a part of the studies conducted by the department, the patients are occasionally provided with the support to do expensive investigations, either at the hospital or outside. However, this depends on the nature of the study. For instance, as part of the study on meningitis, the department provided hearing aids to several children who lost their hearing ability, as an after effect of meningitis.

EQUIPMENT


VirTis
benchtop K
COND
VAC
AUTO
DEFR
MENU
TRIVAC E2
TRIVAC
D 2.5 E
VirTis

Description

Equipments are integrated part of capacity of a laboratory. Therefore with the increase of capacity and expanded collaborations with other organizations, the department gathered many sophisticated equipments. Most of these equipments are gathered as part of the collaborative work with international organizations and agencies. A list of the equipments with relevant information is given below).

GRADIENT THERMAL CYCLER

96 well Gradient PCR with precise Ramping time Used for diagnosis & epidemiology, DNA fingerprinting, detection drug resistance

Manufacturer: Thermo, USA

Courtesy: **Wellcome Trust**

PCR WORK STATION

PCR Master Mix preparation area with UV lamp to decontaminate the area and intense light for precise reagent preparation.

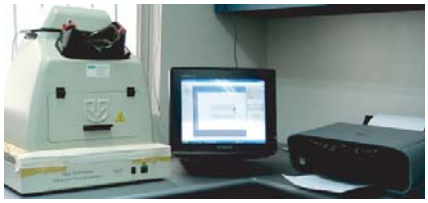
Manufacturer: CBS scientific California

Courtesy: **Wellcome Trust**

GEL DOCUMENTATION UNIT

Instrument used for visualization and documentation of DNA band after gel electrophoresis

Manufacturer:
Camera: Olympus, Indonesia
Illuminator and software: UVP, USA



Courtesy: **Wellcome Trust**

MINI MICRO-CENTRIFUGE

Hi speed (1000-14000 rpm) mini centrifuge machine mostly used in the process of DNA extraction and other molecular procedure.

Manufacture: Eppendorf, Germany.

Courtesy: **Wellcome Trust**

COOL MICRO CENTRIFUGE

Hi speed (1000-18000 rpm) cool (-10°c to 40°c) centrifuge machine mostly used in the process of DNA extraction and other molecular procedure.

Manufacture: Eppendorf, Germany.

Courtesy: **Wellcome Trust**

MICRO CENTRIFUGE

Hi speed (1000-18000 rpm) centrifuge machine mostly used in the process of DNA extraction and other molecular procedure.

Manufacture: Hettich universal 92, Germany

Courtesy: **Thresher**

PULSED FIELD GEL ELECTROPHORESIS (PFGE) CHEF DR-III

Used for molecular fingerprinting form chromosomal DNA.

Manufacture: Bio RAD

Courtesy: **Robert E Black, JHU**

GEL ELECTROPHORESIS

Gel electrophoresis chamber with power unit used for DNA separation on agarose gel

Manufacturer: CBS Scientific

Courtesy: **Wellcome Trust**

LYOPHILIZER VIRTIS

Used for long time preservation of bacteria by freeze drying.

Manufacturer: Virtis

Courtesy: **SNL**

Laminar flow

Clean bench equipped with UV lamp and positive pressure through hepa filter to ensure contamination-free zone.

Manufacturer:
Nuve LN 120

Courtesy: **Wellcome Trust**

-80^{0}C Fridger

Ultra low(-70 to -80) fridge used for preservation of strains and specimens.

Manufacturer: Jouan & Revco

Courtesy: **PneumoADIP, JHU**

-20^{0}C Fridger

Manufacturer: REVCO

Courtesy: **JICA**

DISTILLATION PLANT

High quality distilled and ionized water for precise experiment.

Manufacturer: AQUARIUS GS 100

Courtesy: **JICA**

INCUBATOR

Courtesy: **IPH**

MICROPLATE READER

Used to detect antigen and antibody in clinical specimen.

Manufacturer: Jumper

Courtesy: **GlaxoSmithKline**

HORIZONTAL SHAKER

Courtesy: **PneumoADIP, JHU**

ELECTRONIC BALANCE

Courtesy: DS**H**

VORTEX

Courtesy: **PneumoADIP, JHU**

MICROPIPETTE

Courtesy: **Nagasaki University**

AUTOCLAVE

Courtesy: **JICA**

BRIGHT FIELD MICROSCOPE

Courtesy: **JICA**

Maintenance

Maintenance of equipments is the most difficult part as the hospital maintenance department has no capacity to take care of these sophisticated appliances. The department, therefore, keeps contact with the local agents and gets service from them. However, this is not an easy job as the suppliers are also not very efficient to solve the problems. To minimize the problems with the equipments, department monitors the instruments on regular basis by maintaining log books and keeping performance charts of the specific appliances, like incubators, ultra low freezers, etc. It can be mentioned that all the equipments, including the incubator which was abandoned by the Institute of Public Health in 1983, are functional.

Microbiology Team

The microbiology laboratory runs with minimum number of personnel from hospital. In fact, there has been no addition of man power in the last one decade. However, we have quite a few personnel from the collaborative studies. The department functions in a way that all the personnel, either supported by the hospital or collaborative studies are interactive and they share each others work. Furthermore, we get partial support from the department of pathology.

In the diagnostic side we have two technologists and two lab attendants, who are supported by DSH. On the other hand, research side has a laboratory manager, 2 study physicians, 4 microbiologists, 4 community health workers, 2 technologists, 1 data entry person, and 4 laboratory attendants. It also needs to be mentioned here that a senior consultant supervises all the work and administration of the department.

Mentoring the Graduates

The department has trained a large number microbiology graduates, physicians and technologists. Until now, 7 graduates (microbiologists/doctors/biochemists) have gone abroad, and are working for their Ph.D and post doctoral degrees in different parts of the world. All these graduates started their carrier in this department and had the basic training from this laboratory. In addition to that 3 of our existing personnel had the training from Oxford University of UK, Nagasaki University of Japan and Centre for Disease Control of Atlanta, USA.

Furthermore, several physicians worked in the collaborative clinical research projects of this department. All these doctors got trained on clinical research methodology and good patient care through their work with the international counter parts.

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