6. Microbiology

Division of Microbiology extended microbiological diagnostic support to both intramural and extramural projects of NARI. Opportunistic infections contribute significantly towards the morbidity and mortality in HIV infected individuals. This year we made an attempt to isolate organisms from stool, sputum/oral swab, urine collected from HIV infected patients who attended NARI clinic. As continuation of the study initiated last year we investigated diarrhoeal etiology among the indoor patients admitted to Pune Municipal Corporation’s Naidu hospital. Like in the past we continued to isolate N. gonorrhea strains from gonorrhea patients to study the trend of antibiotic sensitivity pattern in them. Division also supported other projects namely NACO’s community based countrywide STD prevalence study, IIPS Male sexuality project and HPTN 047 project for Chlamydia and gonorrhea diagnosis by multiplex PCR tests. Data on syphilis and Hepatitis C virus serology was analysed while hepatitis C data has already been published syphilis data is being analysed.

Study on Opportunistic enteric parasites in HIV seropositive adult patients hospitalized for diarrhea

Principal Investigator: Dr. R Kairon  
Co-Investigators: Dr. AR Risbud  
Funding support: HPTN  
Initiation: 2002  
Completion: Ongoing

As a continuation of the project initiated last year, 127 samples (blood and stool), were collected from indoor diarrhea patients admitted at Dr. Naidu Hospital of Pune Municipal Corporation. Of the 127 patients studied, 52 (40.9%) patients were HIV seropositive (45 males & 7 females). Enteric parasites identified in 76 samples have been shown in the diagram below. Most common parasites seen this year were E. histolytica (20.4%), Microsporidia (12.9%), Isospora (8.6%) and hookworm (5.5%) compared to Candida (8.4%), hookworm (7.2%) and E. histolytica (6.3%), Isospora (5.2%), Cryptosporidia (5.2%) seen during the year 2002. Vibrio cholerae was isolated and AFB was observed on smears of stool samples from one patient each.
Fig. 6.1: Enteric parasites identified (%) in stool samples (n=76)

Microbiological Investigations form Clinical Progression of HIV infection (CPI) study

Principal Investigator: Dr. AR Risbud
Co-Investigators: Dr. SM Mehendale, Dr. RS Paranjape
Funding: HIVNET, HPTN
Initiation: 2001
Completion: 2004

HBV serology
292 samples from the HIV infected patients in the CPI study were tested for HBs Ag. 14 samples (4.8 %) were found to be positive for HBsAg. 178 samples from the same study were tested for Anti-HBs. 42 (23.5%) of these were found to be positive.

Enteric pathogens
Twenty-eight stool samples were obtained from the HIV infected patients suffering from diarrhea in the clinical progression of HIV infection study. Enteric parasites identified in 10 samples have been shown in the diagram below. AFB was observed on smears of stool samples from three patients.
**Candida isolates from sputum/ oral swabs**
Candida isolates (n=8) obtained over one year from oral / sputum specimens collected from HIV infected patients attending NARI clinics. Isolation and species identification was done for these Candida strains. All the strains were C. albicans.

**Urine Culture**
A total of 12 urine specimens were collected and processed for bacterial culture. E.coli (2), Pseudomonas sp. (1) and Flavobacterium (1) were isolated from these specimens. Antibiotic sensitivity testing was also performed on all these isolates.

**M.TB**
2 Urine and 2 Ascitic fluids were processed for culture for Mycobacterium Tuberculosis. All 4 were smear negative, only 1 culture was positive from ascitic fluid sample.

**Incidence of syphilis and its association with HIV-1 infection**

*Principal Investigator:* Dr. AR Risbud  
*Co-Investigators:* Dr. S.M. Mehendale  
*Funding:* HPTN  
*Initiation:* 2002  
*Completion:* 2003

Individuals who attended NARI STD clinics from 1993 to 2000 were screened at baseline and follow-up visits for serologic evidence of syphilis using the rapid plasma reagin (RPR) antibody test with confirmation by *Treponema pallidum* hemaglutination assay (TPHA). 2324 participants, 172 were found to have clinical or laboratory evidence of incident syphilis during follow-up resulting in a crude syphilis incidence rate of 5.4 cases per 100 py (95% CI, 4.8-6.5 per 100 py). Risk factors for incident syphilis identified in the unadjusted analysis included
earlier calendar year of follow-up, sex work among females, younger age, lower level of education, having a genital ulcer (any cause) at current or prior visit, lack of a recent medical injection and recent HIV-1 infection.

In the multivariate analysis, the risk of incident syphilis was highest in those <20 years old compared to participants older than 30 years. Other independent predictors of syphilis acquisition included a lack of formal education, and recent HIV-1 infection. Syphilis incidence was also higher in the earlier years of the study (1993-96), incidence 7.6 per 100 py (95% CI 6.5-9.2 per 100 py). The relationship between incident syphilis and HIV was investigated in a Cox proportional hazards model. The greatest risk of HIV-1 infection was found among participants within 6 months of syphilis infection (RR 4.44, 95% CI; 2.96-6.65, p<0.001) compared to those with no evidence of syphilis at baseline or follow-up. Participants with a reactive RPR at baseline screening and no clinical or laboratory evidence of primary syphilis did not have significantly increased risk of HIV-1 acquisition during follow-up. A high incidence of syphilis was observed among STD clinic attendees. The elevated risk of HIV-1 infection observed among participants with incident syphilis supports the hypothesis that syphilis enhances the sexual transmission of HIV-1 and highlights the importance of early diagnosis and treatment of syphilis.

HCV Prevalence in STDs Clinic Attendees

Principal Investigator: Dr. A.R. Risbud
Co-Investigators: Dr. R.S. Paranjape
Funding source: HPTN
Initiation: 2001
Completion: 2003

Consecutive 9141 serum samples collected between 1994 and 1999 were tested for anti-HIV and anti-HCV antibody. The anti-HCV antibody prevalence among HIV-infected individuals was 1.5% (95% CI 1.0-2.1%) and that among those not infected by HIV was 0.44 % (95% CI 0.3-0.6%) (p=<0.01). The annual anti-HCV antibody prevalence rate among the STD clinic attendees in general, between 1994 and 1999 was 0.57%, 0.46%, 1.10%, 0.81%, 0.37%, and 0.61% respectively. The anti-HCV antibody prevalence did not change significantly over time a univariate analysis revealed that history of past STD or current STD was not associated with HCV. Whereas, female gender [OR=2.07 (95% CI 1.17-3.66)], prevalent HIV infection [OR=3.38 (95% CI 2.05-5.58)], history of tattoo [OR= 2.18 (95% CI 1.31-3.63)] and being a sex worker [OR= 2.35 (95% CI 1.27-4.35)] were significantly associated with presence of anti-HCV antibody. However, multivariate analysis revealed that prevalent HIV infection and tattooing increased the likelihood of presence of anti-HCV antibodies in subjects attending STD clinics in Pune, India by 3.08 folds [AOR 3.08 (95% CI 1.86-5.11, p=<0.00)] and 1.87 folds [AOR 1.87 (95% CI 1.12-3.13, p=0.017)], respectively.

Our analysis failed to identify any evidence that could support sexual transmission of HCV. Therefore, HIV-HCV co-infections may not be a major concern in India since the present findings suggest that HCV is not efficiently transmitted sexually.
**Resistance pattern in N. gonorrhea**

*Principal Investigator:* Dr. AR Risbud  
*Co-Investigators:* Dr. Rachana Kairon  
*Funding:* HPTN  
*Initiation:* 1996  
*Completion:* Ongoing

A total of 11 Neisseria gonorrhea isolates were obtained in the year 2003. Antibiotic sensitivity testing was conducted by the Kirby Bauer method. MIC for these strains were performed by E test for Ciprofloxacin, Penicillin and Tetracycline and by the disc diffusion method for Ceftriaxone. Overall, resistance to Penicillin, Ciprofloxacin, Ofloxacin and Tetracycline was observed in 11 (100%) strains. Resistance to Ceftriaxone was observed in 2 (18.18%) strains. Eleven penicillin resistant strains were tested for β lactamase production by chromogenic method (nitrocefin test), none of the strains produced β lactamase. Cumulative data on resistance for seven years is presented below.

**Fig. 6.3: Resistance pattern in N. gonorrhea (1996 – 2003)**
Community Surveillance of Neisseria gonorrhoea and Chlamydia trachomatis: Surveillance in Western India

Principal Investigator: Dr. AR Risbud
Co-Investigators: Dr. R.S. Paranjape, Dr. Rachana Kairon
Funding: NACO/WHO & NARI Intramural funds
Initiation: 2002
Completion: 2003

As a continuation of NACO sponsored study on community prevalence of STI, a total of 1325 urine samples were received since January to December 2002 from eight locations in Western India. The samples were pooled (1:5) and tested by multiplex PCR (Amplicor Roche CT/NG detection kit). Samples from the positive pools were tested individually. Of the 1325 samples tested these 14 (1.05%) were positive for NG and 4 (0.30%) were positive for CT. Thus of the 1910 samples received during 2002 & 2003, 22 (1.15%) were positive for NG and 11 (0.57%) were positive for CT.

Fig. 6.4: MPCR results of N. gonorrhoeae & C. trachomatis (2003): Community Center wise results are presented in figure.
Efficacy of DOTS Study

Principal Investigator: Dr. AR Risbud
Co-Investigators: Dr. SP Tripathy
Funding: NARI
Initiation: 2002
Completion: Ongoing

A total of 63 sputum samples were received during the year from the patients screened for the study of Efficacy of DOTS in HIV seropositive and HIV seronegative tuberculosis patients in Pune.

Relationship between sputum smears and cultures

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<th>Condition</th>
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</tr>
<tr>
<td>Smear –ve / Culture positive</td>
<td>13</td>
</tr>
<tr>
<td>Smear –ve / Culture negative</td>
<td>46</td>
</tr>
<tr>
<td>Total</td>
<td>63</td>
</tr>
</tbody>
</table>

Institutional Staff
Dr. Arun. R. Risbud
Mrs. Sangita. V. Kulkarni
Mr. Mycal Periera

Project Staff
Ms. Sarita Umar

Contact Person
Dr. Arun R. Risbud