1) Rapid detection of anti-TB drug resistance in Mycobacterium tuberculosis isolates from HIV infected and uninfected tuberculosis patients in Pune, India	
Principal Investigator	Dr. AR Risbud, Scientist F
Co-Principal Investigator(s)	Dr. SP Tripathy, Scientist F
Other Investigator(s)	Nil
Category / Nature	Basic Science
Collaboration / Participating Centers	Nil
Funding Agency(ies) / Sponsors	Intramural funding, NARI
Budget	Nil
Study Period	2010
Objectives	Nil
Description	PhD Students: Mr. Mycal Pereira Tuberculosis (TB) is still a major public-health concern worldwide and main cause of death by a single infectious agent, namely Mycobacterium tuberculosis. Some recent developments, such as emergence of multidrug resistant (MDR) TB, resistance to at least rifampicin and isoniazid of first-line anti-TB drugs resulting from inadequate therapies and indiscriminate use of antibiotics and HIV/ AIDS pandemic has worsened the TB scenario. Treatment of MDR TB requires the use of more costly and more toxic second-line drugs and is associated with higher mortality rates than drug-sensitive TB, particularly in immunocompromised patients. A delay in the diagnosis of MDR TB associated with standard drug susceptibility testing (DST) methods is likely to contribute to the transmission of resistant isolates. Therefore, there is a great need for use of rapid methods for identification and drug susceptibility testing of M. tuberculosis isolates and hence the need to evaluate these methods for their sensitivity and accuracy in Indian settings prior to implementation. Rapid detection and monitoring of anti-TB drug resistance pattern in TB patients in general and HIV Seropositive tuberculosis patients in particular, would provide important data, which may be crucial for the National Tuberculosis Control Programme. Multidrug resistant tuberculosis (MDR-TB) poses a formidable challenge

	to TB control due to its complex diagnostic and treatment challenges. Conventional methods for mycobacteriological culture and drug susceptibility testing (DST) are slow and cumbersome, requiring sequential procedures for isolation of mycobacteria from clinical specimens, identification and testing of susceptibility to anti-TB drugs. The objective of this work is to evaluate the sensitivity and accuracy of rapid anti-TB drug resistance detection methods; Microscopic Observation of Drug Susceptibility (MODS) and BACTEC MGIT 960 and detection of mutations associated with anti-TB drug resistance in Mycobacterium tuberculosis in Pune, India.
Current Status	A total of 280 sputum samples have been processed under this study and the study is ongoing.
Publications	Nil
Presentations	Nil

2) Quinolone resistance mutations in Neisseria gonorrhoeae	
Principal Investigator	Dr. AR Risbud, Scientist F
Co-Principal Investigator(s)	Nil
Other Investigator(s)	Nil
Category / Nature	Basic Science
Collaboration / Participating Centers	Nil
Funding Agency(ies) / Sponsors	Intramural funding, NARI
Budget	Nil
Study Period	2007 Four years
Objectives	Nil
Description	Ph D student: Mrs. Sangeeta Kulkarni, Technical officer Gonorrhea remains one of the most common sexually transmitted diseases worldwide. Gonorrhea increases the risk of HIV infection and early treatment of gonorrhea is critically important. Gonococcal resistance to antimicrobial agents is an increasing problem in the treatment of gonorrhea. Antimicrobial resistance of Neisseria gonorrhoeae to penicillin and tetracycline is well known and has been increasing over the years. The emergence of resistant strains has led to the increased use of broad-spectrum cephalosporins and fluoroquinolones for the treatment of uncomplicated gonorrhea. However, during the past decade, quinolone – resistant Neisseria gonorrhoeae (QRNG) has been isolated in Asia, Europe, Australia, and North America, threatening the usefulness of quinolone antimicrobials for the treatment of gonorrhea. The treatment of gonorrhea has now become more complicated due to resistance to a variety of antimicrobial agents. In India, penicillin, tetracycline and fluoroquinolone resistance has been reported. Fluoroquinolone resistance in Neisseria gonorrhoeae has been attributed to point mutations in the bacterial genes gyr A and ParC, which code for the target enzymes DNA gyrase and topoisomerase IV, respectively. Previous studies have suggested that gyr A mutations are required for quinolone resistance, which is evident by increased MICs and was clinically expressed as

	treatment failure. In India only one study has been carried out in Delhi reporting that double and triple mutations in gyr A alone or together in gyr A and par C could be responsible for such a high resistance of ciprofloxacin in Delhi. The objective of this work is to identify and characterize mutations in the gyr A and par C genes & comparing these mutations with the level of MICs of fluoroquinolone (Ciprofloxacin, Gatifloxacin, Ofloxacin, Enoxacin, Gatifloxacin and Lomefloxacin) resistance Neisseria gonorrhoeae strains in India
Current Status	Sixty six Neisseria gonorrhoeae clinical isolates were collected from patients attending sexually transmitted disease clinics [34-Delhi, 19-Pune, 5-Mumbai, 2-Nagpur and 6-Hyderabad] during Nov 2006-March 2010. The minimum inhibitory concentration {MIC} of quinolones for these isolates was determined by agar dilution and E-test method. Mutation patterns of the gyr A, par C and mtr were analysed. Mutation in S91F and D95 G/N in gyr A combined with E91G in par C was the most commonly observed mutation.D95G mutation was most prevalent in strains isolated from Delhi while D95N was prevalent in strains isolated from Pune. S91T mutation was reported only from the MSM population from Mumbai. Strains having the A39T or G45D and Y105H alteration in MtrRCDE efflux system were associated with high resistance. Double and triple mutations in gyrA alone or together in gyrA, parC and mtr gene, could be responsible for such a high level quinolone resistance in Indian strains of Neisseria gonorrhoeae.
Publications	• Antimicrobial Susceptibility of Neisseria gonorrhoeae in Pune from 1996-2007. Accepted for publication in Indian journal of STD & AIDS.
Presentations	• Poster presentation titled Quinolone resistance among Neisseria gonorrhoeae isolates in India: Detection of quinolone determining region mutations at International Pathogenic Neisseria conference 11th-16th Sep, Banff, Canada. •Talk on Quinolone resistant mutations in Neisseria gonorrhoeae in India at Consultative Meeting cum Training Workshop on Gonococcal Antimicrobial Susceptibility Programme (GASP) organized by WHO-SEAR GASP Reference Laboratory, at Regional STD Teaching, Training & Research Centre, Safdarjang Hospital, New Delhi from 7th Dec. to 9th Dec 2010.

3) Evaluating Essential Sexually Transmitted Infection Service Package for Sex Workers in India	
Principal Investigator	Dr. A.R Risbud , Scientist 'F'
Co-Principal Investigator(s)	Nil
Other Investigator(s)	Nil
Category / Nature	Multicentric and longitudinal Category: Operational Research
Collaboration / Participating Centers	National AIDS Research Institute, Pune, Family Health International, National AIDS Control Organization, New Delhi.
Funding Agency(ies) / Sponsors	Bill & Melinda Gates Foundation's Avahan project
Budget	$170427
Study Period	Three years
Objectives	1. To validate the essential service package for FSW and MSM; and 2. To monitor the STI prevalence and patterns within the cohort, the community of FSW, MSM and men attending STI clinics. More specifically, the study will: 3. Measure the prevalence of symptomatic and asymptomatic STIs and common RTIs among FSW and MSM at the start of the intervention and 6 months later, and correlate with demographics, behavioral characteristics, clinical manifestations, and symptoms. 4. Measure incidence of symptomatic and asymptomatic curable STIs in the cohort of FSW and MSM, and correlate with demographics, behavioral characteristics, clinical zanifestations, and symptoms. 5. Determine the patterns and etiologies of urethral discharge and genital ulcers among men consulting the STI clinics. 6. Measure gonococcal susceptibility patterns in selected sites.
Description	Avahan is the STI/HIV prevention project in India, funded by the Bill & Melinda Gates Foundation. Avahan's STI strategy aims to reduce the prevalence of common curable STIs that facilitate HIV transmission, and to reinforce general STI and HIV prevention efforts. In coordination with peer-based outreach and community-led approaches, clinics supported by Avahan state lead partner (SLPs) provide an essential service package (ESP) for female sex workers (FSW) and men having sex with

	men (MSM) designed to detect and treat curable STIs and to reinforce condom use. The main clinical components of this essential service package (ESP) are syndromic case management, provision of regular check-ups and presumptive treatment for asymptomatic infections. The Clinic Operational Guidelines and Standards (COGS) COGS recommend that FSW and MSM with STI symptoms should be managed based on syndromic case management using the National AIDS Control Organization (NACO) guidelines. Presumptive treatment of asymptomatic infections at first visit and repeated if FSW and MSM has not come for STI screening for 6 months is intended to treat infections that are missed by other methods and is based on assumptions of high STI prevalence and frequent re-exposure. These protocols have been developed based on the limited data in India and experiences from other countries.
Current Status	Completed
Publications	1. Title: Evaluating Essential Sexually Transmitted Infection Service Package for Sex Workers in India. Anjana Das, Parimi Prabhakar, Prakash Narayanan, Graham Neilsen, Teodora Wi, Sameer Kumta, Gururaj Rao, Raman Gangakhedkar, A. Risbud (2011) Prevalence and assessment of clinical management of sexually transmitted infections among female sex workers in two cities of India. J of Infectious Diseases in Obstetrics and Gynecology (Accepted).
Presentations	• Gururaj Rao, P Prabhakr, A Das, Vijay Nema and Arun Risbud R (2010) Sample preparation alteration enhances performance of the Multiplex PCR assay for diagnosis of Genital Ulcer Disease. 26-28 November-MICROCON-2010. • A Risbud, Gururaj Rao, A Das and P Prabhakar (2010) Performance of APTIMA Combo2 assay for detection of Chlamydia trachomatis and Neisseria gonorrhea in urine and vaginal swab specimens in symptomatic high risk women. 26-28 November-MICROCON-2010. • V Kumar, P Prabhakar, P Narayanan1, G Rao, A Risbud (2010) Etiological diagnosis of genital ulcers in India- is there a need for revision of national treatment guidelines? XVIII International AIDS Conference. 18-23 July-2010.

4) Characterization of oral Candida isolates from HIV-positive and HIV-negative individuals	
Principal Investigator	Dr. Arati Mane
Co-Principal Investigator(s)	Nil
Other Investigator(s)	Dr. Arun Risbud
Category / Nature	STI and OI category
Collaboration / Participating Centers	Nil
Funding Agency(ies) / Sponsors	NARI-Intramural
Budget	Rs. 10,00,000/-
Study Period	Jan 2008 – Dec 2010
Objectives	This project was carried out to study the species distribution, antifungal susceptibility pattern, virulence factors and genotypes of Candida isolates HIV-infected and healthy individuals.
Brief description (one paragraph)	The data showed that *Candida albicans* was the commonest spp. both as colonizer as well as pathogen. Non-albicans isolates like *C. tropicalis, C. lusitaniae, C. glabrata, C. krusei* were increasingly recovered from HIV-positive individuals. An overall 14% resistance to azoles was seen *(Indian J Med Res 2010, 131, 836-838).* The increased expression of virulence factors like proteinase, phospholipase, hemolysis and adherence to buccal epithelial cells in C. albicans isolates compared to Candida non-albicans was shown as a possible explanation of more frequent association of these isolates with severe and disseminated infections *(Mycol Med 2010, 49(5), 548-551).* The significant role of biofilm formation in pathogenesis of non-albicans Candida was seen *(Mycoses 2013, 56, 182 - 186).* The data showed increased expression of virulence factors in isolates from HIV positive individuals compared to HIV negative ones and thus supported the concept preferential strain selection, the molecular dissection of which could open new approaches for therapeutic interventions *(J Med Microbiol 2012, 61, 285-290).* This work has also been awarded the *best paper award at the '4th International Conference on Opportunistic Pathogens in HIV/AIDS'.*
Current status	Completed

Publications	1. Arati Mane and Arun Risbud. Biofilm formation in Candida isolates from HIV infected and uninfected individuals from Pune, India. Mycoses 2013, 56, 182 - 186. 2. Arati Mane, Shraddha Gaikwad, Shilpa Bembalkar, and Arun Risbud. Increased expression of virulence attributes in oral Candida albicans isolates from HIV -positive individuals. J Med Microbiol 2012, 61, 285-290. 3. Arati Mane, Chayya Pawale, Shraddha Gaikwad, Shilpa Bembalkar & Arun Risbud. Adherence to buccal epithelial cells, enzymatic and hemolytic activities of Candida isolates from HIV-infected individuals. Med Mycol 2011, 49 (5): 548-551. 4. Arati Mane, Sapna Panchvalli, Shilpa Bembalkar & Arun Risbud. Species distribution & antifungal susceptibility of oral Candida colonising or infecting HIV infected individuals. Indian J Med Res 2010, 131: 836-838.
Presentations	1. "Asymptomatic oral colonization and virulence determinants of Candida spp. from HIV infected individuals"; Arati Mane , Sapna Panchvalli, Shilpa Bembalkar & Arun Risbud presented at '33rd Annual Conference of Indian association of Medical Microbiologists (MICROCON)' in October 2008 held at AFMC, Pune. 2. "Characterization of oral Candida isolates from HIV positive and healthy individuals"; Arati Mane, Shraddha Gaikwad, Shilpa Bembalkar, and Arun Risbud presented at the'4th International Conference on Opportunistic Pathogens' in September 2009, New Delhi'.

5) Role of induced sputum as a non-invasive tool for diagnosis of pulmonary infections in HIV positive patients	
Principal Investigator	Dr. Arati Mane
Co-Principal Investigator(s)	Nil
Other Investigator(s)	Dr. Arun Risbud
Category / Nature	STI and OI category
Collaboration / Participating Centers	Nil
Funding Agency(ies) / Sponsors	NARI-Intramural
Budget	Rs. 10,00,000/-
Study Period	April 2010 – August 2012
Objectives	This study is being carried out to isolate the etiological agents causing pulmonary infections in HIV infected individuals and compare the diagnostic yields of induced sputum and bronchoalveolar lavage (BAL) samples.
Brief description (one paragraph)	In this study we identified the spectrum of etiological agents causing pulmonary infections in HIV infected individuals and compared the diagnostic yields of induced sputum (IS) and bronchoalveolar lavage (BAL) samples. A total of 110 HIV-positive patients presenting with respiratory symptoms were enrolled. Of the 110 participants, 76 (63.6 %) were males and 40 (36.7 %) were females with median age of 34.2 years (range, 19 - 66 years), medianCD4 count of 181 cells/mm^3 (37- 489), while 42.7% were on antiretroviral treatment. Definitive etiological diagnosis was possible in 92 (83.6 %) patients, out of which monomicrobial etiology was seen in 74 (80.4 %) patients. The causative agents were pyogenic bacteria in 47/110 (42.7 %) patients, mycobacteria in 32/110 (29.1 %), atypical bacteria in 3/110 (2.7%), P. jiroveci in 14/110 (12.7 %) and viruses in 8/110 (7.3 %) patients. The percent agreement (by kappa statistics) between IS and BAL samples for detection of pyogenic bacteria was 92%, Pneumocystis 100%, Mycobacterium tuberculosis (ZN smear) 79%, Mycobacterium tuberculosis (culture) 94 %, indicating that IS is as good as BAL for detection of pulmonary pathogens leading to an option of safer sample collection procedure.

Current status	Completed
Publications	Manuscripts are under review.
Presentations	"Induced sputum versus Bronchoalveolar lavage for diagnosis of pulmonary infections in HIV positive patients"; ***Arati Mane****, Shraddha Gaikwad, Pankaj Gujar , Ghorpade Shivhari , Rahul Lokhande , Arun Risbud* presented at '35th Annual Conference of Indian association of Medical Microbiologists (MICROCON)' in November 2010 held at Kolkata.