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Non rotaviral etiological agents of viral gastroenteritis in Kolkata, India

Viral gastroenteritis primarily causes morbidity and affects the younger age group, but lack of proper management to control dehydration can claim the lives of affected children. The poor sanitation and non-hygienic conditions prevailing in the developing countries has taken a huge toll through the spread of viral infections. The present scenario of vaccinating children against rotavirus infection has brought about distinct improvement in those countries where the vaccine is included in their immunization schedule. There are no vaccines available to date for protection against non rotaviral etiological agents.

Torovirus

The use of electron microscopy enabled detection of novel viruses associated with viral gastroenteritis. Toroviruses are classified under the order *Nidovirales*, family *Coronaviridae*, subfamily *Torovirinae*; these viruses were first reported in 1972 from horses in Berne –Switzerland as Berne equine virus and later as Breda virus (BRV) from calves in Iowa, Ohio, USA in 1982. (Woode et. al. 1982; Weiss et. al. 1983; Woode 1987, Horzinek et. al. 1987). The torovirus particles having pleomorphic appearance with short fringe (7-9 nm long) and approximately 90nm in diameter (Fig 1a) were detected in the faecal specimens of two diarrhoeic children in Kolkata. Further

immune electron microscopic examination showed that these virus particles were agglutinated by immune sera raised against Breda I and Breda II viruses of calves (Fig 1b). This observation was reported as electron microscopic evidence of torovirus like particles among children with diarrhoea as it suggested that torovirus may be another new viral pathogen of humans in Kolkata, India (Krishnan and Naik 1997). No other co-infection with hitherto known viral, bacterial or parasitic pathogens was detected in these samples.

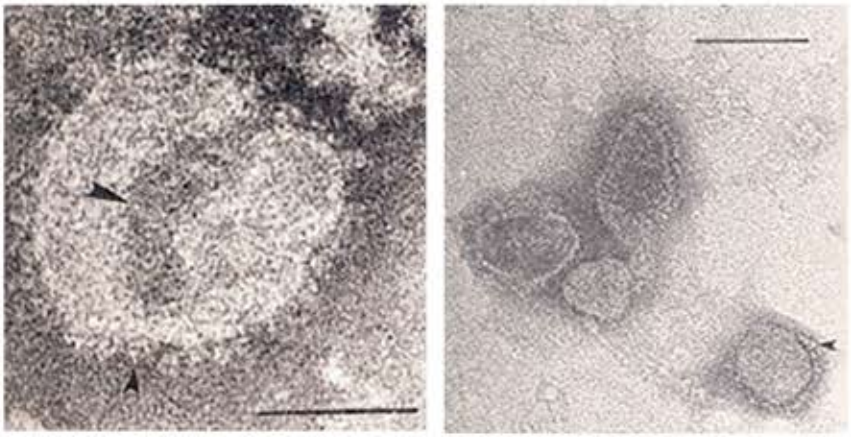


Fig 1a. Negatively stained Breda virus partide, pleomorphic in shape surrounded by peplomers(smaller arrow) and showing the toroidal outline (larger arrow).Bar denotes 100 nm.

Fig 1b. Immune electron microscopy showing agglutination of Breda virus-like partides after treatment with antisera to Breda virus Type II. Arrow indicates peplomers. Bar denotes 100 nm.

(Krishnan and Naik 1997)

Group B Adult rotavirus (ADRV)

Fig 2. Gel electrophoresis showing the genomic dsRNA pattern of a Group A rotavirus in comparison to that of the Group B rotavirus. (Krishnan et al 1999)

Group A Rotavirus has been recognised as the major causal agent since it was first reported (Bishop et al 1974). Non Group A rotaviruses associated with acute watery diarrhoea are the Group B rotaviruses (also referred to as 'adult diarrhoea rotavirus', ADRV). The rotavirus particles have a wheel shaped appearance and are about 70nm in diameter. The studies to understand the antigenic nature of the virus showed that Group B Rotaviruses (ADRV) were responsible for adult diarrhoea in very large numbers among the population in China during 1982-'83 (Hung et. al. 1984), but the Adult Diarrhoea Rota Virus (ADRV) was never reported thereafter outside China for nearly sixteen

years. The emergence of adult diarrhoea rotavirus in Kolkata, India was the first instance that was recorded, of five cases of ADRV outside China during the surveillance in 1987–'88, as shown in Fig 2. (Krishnan et. al. 1999). The ADRV has subsequently been detected in three cases from Pune (Kelkar and Zade 2004) and one case from Madhya Pradesh (Malik et. al. 2011) and also from Bangladesh.

Astrovirus

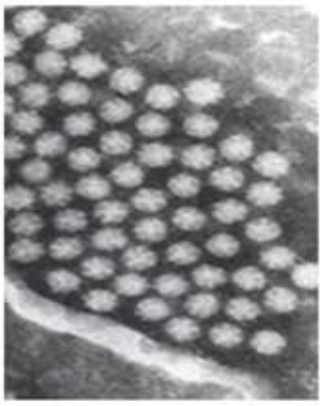
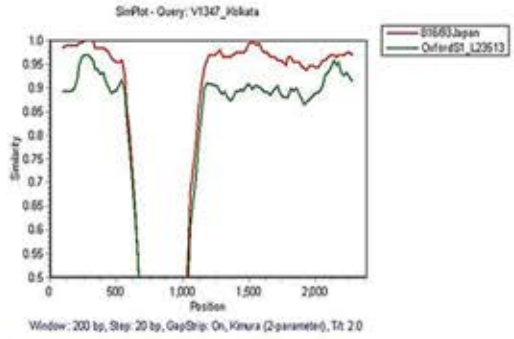


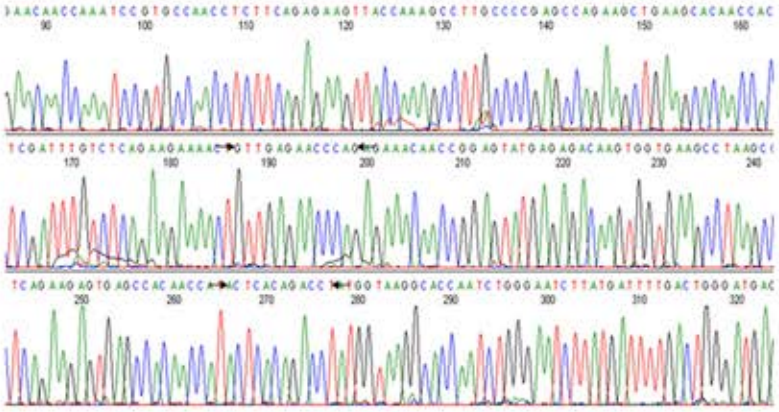
Fig 3. Negatively stained astrovirus particles

Astrovirus infection among acute watery diarrhoea cases was detected by electronmicroscopy (Fig 3.) as the virion has a characteristic five or six pointed star-like appearance (astron means 'star' in Greek). The virion has icosahedral symmetry and is non-enveloped. The genome is single stranded with three open reading frames (Mendez and Arias 2013). Astroviruses belong to the family *Astroviridae* and have been characterised into two genera viz. Avastrovirus (infecting birds) and Mamastrovirus (infecting mammals) and are reported to infect many host species (Krishnan 2014). The viral antigen was also screened by ELISA to understand the prevalence and seasonality in Kolkata, India. Astrovirus infections were detected sporadically throughout the year and were associated with varying degree of dehydration and acute watery diarrhoea (Bhattacharya et. al. 2006). In 52% of astrovirus positive cases, the virus was detected as the sole agent; mixed infections were also detected with other diarrhoeic pathogens such as rotavirus (32%), picobirnavirus (2%), rotavirus and picobirnavirus (2%), picobirnavirus and Enterotoxigenic *E.coli* (ETEC) (2%), rotavirus and ETEC (2%), rotavirus and Enteroaggregative *E.coli* (EAEC) (2%), Enteropathogenic *E. coli* (EPEC) (2%), *Shigella flexneri* type 3a(2%) and Ascaris (2%).To understand the molecular epidemiology of human astrovirus infections in Kolkata, India, RT-PCR was also carried out using specific primers, viz. Mon340 (+) and Mon348 (-) targeting a highly conserved domain of ORF1a (289 bp) of human astroviruses in Kolkata, India that showed maximum homology to the astrovirus strain ("5-158") from Seoul (98%).

The complete genome was amplified and sequenced to study the phylogenetic relationship of the Kolkata strains with hitherto known astroviruses. The Kolkata strain V1347/04/IND showed a unique stretch of sequence that was further confirmed by SimPlot.



Similarity plot (SimPlot) analysis for the query strain V1347/IND/04 vs. serotype 1 strain "816/93" from Japan and the Oxford serotype 1 strain (L23513). Analysis was performed on a window size: 200bp; step size: 20bp; using Kimura 2-parameter.



Unique nucleotide stretch from chromatogram 107-123 and from 184-198 (indicated in arrows) as observed in the fragment amplified with primer pair Prot9[+], P8[-] for the strain V1347/04/IND

Emergence of unique variants and inter-genotype recombinants of human astroviruses infecting infants, children and adults in Kolkata, India was reported by Pativada et al (2011). Astrovirus strains that were analyzed for their sequences of overlap region between ORF1b (RdRp) and ORF2 (capsid) were found to have close genetic relatedness to the Japanese strain HAstV_G1 [AB009985]. Additionally, the IDH2211 Kolkata strain showed a close genetic match with the Thai HAstV_G3 strain [EU363889].

HAstV-positive cases with ORF1a [HAstV genotype G2 or G8] and ORF2 [HAstV genotype G1, G2, or G3] were detected as sole or mixed infection among infants, children and adults in Kolkata with severe illness owing to acute gastroenteritis that required hospitalization for treatment between 2007 and 2009. In course of the study two conserved genomic fragments viz. 289bp of ORF1a and 449bp of ORF2 was amplified by RT-PCR among astroviruses that indicated interesting recombination event representing new and novel genetic variants (n=5) within eight different genotypes of astroviruses known to date. The twelve interesting recombinants were of type HAstV _ ORF1a _ ORF2 as HAstV _ G8_ G2 (n=1), HAstV _ G8_ G1 (n=10) and HAstV _ G2_ G3 (n=1) (Pativada et al 2012).

To examine the evolutionary pressures influencing the evolution of human astroviruses we further implemented evolutionary genetics analysis. Maximum recombination break points detected in Kolkata strain IDH1300 were 8 and a single break point location was detected at 1205 nt position. Partition-wise phylogenetic analyses of the IDH1300 Kolkata strain did not show close homology to the reference strains. Further phylogenetic analyses of full length ORF1b region of the human astrovirus strains showed that they formed a close cluster with each other and displayed a separate lineage in comparison to other human astrovirus strains worldwide. These novel human astrovirus strains showing multiple recombinations within highly conserved ORF1b that were detected from hospitalized acute watery diarrhea cases in Kolkata, India was reported by Pativada et. al. (2013).

Noroviruses

Noroviruses are an important cause of viral gastroenteritis and are chiefly implicated in food borne gastro-enteritis. Noroviruses are one of the most important genus in the *Caliciviridae* family where the virion has typical cup shaped depressions on its surface (calyx means 'cup'). There are two important genogroups of Noroviruses *viz* Genogroup I and Genogroup II respectively (Kim 2013).

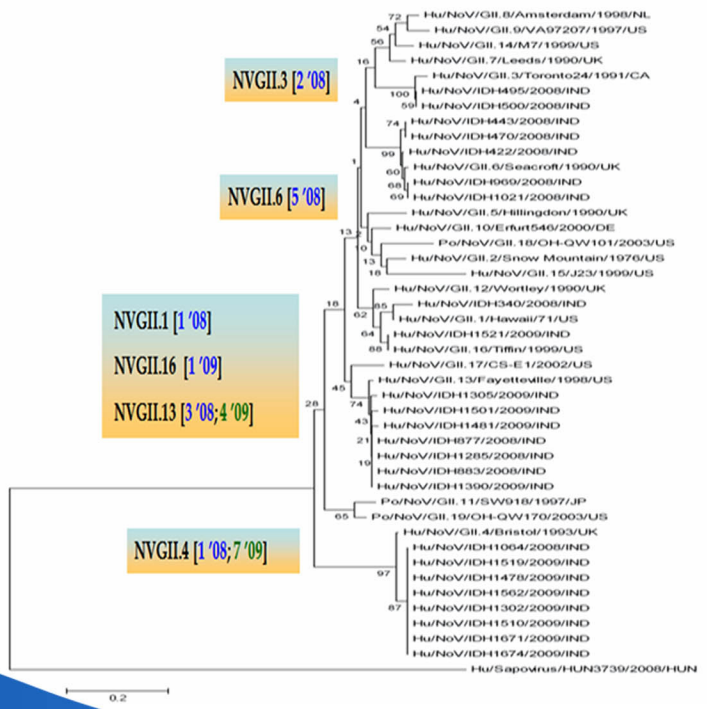
A novel intergenogroup recombinant Norovirus strain was reported from Kolkata, India (Nayak et al 2008). The intergenogroup recombinant norovirus strain was detected in the faecal specimen of a 17 year old male, who had suffered from acute watery diarrhea and severe dehydration.

Strains	RdRp Sequence	Capsid Sequence
AY038598 (NLV/VA98115/98/GI)	**86.2%**	65.7%
AB187514 (Hu/GI/Otofuke/79/JP)	85.4%	65.2%
U04469 (Desert Shield virus DSV395/93/GI)	76.9%	65.2%
AF414405 (NLV/Little Rock/316/94/US/GI)	84.5%	66.0%
EF126964 (Hu/GII.4/Terneuzen70/06/NL)	62.6%	**99.0%**
AB220924 (Norovirus Hu/Chiba/04-899/04/JP/GII)	63.9%	97.9%
AY502023 (Hu/NoV/Farmington Hills/02/USA/GII)	64.8%	98.2%
AY587989 (Hu/NLV/Oxford/B2S16/02/UK/GII)	64.7%	97.5%

Further sequence analysis confirmed that this novel recombinant strain [Hu/Koll/NLV/L8775/AB290150/2006/India] was having a polymerase gene fragment that closely resembled Norovirus (NoV) genogroup-I genotype-3 virus (HuCV/NLV/GI.3/VA98115/AY038598/1998/USA) and the capsid gene resembled NoV genogroup-II genotype-4 virus (NoV/Hu/GII.4/Terneuzen70/EF126964/2006/NL). The crossing over and recombination was observed at nucleotide (nt) 790 of NoV GI VA98115 strain and nt 808 of NoV GII Terneuzen70 strain. In both parent strains conserved nucleotide sequence and hairpin structure (DNA secondary structure) were reported at the junction point of ORF1 and ORF2, exhibiting the mechanism of recombination in these viruses.

The HuCVs were detected by reverse transcription-polymerase chain reaction (RT-PCR) of the partial RNA dependent RNA polymerase gene (RdRp) and capsid gene and confirmed by sequencing. The sequences were analyzed and the recombination point was detected. The emergence of new variant of GII.4/2007, three novel NoV GII inter-genotype recombinant strains of NVGII causing acute watery diarrhoea among children in Kolkata, India indicated the remarkable genetic diversity of the HuCVs circulating in Kolkata, India viz.12 NoV cases (54.5%) were GII.4 and six cases showed 99% identity with the new variant Japanese strain-Hu/NoV/GII.4/OC07138/JP. Three novel NoV GII inter-genotype recombinant strains V1628/IND, V1656/IND and V1737/IND were also detected. The RdRp region of V1628 showed

A new variant of Norovirus GII.4/2007 and inter-genotype recombinant strains were reported from Kolkata, India. Journal of Clinical Virology 2009



96% identity with Pont de Roide 673/FRN whereas capsid region resembled GII.7/Osaka F140/JPN strain (98%); the strain V1656 showed 98% identity with RdRp region of GII.4/Monastir 375/TUN but capsid region resembled GII.8/Leverkusen267/DE (91%); the strain V1737 showed 88% identity with RdRp of GII.5/Minato 6/N1/6/JPN whereas capsid region resembled the GII.12/Gifu 96/JPN (93%). During characterization of Caliciviruses two strains of NoVGIIb and one strain of each NoV GI.1/V1622/06/IND, GI.3/V1707/07/IND, GII.3/V1668/IND, GII.16/V1729/IND, Sapovirus GII.1/V1716/IND were also detected (Nayak et. al. 2009).

Partial RNA dependent RNA polymerase gene (RdRp) sequences corresponding to the Norovirus GII positive samples showed homology to the sequences of Djibouti (horn of Africa), Brazil, Italy, Japan and US norovirus strains indicating emergence of divergent Noroviruses among children from Kolkata, India (Nataraju et. al. 2010); RT-PCR with specific primers targeting region C of the capsid gene of noroviruses (NoVs) and sequencing of NVGII strains showed clustering with GII.4 NoVs followed by GII.13 and GII.6 NoVs. (Nataraju et. al. 2011a).

The emergence of two recombinant NoV strains (Hub/NoV/ IDH1501/2009/IND and Hu/NoV/IDH1873/2009/IND) along with other interesting GII NoV strains was reported (Nataraju et. al 2011b). Similarity plot and phylogenetic analysis confirmed the strain Hu/NoV/IDH1501/2009/IND as a NoV recombinant strain with genes for RNA dependent RNA polymerase (RdRp)GII.1-like and capsid GII.13-like; the strain Hu/NoV/IDH1873/2009/IND was a NoV recombinant strain with its RdRp gene GII.5-like and capsid gene being GII.13-like respectively.

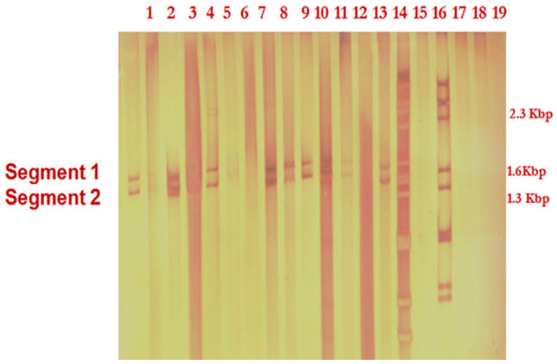
Picobirnavirus

The genus, Picobirnavirus (PBV), Spanish 'pico'='small', birna for 'bipartite RNA' genome, belongs to the family *Picobirnaviridae* under the proposed order *Diplornavirales*. Picobirnaviruses are a group of unclassified, non-enveloped, small spherical viruses, 35-41 nm in diameter without any apparent surface morphology. Picobirnaviruses (PBVs) showing bisegmented small RNA genome profile (1.75 and1.55kbp for segment 1 and 2, respectively) were detected by Polyacrylamide gel electrophoresis of genomic RNA extracted from faecal specimens of acute watery diarrhoea cases, in Kolkata, India and silver staining. The short genome profile PBVs associated with acute watery diarrhoea were another emerging diarrhoeagenic virus in Kolkata, India. (Bhattacharya et. al. 2007; Krishnan 2010).

Varying degrees of dehydration among infected cases had necessitated their visit to hospital for further treatment and management of acute watery diarrhoea. PBV infection was associated with co-infection of rotavirus (n=3), astrovirus (n=3) and with both in one case. There was a single instance of co-infection with *Salmonella* spp. (n=1).

PAGE gel of the picobirnavirus positive strains with 'small profile' detected from diarrhoeic children

Segment 1 and 2 were seen just above rotavirus segments 5,6 [1.3 Kbp and 1.6 Kbp approximately]

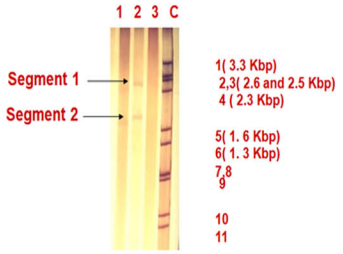


It was interesting to note that both Genogroup I and Genogroup II picobirnaviruses were associated with acute watery diarrhoea by RT-PCR and sequencing experiments. Molecular characterization using reported primers PicoB25-PicoB43 for Genogroup I and PicoB23-PicoB24 for Genogroup II in RT-PCR showed the presence of Genogroup I PBVs and Genogroup II PBVs while some could not be amplified with these primers. Sequence analysis of Genogroup I amplicons indicated remarkable sequence heterogeneity. After more than a decade, four PBV positives of Genogroup II were detected in Kolkata, India. Phylogenetic analysis showed varying degree of genetic diversity amongst PBV strains from Kolkata and other countries.

PBVs showing large genome profile were also detected among children from a slum community in Kolkata, India (Bhattacharya et. al. 2006). They showed characteristic bisegmented double stranded RNA genome of 'large profile' (2.3-2.6 kbp for the larger and 1.5-1.9 kbp for the smaller segment, respectively).

PAGE gel of a picobirnavirus strain with 'large profile' detected in Kolkata

Segment 1: 2.3-2.5 Kbp; Segment 2: above 1.6 Kbp.



Faecal specimen from four asymptomatic cases (P597_02_IND, K135_02_IND,A373_03_IND, A356_03_IND) and one diarrhoeic case (K135_03_IND) had genogroup I picobirnaviruses (1-CHN-97 like) showing amplicons within the 201 bp region, with primers PicoB25-PicoB43, targeting the conserved domain of RNA-dependent RNA polymerase (RdRp) gene. It was interesting to note that only the PBV strain P597_02_IND from Kolkata with large genome was closely related to a reported strain (similarity with 2-GA-91 from USA was 87% at the nucleotide level and 90% at the amino acid level). Sequence analysis showed three conserved amino acid domains as well as a highly conserved D-S-D motif characteristic of RNA-dependent RNA polymerase gene of bisegmented, double stranded RNA viruses. Sequence data of the picobirnavirus A356_03_IND indicated strong heterogeneity with all other picobirnavirus strains sequenced till date.After nearly a decade a genogroup II picobirnavirus strain (R227_03_IND) was isolated from a diarrhoea case in the community, with small genome profile and amplified with specific primers PicoB23-PicoB24; but the sequence data showed that it was divergent from the hitherto reported prototype strain 4-GA-91 of genogroup II human picobirnaviruses.

PBV infections have been reported from diarrhoeic animal species and humans as well as from asymptomatic cases. The detection of Picobirnaviruses (PBVs) in diarrhoeic faecal specimens from children aged <5 years was suggestive of zoonotic transmission from an urban slum community in Kolkata between July and October 2007. The Picobirnaviruses showed either large profile or small profile for their bisegmented genomic double-stranded RNA (dsRNA). Reverse transcription polymerase chain reaction (RT-PCR) showed amplicon of 201bp with genogroup I primers [PicoB25(+) and PicoB43(-) specific for RNA dependent RNA polymerase (RdRp) gene fragment encoded by genomic segment 2 and these were sequenced [GPBV1-5, 7 and 8]. Sequence analyses showed that four PBV strains [GPBV1-3 and 8] resembled different clones of porcine PBV strains (D4, D6 and C10) reported in 2008 from Hungary and two PBV strains [GPBV4 and 7] resembled human PBV strains (P597,Kolkataand 2-GA-91, USA) with the maximum nucleotide (nt) identity ranging from 78% to 92%. One strain GPBV5 clustered with human PBVs and porcine PBVs that were reported from Hungary, Venezuela and Argentina showing close homology to human-like PBVs (Ganesh et. al 2011).

There was an instance when multiple strains of Picobirnavirus causing mixed infection in a diarrhoeic child was observed in the PAGE experiment. The genomic RNA profile from the faecal specimen showed three sets of PBV with large profile bisegmented genomic RNA with slight variation in migration pattern. Molecular cloning experiments confirmed that PBV/ Human/INDIA/GPBV6/2007 had mixed infection comprising four different strains of PBV genogroup I [GPBV6C1P-GPBV6C4P] and one PBV genogroup II strain [GPBV6G2P]. Sequence comparison and phylogenetic analysis of gene segment 2 of GPBV6 clones (C1, C2, C3 and C4) revealed low nucleotide identities (59-63%) and distant genetic relatedness to other human and porcine genogroup I picobirnaviruses. The strain GPBV6G2P represented another PBV genogroup II strain after prototype strain 4-GA-91/USA as genogroup II PBVs have seldom been reported to date, except from Kolkata, India and Netherlands. Thus, the first instance of detection of multiple strain (mixed) infection of picobirnavirus [genogroups I and II] from a diarrhoeic child in a slum community of Kolkata, India showed the emergence of prototype Genogroup II-like strain in Kolkata, India. (Ganesh et. al. 2010).

Conclusion

The surveillance studies have provided invaluable opportunity to understand the association of different etiological agents besides Group A rotavirus with acute watery diarrhoea. Viral gastroenteritis is a major public health problem and the lack of suitable vaccines continues to cause unabated infections among children and other age groups. The need to provide adequate measures to control infections and development of vaccines for specific viruses associated with gastroenteritis is necessary to alleviate the problem and bring forth 'healthy childhood' to the children in developing countries.

Acknowledgements

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---------- Dr. Triveni Krishnan---------
Scientist F
Virology Division

NICED Library News

NICED Library received a fund of amount Rs. 50 lakhs from Indian Council of Medical Research for the year 2016-2017. This fund has been used completely to purchase books and online journals. The list of books and journals added to the resources of the library are given below:

Newly added books

1. Harper's illustrated Biochemistry
2. Health Policies & Programmes in India
3. Community Medicine With Recent Advances
4. Roitt's Essential Immunology
5. Biomedical Informatics: computer applications in health care and biomedicine
6. Biostatistics
7. Pediatric Gastroenterology & Liver Disease
8. Nelson Text Book of Pediatrics (2v set)
9. Park Text book of Preventive & Social Medicine
10. Microbiology: Principles & Explorations
11. Con's Current Therapy 2017
12. Koneman's Colour Atlas & Textbook of Diagnostic Microbiology
13. Cell Biology
14. Manson's Tropical Diseases
15. Applied Demography and Public Health in the 21st century
16. Permutation Statistical Methods: an Integrated Approach
17. Landmark Experiments in Molecular Biology
18. A Historical Introduction to Mathematical Modeling Of Infectious Diseases

19. Analyzing and Modeling Spatial and Temporal Dynamics of Infectious Diseases
20. Statistical Methodologies with Medical Applications
21. Animal Cell Culture
22. Gene Cloning & DNA Analysis
23. Stress and Environmental Regulation of Gene Expression and Adaptation in Bacteria
24. Clinical Virology Manual
25. Concepts of Genetics, Global edition
26. Big Data Analysis for Bioinformatics and Biomedical Discoveries
27. Practical Manual of Biochemistry
28. Zubay's Principles of Biochemistry
29. Molecular Medical Microbiology
30. Polymers and Nanomaterials for Gene Therapy
31. Janeway's Immunobiology
32. Microbe
33. Diagnostic Medical Parasitology
34. Kuby's Immunology
35. Manual of Clinical Microbiology
36. Manual of Environmental Microbiology
37. Manual of Molecular & Clinical Laboratory Immunology
38. Medical and Health Genomics
39. Molecular Biology: Structure & Dynamics of Genomes & Proteomics
40. Molecular Microbiology: Diagnostics Principles and Practices
41. Principles of Virology
42. Protein concept in Biochemistry
43. Virulence Mechanisms of Bacterial Pathogens
44. Zoonoses: Infectious Diseases transmissible from Animals to Humans
45. Swamy's compilation of delegation of financial powers
46. Swamy's The Prevention of Corruption Act
47. Swamy's compilation of FRSR (part I, Part II, Part III, Part IV)
48. Swamy's pension compilation incorporating CCS
49. Swamy's compilation of HBA rules
50. Swamy's compilation of Central Treasurey rules
51. Swamy's CAT
52. Swamy's compilation of Central Govt. Account Rules
53. Swamy's compilation of Reservation and Concession+ Supplement to C45
54. Swamy's compilation of RTI
55. Swamy's Annual Orders on Service Matters 2013, 2014, 2015
56. Swamy's Manual on disciplinary proceedings for Central Govt Staff complete with rules and orders
57. Swamy's Complete Manual on Establishment and Administration
58. Swamy's Manual on office procedures
59. Swamys Master manual for DDOs and Heads of Offices (Part I, Part II)
60. Swamy's Payrules made easy
61. Swamy's Compilation of General Financial Rules, 2005 Incorporating Compendium of Rules on Advances to Government Servants

62. Current Protocols in Microbiology
63. Current Protocols in Molecular Biology

Online journals for 2017

1. Nature Immunology
2. Nature Reviews Microbiology
3. Mucosal Immunology
4. Gastroenterology
5. Infection Genetics & Evolution
6. International Journal of Medical Microbiology
7. Journal of Clinical Epidemiology
8. Microbes and Infection
9. Protein Expression & Purification
10. Trends in Microbiology
11. Trends in Parasitology
12. FEMS Microbiology Reviews
13. FEMS Immunology & Medical Microbiology now Pathogens and Diseases
14. Cellular Microbiology
15. Vaccine
16. Lancet Infectious Diseases
17. Cell Host & Microbe
18. Antimicrobial Agents and Chemotherapy
19. Applied & Environmental Microbiology
20. Clinical & Vaccine immunology
21. Clinical Microbiology Reviews
22. Infection & Immunity
23. Journal of Bacteriology
24. Journal of Clinical Microbiology
25. Journal of Virology
26. Microbiology and Molecular Biology Reviews
27. Molecular & Cellular Biology
28. Ecosal Plus
29. American Journal of Epidemiology
30. Journal of Immunology
31. Journal of Medical Microbiology
32. Epidemiology & Infection
33. Microbiology Spectrum
34. Science and Culture (Print Journal)