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Contents

Sr. No.	Title	Page No.
1	Message from the Chancellor Dr. Sanjay D. Patil	i
2	Message from the Vice Chancellor Dr. Prakash B. Behere	ii
3	Message from the Pro-Vice Chancellor Prof. Dr. Shimpa Sharma	1
4	Message from the Dean Dr R K Sharma	2
5	Message from the Research Director Dr. C. D. Lokhande	3
6	Message from Convener Dr. Roma A. Chougale	4
7	Message from Organizing Secretary Dr. V. S. Vatkari	5
8	All You Want To Know About Nipah Virus Dr. Praveen Shahapur	6
9	Arthropod Borne Virus Disease (Arbovirus Disease) in Maharashtra with special reference to Dengue Dr. Raju Jotkar	16
10	Updates on Chikungunya virus - Diagnosis, molecular epidemiology & treatment Deepti Parashar	23
11	Molecular Epidemiology of Dengue Viruses Circulating in India Alagarasu Kalichamy	24
12	Overview of Arboviruses & Nipah Virus Dr.V.R.Wagh	25
13	Radiographic study of anatomical basis of Coracoacromial arch impingement due to varied morphology of acromion process Aruna Y Yadav, AD Patil	30
14	Iridocorneal Endothelial Syndrome with secondary glaucoma Dr. Milind Sabnis	31
15	Isolation and Antibiotic Susceptibility Pattern of Coagulase Positive <i>Staphylococcus Aureus</i> from Various Clinical Specimens at Tertiary Care Hospital, Jaipur Dr Maina raigar, Dr Aruna vyas	32

16	Variation In Branching Pattern of Coeliac Trunk and Its Clinical Significance D.T. Wagh	33
17	Intra Abdominal Hypertension After Emergency Laparotomy Dr. Amarnath Basu	34
18	Rule Of 500 Dr. Chaitanya Patil	35
19	Utility of Haematological parameters and C-Reactive Protein levels in early diagnosis of Neonatal sepsis Dr. Pragati Narayanakar, Dr Sharada Metgud	36
20	Index Finger Pollicization For Traumatic Thumb Amputation Dr. Rajat Kumar Singh Dr. Mayuresh Deshpande	37
21	Steatocystoma simplex on the scalp Dr. Rajat Kumar Singh Dr. Mayuresh Deshpande	38
22	Role of Sympathetic Block and Ozone Therapy as A Combination Therapy in Treatment of Diabetic Foot Ulcers Dr. Narendra Bhoir, Dr. R. M. Kulkarni	39
23	Septicemia Due to <i>Propionibacterium Propionicum</i> In Three Month Old Female Child - A Case Report Dr Neeta Jangale, Dr Hemangi walke, Dr Smita Pokharnikar	40
24	A Study on Outcome of Surgical Management of Tympanic Membrane Retractions Dr Deepal Kundu	43
25	Study Of Bacterial And/Or Fungal Infections In Clinically Diagnosed Cases Of Acute Conjunctivitis Dr. Sneh Rauth	45
26	Clinical Study of Mucormycosis –A Deadly Disease on Rise Dr. Potdar	46
27	Status of Ossicular Chain in Chronic Otitis Media Dr. Roshni Mohanty	48
28	Isolation and Antibiotic Susceptibility Pattern of Coagulase Positive from Various Clinical Specimens At Tertiary Care Hospital, Jaipur Dr Maina Raigar, Dr Aruna Vyas, Dr. Rajani Sharma, Dr. R.K. Maheshwari	49
29	Wet Mount Microscopy and Gram Staining in The Diagnosis of Urinary Tract Infection Ravindra V. Gawande	51
30	Craniofacial Measurements in Maharashtra Population: A Cephalometric Study Supriya P. Satpute, Vasudha R. Nikam ² , Tohid Mujawar, Manjiri Desai	52
31	Reliability of Stature Estimation From Facial Parameters Amongst Sangli District Population Dr. Vaishali A. Mane, Dr. Ashalata D.Patil. Dr. A.Y. Mane	53

- 32 **Epidemiology and sero-positivity of dengue fever cases in tertiary care hospital of western Maharashtra, India** 54
Santosh S Patil
- 33 **To study whether our medical colleges inculcate health promoting life style among Medical students – A cross sectional study from a medical institute from western Maharashtra** 57
Dr. Deepak W. Deshkar, Ms. Shruti D. Deshkar, Dr. Priti D. Deshkar, Mr. Janardhan V. Narute, Mr. Vijaykumar D. Somvanshi
- 34 **Study of Bloodstream Infections in Paediatric ward in a Teaching Hospital, Kolhapur, Maharashtra** 61
Dr Mrs. Vishwashanti S Vatkar, Dr Mrs. R A Chougale
- 35 **Prevalence of Amp C β -lactamase production in gram negative uropathogens** 64
Dr. Anjali Sunildatta Jog
- 36 **Evaluate the Awareness of Hand washing by using Semi- Structured Questionnaire from a tertiary care hospital, Kolhapur** 70
Roma A Chougale, Arun Kumar P
- 37 **Incidence of *Pseudomonas Aeruginosa* in Post – Operative Wound Infections in A Teaching Hospital in Western Maharashtra** 75
Dr.Veerendra Patkar, Dr.Deepak Deshkar, Mr. Janardhan Narute and Mr.Vijaykumar Somvanshi
- 38 **Knowledge, attitude and practices regarding prevention of dengue and malaria among residents of urban area of Kolhapur** 80
Dr. Pallavi A. Potdar, Dr. T. A. More, Dr. Anjali Wagh
- 39 **Comparative study of NAFLD fibrosis score and APRI score in ultrasonographically diagnosed NAFLD with type2 diabetes patient** 81
Dr. Onkar Kakare, Dr.Shimpa Sharma
-

NATIONAL CONFERENCE ON RECENT UPDATES ON NIPAH AND ARBOVIRUSES – INDIAN SENARIO (NCRUNA – 2018)

**18TH AUGUST 2018 HELD AT AUDITORIUM D.Y.PATIL
MEDICAL COLLEGE, KOLHAPUR**

Organized by
**D. Y. Patil Education Society,
Kolhapur Institution deemed to be University,
Department of Microbiology.**



Message form the Chancellor



I am extremely pleased to know that the Department of Microbiology of our college is organizing a national conference on 'RECENT UPDATES ON NIPAH & ABROVIRUSES – Indian Scenario'. Recently there was an outbreak of NIPAH viral infection in Kerala. Presently there is an outbreak of Dengue fever in our own district of Kolhapur. It is essential for all the medical fraternity to be updated so as to face these medical outbreaks effectively.

This is the first such national conference organized not only in our college but also in Kolhapur district.

As rightly said

**"Reading "maketh a full man.
"Conference" a ready man,**

I wish the department of Microbiology and the organizing Committee best of luck for the conference.

Dr. Sanjay D. Patil,
Hon.' Chancellor

D. Y. PATIL EDUCATION SOCIETY, KOLHAPUR

(Institution Deemed to be University)

(Declared u/s 3 of the UGC Act 1956 vide Notification No. F.9-26/2004-U.3 dt. 01-09-2005 of the GOI)

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M.D.(Psy.) FNAMS, FIIOPM

Vice-Chancellor

Prof. of Psychiatry

Recipient Dr.B.C.Roy National Award



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MESSAGE



It gives me a great pleasure to write this message for the Department of Microbiology who is organizing "National Conference on Recent Updates on Nipah & Arboviruses-Indian Scenario (NCRUNA)", on 18th August 2018. The conference will provide a platform to bring together the experts from this field involving Doctors, Clinician, Academician, Experts from all over the state. The interactions amongst the participants and resource persons will help to broaden their expertise in variety of areas of their interests and also to bring out the variety of new methods with their very interesting and useful applications which will lead in the way to build the new ideas. As this is relatively new virus, the outcome of this update will bring new solutions as well

I am aware of the fact that such type of innovative programs has always been organized in past by Dept. of Microbiology. I take this opportunity to appreciate the efforts of the members of the association for organizing such an important Conference.

I wish a grand success to this Conference.

Dr. Prakash B. Behere

M.D.(Psy.) FNAMS, FIIOPM

Vice-Chancellor,

Message from the Pro-Vice Chancellor



Congratulations to the Department of Microbiology, D Y Patil Medical College for organizing the national conference on 'Recent Updates on Nipah & Arboviruses – Indian Scenario' to be held on 18th August 2018.

I welcome all the delegates to our campus for this occasion. This conference is in keeping with the high priority given by the University to research in emerging areas and to the dissemination of new knowledge to relevant stakeholders.

The WHO and most experts in the field believe that the source of the next human pandemic will be zoonotic with wildlife being the source of concern. In recent years SARS, Swine Flu, Avian bird flu, MERS, Ebola, Zika and other infections have created major public health situations. Emerging diseases are a challenge faced by all delivering health care services.

Against this backdrop, a conference focused on the recent emerging or re-emerging zoonotic infections is a welcome and most needed event. The emphasis on the status of these diseases in our country will be invaluable to researchers, clinicians and diagnosticians as well as to students. The distinguished speakers who have consented to address the delegates are both experienced and respected in their fields. This promises all attendees an enjoyable and productive experience.

I would like to again congratulate the faculty and staff of Department of Microbiology on this event and wish them the very best.

Prof. Dr. Shimpa Sharma

Pro-Vice Chancellor,

D Y Patil Education Society, Institution Deemed to be University
Kolhapur.

Message from the Dean

'National Conference on Recent Updates on Nipah & Arboviruses – Indian Scenario' is being organised by the Department of Microbiology of DY Patil Medical College.

The topics being deliberated are very appropriate keeping in view the emergence of new viruses leading to increased mortality and morbidity.

Eminent speakers are going to enthral the audience by their immense knowledge and experience in this field. They are going to provide insight not only to the molecular basis but also to the clinical implications.

The faculty of the Microbiology Department has worked as a very hard as a well coordinated team. I wish them all and this National Conference a great success.

Dr. R. K. Sharma

Dean

D. Y. Patil Medical College, Kolhapur.

Message from the Research Director



It's a matter of great delight for me as a Research Director to see that the department of Microbiology is hosting its first national conference. The theme of the conference "Recent Updates on Nipah & Arboviruses – Indian Scenario" is most appropriate in the time when we recently faced the Nipah virus outbreak in Kerala and the present ongoing epidemic of Dengue virus in Kolhapur district. Search and research in an intrigal part of a university.

The organizers have taken great efforts to convey this to the delegates by inviting eminent personalities from the Maharashtra health services and scientists from national institute of virology.I wish the occasion great success.

Dr. C. D. Lokhande

Research Director

D. Y. Patil Education Society, Kolhapur.
Institution Deemed to be University.

MESSAGE FROM CONVENER

I am delighted to welcome all the delegates and dignitaries to the first Microbiology Conference in Kolhapur City, Recent Updates on Nipah and Arbo viruses- Indian Scenario on 18th August 2018. The recent outbreak of Nipah virus has created havoc in Kerala this year. Arbo virus epidemics like dengue, Chikungunya, Japanese encephalitis, Kyasnur Forest Disease (KFD), Zika virus have also left our Indian public in fear.

To face this challenge, we have organized this one-day National conference. This conference has brought together delegates from all over India i.e. Maharashtra, Gujrat, Rajasthan, Karnataka etc.

Scientists from National Institute of Virology, Ex-president of IAMM & Head SDM Medical college Dharwad; Assistant Director, Joint Director and Deputy director of Health services- Govt of Maharashtra, DHO- Kolhapur region, Pathologists, Microbiologists, Medicine & Community medicine faculty, Ophthalmologists, Gynecologists, ENT & General surgeons, Pediatricians as well as all the pre & paraclinical departments, Medical officers and Consultants, Faculty, post graduate & undergraduate students, PhD, MSc, DMLT, etc. will have detail discussions during the conference.

I am sure you will all have a fruitful time with us and sweet memories of our conference and Kolhapur city.

The conceptualization, planning and publishing of this NCRUNA conference proceeding is the outcome of countless people. We are indebted to the dedication shown by numerous zealot colleagues. However I take this opportunity to wholeheartedly acknowledge the efforts of some who left an undisputed imprint on the quality of this effort. I hope you will like the articles published in this proceeding

Dr. Roma A. Chougale,
Convener- NCRUNA-2018
& Guest Editor NCRUNA Proceedings
Prof & Head of Microbiology
D.Y.Patil Medical College,
Kolhapur.

Message from organizing Secretary



It is my privilege to welcome all the dignitaries and the delegates to this city of Kolhapur, on the occasion of the 'National Conference on Recent Updates on Nipah & Arboviruses- Indian Scenario'.

We designed the theme of the conference to enrich the participants to resolve the burning problems related to Nipah and Arboviruses.

The talent of young scientists will also be tested at the same time, who will be presenting their research work in the form of Oral & Poster presentations.

The encouraging support from the President & Chancellor of the D Y Patil Deemed To Be University, the Vice chancellor, the Pro vice chancellor, the Dean and the enthusiasm of the committee members shall help to make this academic event successful.

I wish each one of you a pleasant stay here with us and enjoy the academic feast and delicious food. I hope that you carry the pleasant memories of the conference along with you,

Dr V S Vatkari

Associate Professor,
Microbiology Department
Organizing Secretary,
NCRUNA-2018

All You Want To Know About Nipah Virus

Dr.Praveen Shahapur

Professor

Department of Microbiology

B. M. Patil Medical College, Vijapur, Karnataka.

The 2018 Kerala Nipah virus outbreak was an alarming signal to medical fraternity to be prepared for such new microbial outbreaks in future. The outbreak control is mainly depending on two simple public health measures (i) isolating symptomatic individuals and (ii) tracing and quarantining their contacts. These two measures were implemented vigorously and outbreak was contained in Kerala within 40 days after index case was reported.

The knowledge of the Nipah virus is must because, there is no treatment or vaccine available for the same and it spreads from Person to person and Nosocomial and the Virus has High case fatality rate.

History

Nipah virus was initially isolated and identified in 1999 during an outbreak of encephalitis and respiratory illness among pig farmers and people with close contact with pigs in Malaysia and Singapore. Its name originated from Sungai Nipah, a village in the Malaysian Peninsula where pig farmers became ill with encephalitis. The causative agent was isolated from cerebrospinal fluid of a human fatal case and shown to be closely related to Hendra virus

Epidemiology

In the 1999 outbreak, Nipah virus caused a relatively mild disease in pigs, but nearly 300 human cases with over 100 deaths were reported. In order to stop the outbreak, more than a million pigs were euthanized, causing tremendous trade loss for Malaysia. Since this outbreak, no subsequent cases (in neither swine nor human) have been reported in either Malaysia or Singapore

In 2001, NiV was again identified as the causative agent in an outbreak of human disease occurring in Bangladesh. Genetic sequencing confirmed this virus as Nipah virus, but a strain different from the one identified in 1999. In the same year, another outbreak was identified retrospectively in Siliguri, India with reports of person-to-person transmission in hospital settings (nosocomial transmission). Unlike the Malaysian NiV outbreak, outbreaks occur almost annually in Bangladesh and have been reported several times in India.

Indian outbreaks

Total three outbreaks were reported from India. First one is in February 2001, an outbreak of febrile illness with neurological symptoms was observed in Siliguri, West Bengal. Second was in April 2007 from Nadia district of West Bengal, around 30 cases of fever with acute respiratory distress and/or neurological symptoms were reported and five cases were fatal. All five fatal cases were found to be positive for NiV. Last outbreak was reported in May 2018; it was localized in [Kozhikode](#) and [Malappuram](#) districts of Kerala and claimed 17 lives. The outbreak was contained and declared over on June 10, 2018. The transmission in India was mainly by consumption of fruits or fruit products (such as raw date palm juice) contaminated with urine or saliva from infected fruit bats, human-to-human through close contact with people's secretions and excretions and also Nosocomial.

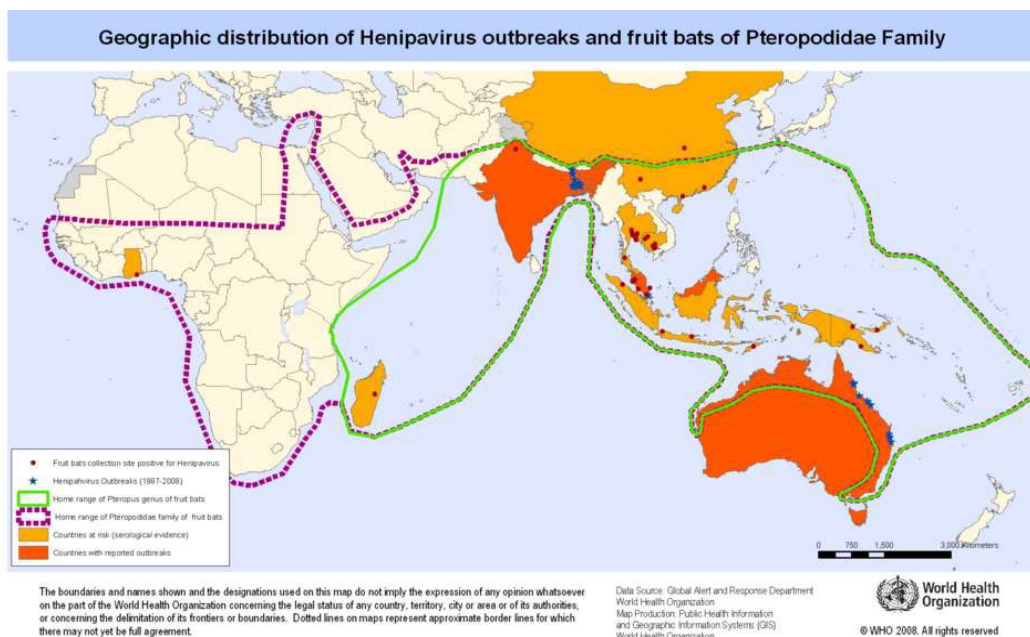
Reservoir

The primary reservoir for Nipah virus are flying foxes (also known as fruit bats) of the genus *Pteropus*. Transmission of Nipah virus from bats to swine has not been shown conclusively; however, there are various biologically plausible means for infected secretions of primary hosts to enter pigs, including direct contact with infected secretions contaminated fruit or dead bats. Scavenging animals may also play a role in the transport of virus into proximity of pigs. Flying foxes are able to carry the virus without being affected by it. Investigations of potential secondary hosts (peridomestic species) have also been conducted. Rats, house shrews, dogs, and chickens have been tested, but no indication of a secondary host has been found.

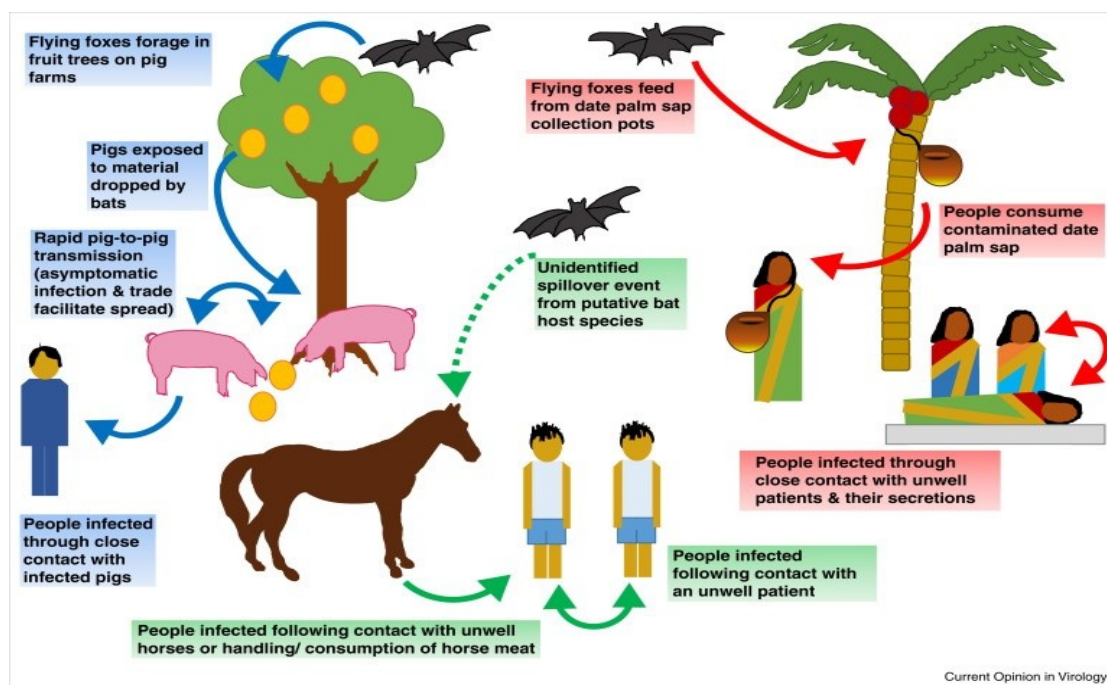
Among the many species of fruit bats found across India, *Pteropus giganteus* (greater Indian flying fox), *Eonycteris spelaea*, *Cynopterus*, *Scotophilus kuhlii* and *Hipposideros larvatus* are known to carry Nipah virus.

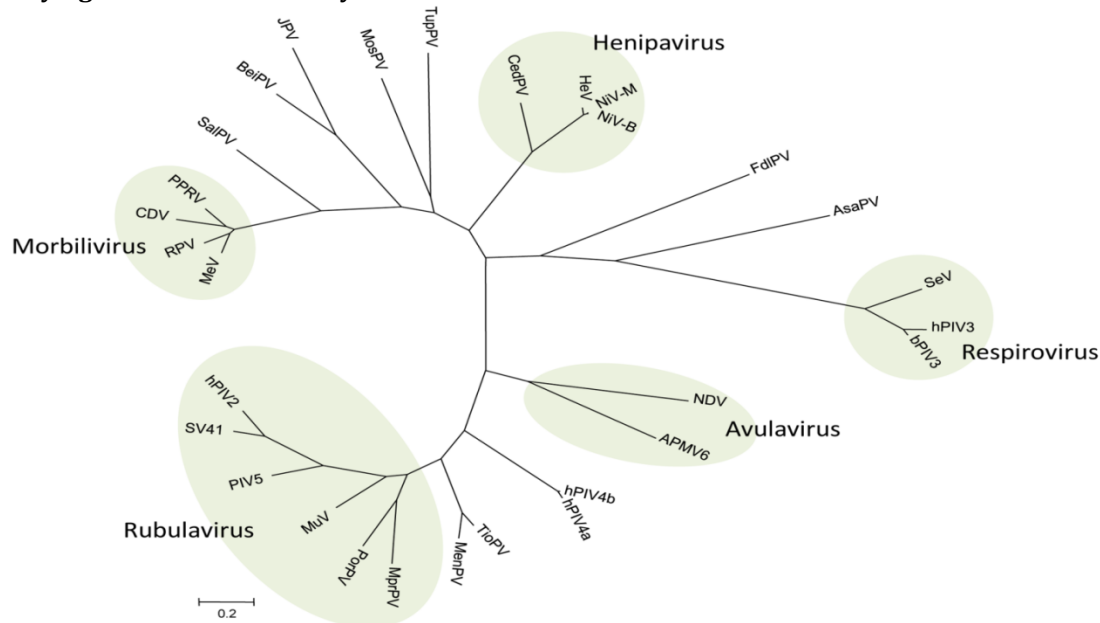
Pteropus giganteus

The Indian flying fox is India's largest bat and one of the largest bats in the world, weighing up to 1.6 kg and males are generally larger than females. The [wingspan](#) ranges from 1.2–1.5 m (appr. 5 ft) and body length averages 15.5–22.0 cm (6.1–8.7 in).

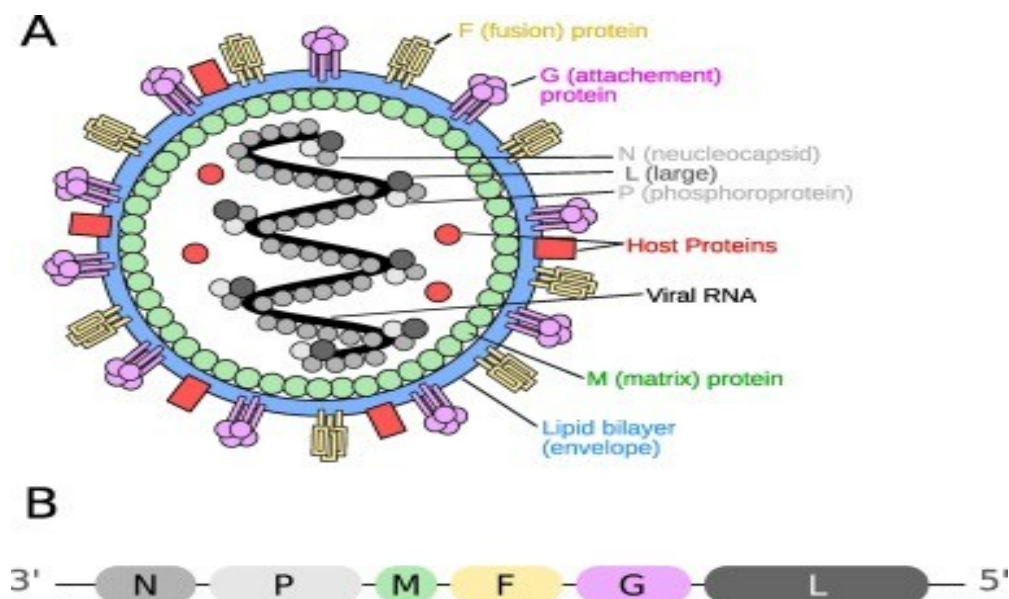


Transmission



Phylogenetic tree of Paramyxovirus:**Morphology of Virus**

NiV has an envelope with filamentous nucleocapsids [1], the genome consists of a single-stranded negative-sense RNA of approximately 18.2 kb. The genome encodes for six major structural proteins: nucleocapsid (N), phosphoprotein (P), ma-trix protein (M), fusion protein (F), glycoprotein (G), and large protein or RNA polymerase (L)



The NiV attachment glycoprotein G and fusion protein F are essential for virus binding and entry and as such are the primary targets for protective antibody responses. The 83% and 88% amino acid identity between HeV and NiV G and F, respectively, results in antigenic cross-reactivity between these viruses. Nipah Virus has two strains: 1. Malaysia (NiVM). 2. Bangladesh (NiV B)

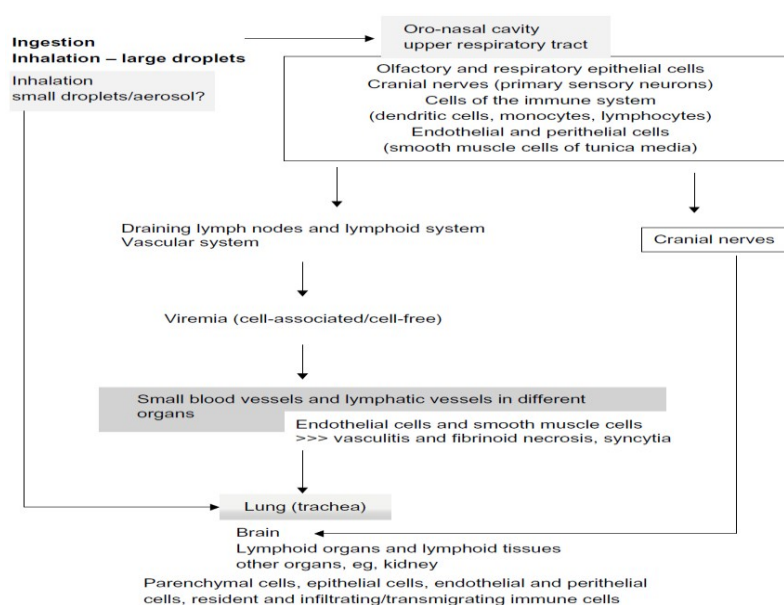
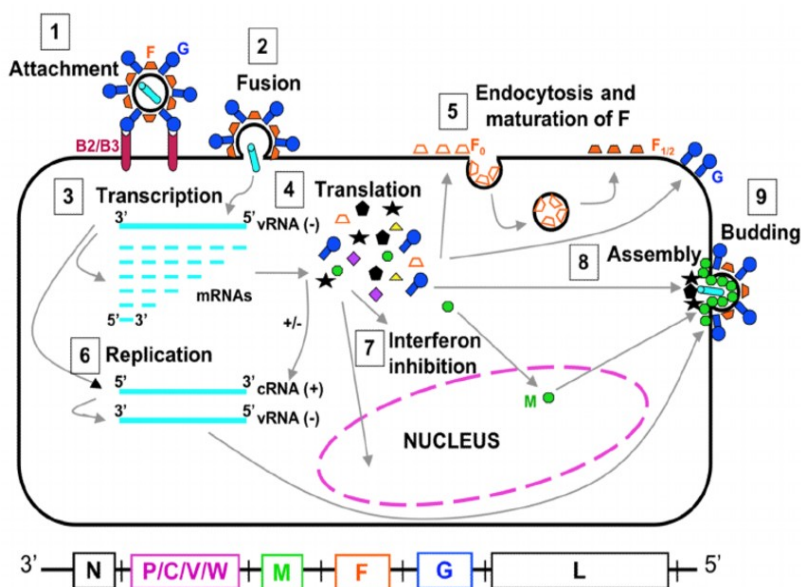
"Sequencing of the genetic make-up of the virus revealed that Indian Nipah virus genome is genetically similar to Bangladesh strain. In African green monkeys (AGM), NiV-B was uniformly lethal but only 50% of NiV-M-infected animals succumbed to infection. Histopathology of lungs and spleens from NiV-B-infected AGMs was significantly more severe than NiV-M-infected animals. These data showed that NiV-B is more pathogenic than NiV-M in AGMs.

Pathogenesis

NiV infection into human population occurs by

1. Spill-over from bats
2. Transmission via an intermediate animal host
3. Bat-to-human transmission
4. Human-human transmission

The viral G protein attaches to the host cell ephrin B2 and/or B3 receptor, and activates the F protein to initiate viral envelope and host membrane fusion and viral entry



Pathophysiology

Incubation period: from 4-14 days. Infected person may develop symptoms such as acute respiratory and neurological illness. virus believed to infect respiratory tract epithelial tissue resulting in shedding of epithelial lining along with nasopharyngeal secretion.

Patients develop symptomatic respiratory infection in early stage of infection.

During late stage, virus spread to lungs endothelium resulting in endothelial syncytium and mural necrosis. Nipah virus can then enter the bloodstream and disseminate throughout the host in either free form or by binding host leukocytes. Nipah virus has been shown to bind to CD3+ leukocytes without entry or replication of the virus. Other target organs of Nipah virus are the brain, spleen and kidneys.

Entry of Nipah virus into the CNS is thought to occur through two distinct pathways: 1. Anterogradely via the olfactory nerve and /or via the hematogenous route through the choroid plexus and cerebral blood vessels.

- Infection of the CNS in humans is characterized by vasculitis, thrombosis, parenchymal necrosis, and presence of viral inclusion bodies.
- Tissue gene expression studies with humans have also found that ephrinB3 is expressed in the pons region of the brain stem. These findings underscore the biological relevance of ephrinB3-mediated usage, as severe brain stem dysfunction is the major clinical defining feature of fatal Nipah viral encephalitis.
- In addition, histological data from fatal cases of NiV infection found abundant NiV antigen staining in the pons region of the brain stem
- ephrinB3-mediated entry may actually be the ultimate cause of death in acute Nipah viral encephalitis.

Clinical Features

- Typically patients present with a sudden onset, non-specific flu-like or febrile illness, sometimes with gastrointestinal symptoms.
- Fever and headache, Myalgia, Sorethroat, Encephalitis, dizziness, drowsiness, vomiting, Seizures progresses to coma in 24-48 hours. Respiratory difficulty and Relapsing neurologic symptoms can also occur. Complications like, Septicemia, GI bleeding, Renal impairment, Mortality has also varied between outbreaks but is high overall 40 to 75%.
- Neurological sequelae may occur in survivors, including relapsing encephalitis with delayed reactivation of latent virus infection.
- Residual neurological deficits

Laboratory Diagnosis

- ❖ Nipah is classified as a biosafety level 4 (BSL4) agent. Work be carried out only in a physical containment level 4 (PC4) facilities. Special precautions must be undertaken in the collection, submission and processing of samples.

Samples: Nasal secretion, blood, contaminated fruits or infected animals. ELISA, RT-PCR, Virus culture are mainstay in diagnosis.

Safety precautions

- (A) Wear full set of Personal Protective Equipment and identify the labeled sample tubes.
- (B) Seal the neck of the sample vials using parafilm to prevent leakage during transit.
- (C) Cover the sample vials using absorbent material to contain leakage, in the event of a breakage.



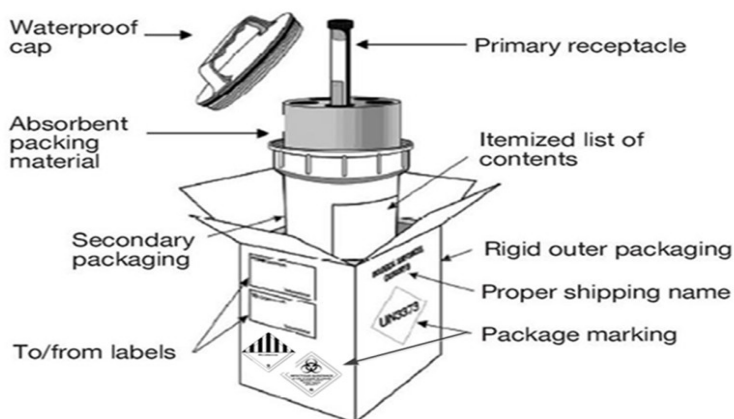
(A) Option 1: Using a cryo-box as a secondary container. (Seal the lid of the box after arranging the samples, using cello.)

(B) Option 2: Using a 50-mL centrifuge tube as a secondary container. (Seal the neck of the tube using cello.)

[Note: Sample vials can also be placed inside a zip-lock pouch, covered in absorbent material and secured by heat-sealing or rubber bands. Then, the zip-lock pouch should be placed inside another plastic pouch and secured.]

(C) Placing the centrifuge tube inside a zip-lock pouch

(D) Placing the zip-lock pouch inside a sturdy plastic container.



“BIOLOGICAL SUBSTANCE, CATEGORY B”

TESTS

Technique	Advantages	Disadvantages
ELISA (IgM)	May provide Nipah diagnosis if performed between 4 days and 3 months after exposure	
ELISA (IgG)	May provide Nipah diagnosis	Requires a second serum sample (convalescent serum)
Nucleic acid-based assays	Becoming the clinical diagnostic standard Performed rapidly	
Viral culture	Virus isolation important when a new case or outbreak occurs	Requires too much time to be of use in clinical setting Requires BSL4 precautions

ELISA

- ELISAs developed for serology testing (IgM or IgG) may use infected cell lysate antigens for coating the plates. However, their use is limited to BSL4 laboratories.
- NiV recombinant proteins, and the N protein in particular, can be used.
- Monoclonal antibody-based antigen capture ELISAs for virus detection and for differentiation between NiV and HeV.
- One study concluded in 92% assay sensitivity and specificity of a NiV-N protein-based IgM capture ELISA, but used as a reference method the ELISA developed at CDC.

RT PCR

- Advantage of not propagating live infectious virus. The one-step quantitative (qRT-PCR) assay targets the intergenic region between the F and G proteins which are only present in full-length virus genomes
- one-step assays to detect NiV are designed to target the viral N gene that is highly expressed during NiV replication. Targeting highly expressed genes can lead to an increase in assay sensitivity

NiV-M and NiV-B genome: Particle:PFU ratio

Virus	Genome	Particle	PFU
NiV-M	1.04×10^3	5.69	1
NiV-B	1.63×10^3	3.07	1

<https://doi.org/10.1371/journal.pone.0199534.t005>

Determination of the limit of detection of the NiV-M and NiV-B qRT-PCR.

Assay	Target Titer (PFU/mL)	Calculated Viral RNA copies/mL	Replicate Ct Values/RNA Extraction		
			1	2	3
NiV-M	10	1.04×10^4	37.5, 37.4, 37.2	37.2, 36.4, 37.4	36.9, 37.1, 37.2
	5.6	5.82×10^3	39.6, 39.2, 39.	39.8, 38.4, 38.3	39.2, 38.5, 38.5
	3.1	3.22×10^3	38.8, 39.2, 38.8	39.0, 39.9, 38.4	38.0, ND, 39.6
	1.7	1.77×10^3	ND, 39.6, ND	39.6, ND, ND	ND, ND, ND
	1.00	1.04×10^3	ND, ND, 36.9	ND, ND, ND	ND, ND, ND
NiV-B	10	1.63×10^4	38.6, 36.6, 37.5	36.9, 37.0, 36.8	36.7, 36.7, 38.1
	5.6	9.13×10^3	38.3, 38.5, 38.2	38.9, 39.9, ND	ND, 39.1, 39.0
	3.1	5.05×10^3	ND, 39.4, ND	39.1, 39.1, 39.1	39.3, 38.0, 39.6
	1.7	2.77×10^3	ND, ND, ND	ND, ND, ND	ND, ND, ND
	1.00	1.63×10^3	ND, ND, ND	ND, ND, ND	ND, ND, ND

Ct, cycle threshold; ND, not detected

<https://doi.org/10.1371/journal.pone.0199534.t006>

Determination of cross-reactivity of the NiV-M and NiV-B assays

	Test Sample	NiV-M Assay	NiV-B Assay
Virus Stocks	NiV-M	D	ND
	NiV-B	ND	D
	HeV	ND	ND
	MeV	ND	ND
	RSV	ND	ND
	EBOV	ND	ND
	LASV	ND	ND
Cell Lines	293T	ND	NT
	Vero E6	ND	ND
	MA104	ND	NT
	CHO	ND	NT
	NR596	ND	ND
	BSC1	ND	NT

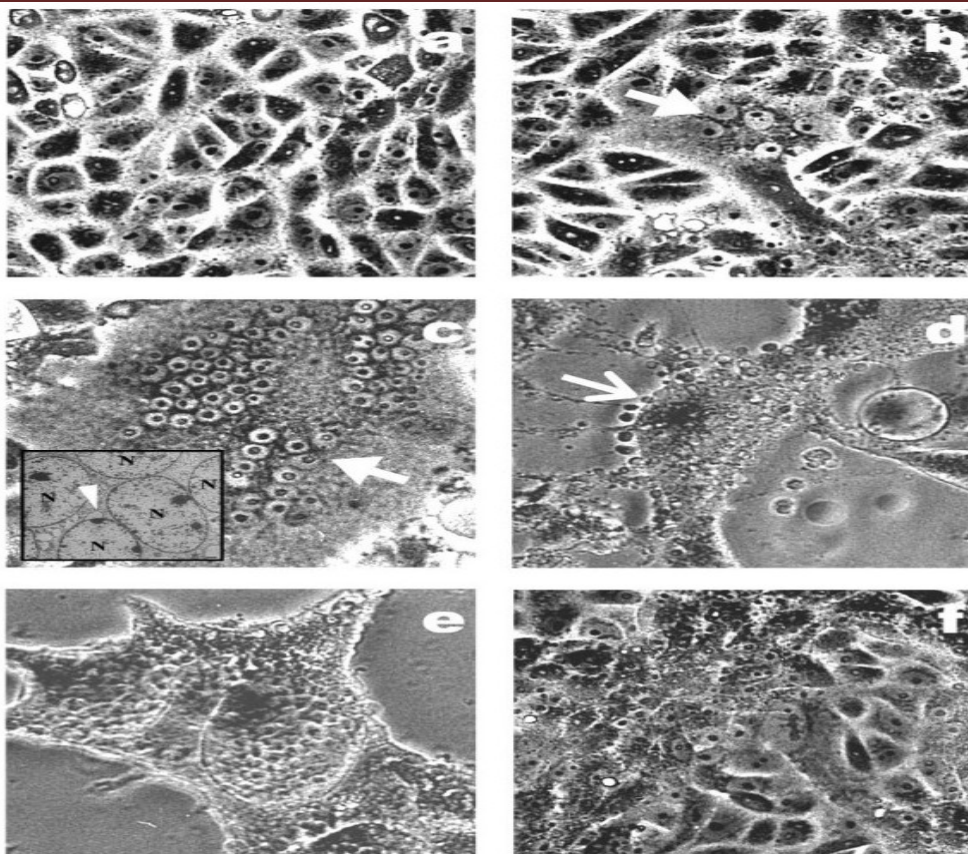
D, Detected; ND, not detected; NT, not tested

<https://doi.org/10.1371/journal.pone.0199534.t007>

Virus Isolation

Virus isolation : by cell culture. Brain, lung, kidney and spleen samples transported at 4°C in 48 hours or frozen if over 48 hours. African green monkey kidney (Vero), rabbit kidney (RK-13) cells are preferred cell lines.

Cytopathic effect (CPE) usually develops within 3 days. Monolayers are examined for the presence of syncytia after incubation for 24–48 hours at 37°C. Henipavirus-induced syncytia are characterised by presence of large multinucleated cells containing viral antigen



Changes in Vero cell morphology following Nipah virus infection. Cell fusion and syncytial formation were observed at eight hours PI (b, thick arrow). Multinucleated giant cells were noted to increase in frequency at 32 hours PI (c, thick arrow). Evidence of apoptosis with the presence of blebbing cell and apoptotic bodies was noted at 48 hours PI (d, thin arrow). At 64 hours PI onwards, cells started to detach from the surface of the tissue culture flask (e). The inset in (c) is an electron micrograph showing multinucleated cells (N) at 32 hours PI and the presence of nuclear invagination (thin arrowhead). The mock-infected Vero cells at 72 hours PI is shown in (f).

Treatment

Drugs: Ribavirin, Ribavirin and chloroquine, HR2-based fusion inhibitor (NiV-Fc2)

Biologicals: Monoclonal antibodies beneficial for post-exposure prophylaxis

m102.4 mAb-Neutralizing and cross-neutralizing activity against both NiV and HeV viruses

Prevention

Avoid close (unprotected) physical contact with infected people

- Wear NH95-grade and higher masks
- Wash hands regularly with soap
- Avoid consuming partly eaten fruits or unpasteurised fruit juices
- Avoid being around animal pens
- Boil freshly collected date palm juice before consuming
- Thoroughly wash and peel fruits before consuming
- Maintain your and children's personal hygiene

Hand hygiene, Gloves, Facial protection (eyes, nose, and mouth), Gown, Prevention of needle stick and injuries from other sharp instruments, Respiratory hygiene and cough etiquette, Environmental cleaning,

Hand hygiene Summary technique: Hand washing (40–60 sec): wet hands and apply soap; rub all surfaces; rinse hands and dry thoroughly with a single use towel; use towel to turn off faucet.

Hand rubbing (20–30 sec): apply enough product to cover all areas of the hands; rub hands until dry.

Summary indications: Before and after any direct patient contact and between patients, whether or not gloves are worn.

- Immediately after gloves are removed.
- Before handling an invasive device.
- After touching blood, body fluids, secretions, excretions, non-intact skin, and contaminated items, even if gloves are worn.
- During patient care, when moving from a contaminated to a clean body site of the patient.
- After contact with inanimate objects in the immediate vicinity of the patient.

Persons with respiratory symptoms should apply source control measures

Cover their nose and mouth when coughing/sneezing with tissue or mask, dispose of used tissues and masks, and perform hand hygiene after contact with respiratory secretions.

- Health-care facilities should: Place acute febrile respiratory symptomatic patients at least 1 metre (3 feet) away from others in common waiting areas, if possible.
- Post visual alerts at the entrance to health-care facilities instructing persons with respiratory symptoms to practice respiratory hygiene/cough etiquette.
- Consider making hand hygiene resources, tissues and masks available in common areas and areas used for the evaluation of patients with respiratory illnesses.

Vaccines

- All R&D activities for NiV vaccines are in the pre-clinical stage.
- These vaccines are based on the F and/or G glycoproteins, and essentially target the induction of NiV neutralizing antibodies

Advantages and disadvantages of the different vaccine platforms under evaluation for Nipah (human vaccination)

Platform	Advantages	Disadvantages
Vaccinia-based vectors	Promising data in animal models of protection MVA strain well known in humans	2-dose schedule Concerns linked to anti-vector immunity
VSV-vectored vaccines Vesicular Stomatitis Virus	Promising data in animal models of protection Efficacy in animals vaccinated 1 day prior to challenge Platform used successfully in the context of an Ebola outbreak Single dose schedule	Neurotropism issue identified with vector expressing a combination of F and G
Measles-vectored vaccine	Promising data in animal models of protection Vector consisting of the measles vaccine strain	2-dose schedule Suboptimal protection observed in AGM model Pre-existing immunity against the vector in human populations
VEE replicon Venezuelan Equine Encephalitis virus	Self-limiting infection Phase 1 clinical data available for VEE combined with HIV or prostate antigen	No protection data in NiV challenge model available 3-dose schedule
Subunit vaccine	Promising data in animal models of protection Expected safety	Need for an adjuvant, impacting perception of safety and potentially complicating clinical development Need for several injections less compatible with use in emergency setting

Bioterrorist Agent

The Center of Disease Control (CDC) has declared it a biosafety level 4 agent.

This the highest biosafety level category, home to agents which can be distributed via aerosol transmission and have no treatment or vaccine.

The CDC has also tagged the Nipah virus a Category C bioagent.

The third highest priority agent category in regards to biological warfare.

The availability, simplicity to produce and disperse, and high mortality rate of the Nipah virus make it possible for it to be used as a weapon of biological warfare.

Arthropod Borne Virus Disease (Arbovirus Disease) in Maharashtra with special reference to Dengue

Dr. Raju Jotkar

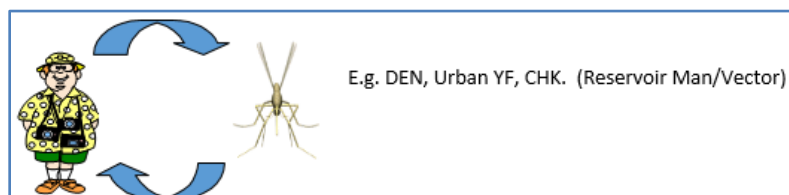
Asst. Director of Health Services,
Mumbai, India.

1. Arbovirus diseases-Overview

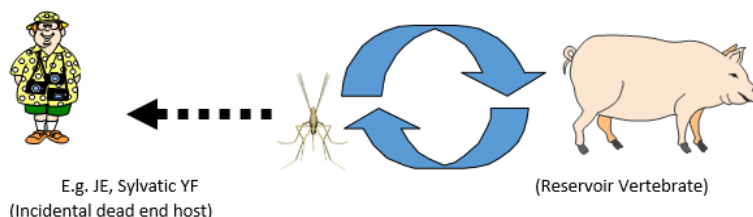
- Arbovirus -acronym ARthropod-BORne virus.
- Arboviruses can affect both animals (+humans), and birds
- In humans, symptoms of arbovirus infection generally occur 3–15 days after exposure to the virus and last 3 or 4 days.
- Substantial proportion of subclinical as well as asymptomatic human cases challenge the surveillance data capture
- Most common clinical features of arbovirus infection are
 - General: fever, headache, and malaise,
 - Encephalitis /meningitis if virus is neuro-invasive
 - Hemorrhagic fever.
- It is documented that 500 arboviruses exist of which
 - 100 infect Human being, of which
 - 40 are detected in India, of which
 - 10 cause human disease

1.1. Maintenance of infection cycle dynamics

1. Man – Vector – Man



2. Vertebrate/Ardeid Bird – Vector - Vertebrate/Ardeid Bird - Vector- Man



1.2. Dominant mode of spread of Arbovirus : Bite by infected vector, with occasional spread through

- Blood transfusion (if Viremia stage)
- Organ transplant (if Viremia stage)
- Sexual contact (if Viremia stage)
- Vertical transmission from mother to fetus/neonate (if Viremia stage)
- consuming fruit contaminated by infected fruit bats (Nipah)

Human to Human spread is documented only for Nipah.


1.3. Preliminary provisional diagnosis of arbovirus infection based on

- clinical presentations of symptoms,
- places and dates of travel, activities, and
- epidemiological history of the location where infection occurred

Definitive confirmatory [diagnosis](#) is typically made in a [laboratory](#) by

- **Serology** - usually used to make a diagnosis of arbovirus infections.
- **Culture** - a number of cell lines may be used, including mosquito cell lines. However, it is rarely carried out since many of the pathogens are group 3 or 4 pathogens.
- **Direct detection tests** - e.g detection of antigen and nucleic acids are available but again there are safety issues

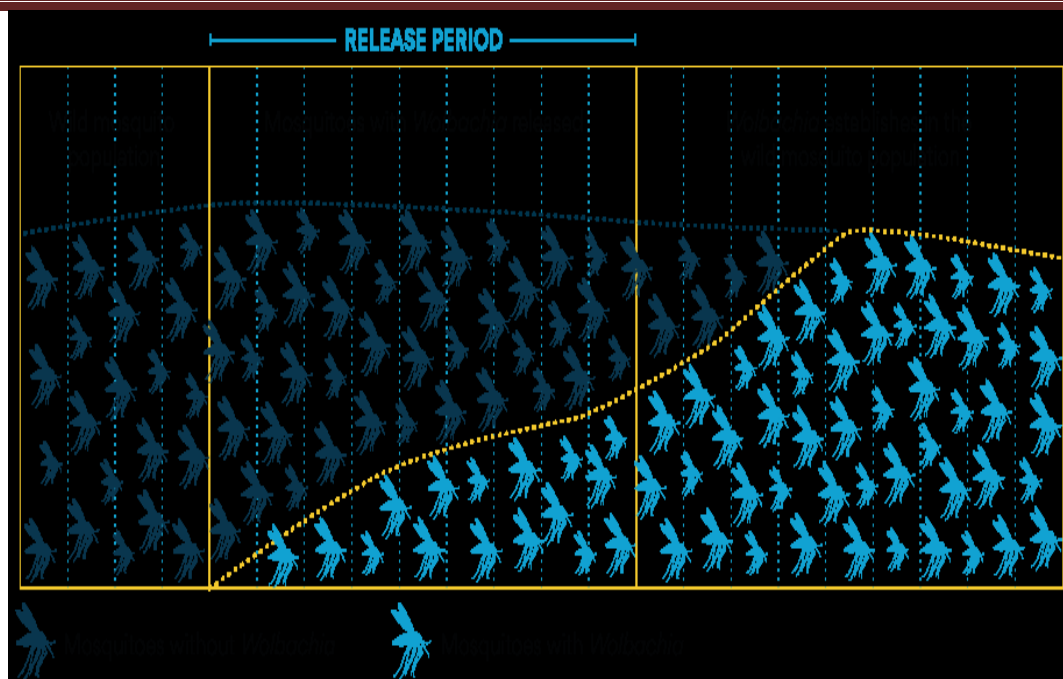
1.4. Arbovirus diseases



No	ARbovirus	Reservoir	Vector	Disease	Prevention and control
1	CHKV- Morbidity only in old age	Monkey	Mosquito (Aedes Aegypti) 	Chikungunya fever	Integrated Vector Management Antilarval measures- Abate, Dry day, cover artificial collection of water , Predator-prey systems (fish, insects, amphibians, copepods) -50 % reduction Antiadult: Source reduction, aduliciding e.g. fogging aerosol ULV, IRS, Personal protection: repellents, protective clothing, Insecticide treated nets, Housing improvement, Biological methods- Sterile male, Wolbachia Vaccine to YF (Mandatory as per international travel guidelines)
2	DENV 1,2,3,4 Sequence 1/2=500 fold DHF 3/2=150 Fold 4/2=50 Fold DHF	Monkeys, Man		DF, DHF, DSS Secondary infection elicit DHF/DSS	
3	ZIKA	Man		Zika fever, (Microcephaly, GBS)	
4	YF	Monkeys, Man		Yellow fever	


Source: Arboviral Infections in India (2015) by Mroor, Devi , Indian Journal of Health Science and Care Vol.2, No.3, Nov 2015,

WHO categorized CHK and DEN as “Neglected Tropical Diseases (NTD)” -in order to package the interventions.

The World Mosquito Program’s Wolbachia method Release of either “both sex mosquitoes” or “male only mosquitoes” with Wolbachia bacteria



No.	ARbovirus	Reservoir	Vector	Disease	Prevention and control
5	JE B-High mortality in age grp. 5-10	Wild birds, Pigs	Mosquito (Culex Vishnoi) 	Encephalitis	<ul style="list-style-type: none"> Mosquito control by Using a/c, installing window/door screens, aerial fogging with ULV in rice growing area Piggeries away from residential area personal protection: repellents, wearing long-sleeved shirts and long pants, and limiting outdoor exposure Vaccine-0,7,28 d (primary) or 2 doses 4 wks apart for 1-15 years
6	West Nile fever	Birds, Pigs, Horse		West Nile fever	
7	KFD	Forest birds, Animals	Tick 	Hemorrhagic fever	<ul style="list-style-type: none"> Control ticks: Aircraft mounted pesticide spray Personal protection: avoid sitting/lying on ground, DEET/DMP Vaccinate

					susceptible
8	Chandipura	Man	<p>Sandflies/Mosquito</p> 	Chandipura	<ul style="list-style-type: none"> • Antiadult: aerosol ULV • personal protection: repellents, wearing long-sleeved shirts and long pants, and limiting outdoor exposure
9	NIPAH	Fruit bat/Pig	- (Transmission to humans thro' ingestion of Contaminated fruit, unpasteurized date palm juice, direct contact with infected bats, infected pigs, or from other NiV infected people.)	Acute respiratory syndrome and fatal encephalitis	<ul style="list-style-type: none"> • avoiding exposure to sick pigs and bats in endemic areas and not drinking raw date palm or consuming fruits • reinforcing standard infection control practices to avoid human-to-human infections in hospital settings

Source: Arboviral Infections in India (2015) by Mroor, Devi , Indian Journal of Health Science and Care Vol.2, No.3, November 2015, pp-192-202, WHO, CDC (2018)

Arbovirus	Clinical signs	Severity	Treatment
CHK	Fever, myalgia, arthralgia, headache	Mild to fatal	Symptomatic, fluid therapy,
DENV 1, 2, 3, 4, Sequencing 1/2=500 fold DHF 3/2=150 Fold 4/2=50 Fold DHF	Fever biphasic, headache (retroorbital pain), joint pains, petechial hemorrhage, low BP,	Mild to fatal <i>Sequence of infecting virus and and longer gap between 1st and secondary infection may decide DHF/DSS</i>	Symptomatic, fluid therapy, (DF-US\$ 4.29, DHF-139 US\$) Platelets
J. E. B	Fever, incoordination, convulsions, death	Mild to fatal, recovery with neurodeficit	Symptomatic, antipyretic, anticonvulsants, Iv isotonic fluid/ mannitol,
West Nile	Fever, convulsion, death	Mild to fatal, recovery with amnesia	Symptomatic, fluid therapy,
Kyasanur Forest disease	Biphasic Fever, dehydration, encephalitis, epistaxis, diarrhora, death	Mild to fatal	Symptomatic, supportive for dehydration and hemorrhage
Chandipura encephalitis	Fever, convulsion, headache death	Mild to fatal	Symptomatic, fluid therapy,

1.5. Vaccines are available for the following arbovirus diseases:

- Japanese encephalitis B- Primary as well as High Risk
- KFD- High Risk
- Yellow fever- Mandatory for travelers to Africa and Latin America

Vaccines are in development for the following arbovirus diseases:

- Zika Virus
- Dengue fever
- Eastern Equine encephalitis
- West Nile

2. Dengue an outbreak prone mosquito borne viral disease.

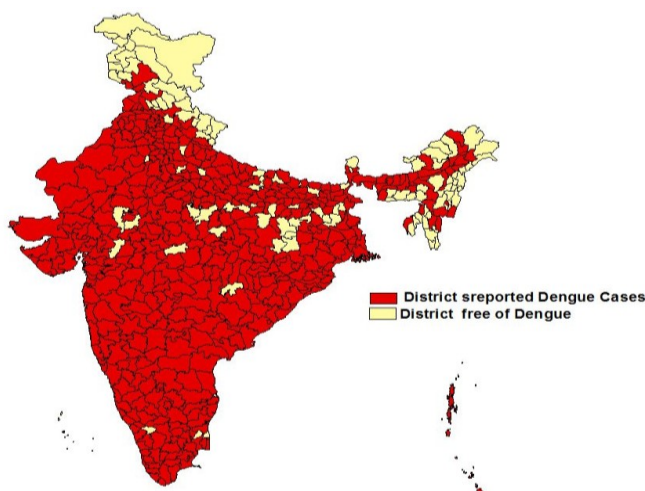
- Caused by four antigenically distinct but closely related viruses - serotypes (DENV 1 to 4) belonging to family *Flaviviridae* (Flavivirus).
- A 50-nm single strand RNA virus enveloped with a lipid membrane
- Nucleotide sequencing reveals existence of multiple genotypes for each serotype
- Serotypes = Genotypes /subtypes
 - DENV-1 = 3
 - DENV-2 = 6
 - DENV-3 = 4
 - DENV-4 = 5
- Over 2.5 billion people – over 40% of the world's population are at risk from dengue.
- Now approximately 128 countries are affected with dengue infection.
- About 52% of the population in WHO SEAR is estimated to be at risk with 10 out of the 11 Member States (with the exception of DPRK) being endemic.

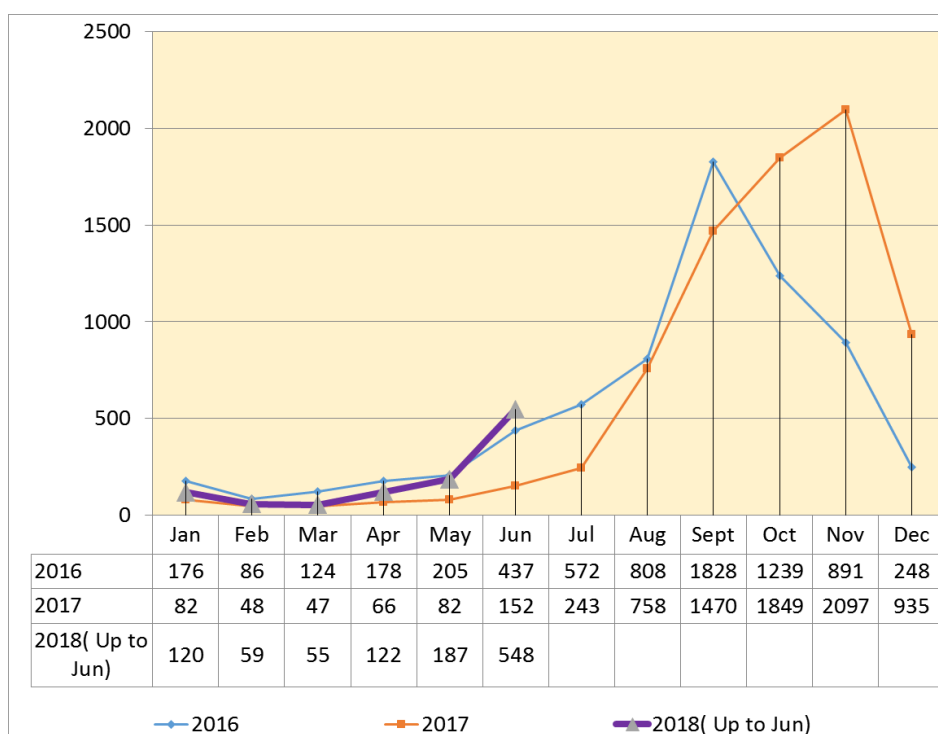
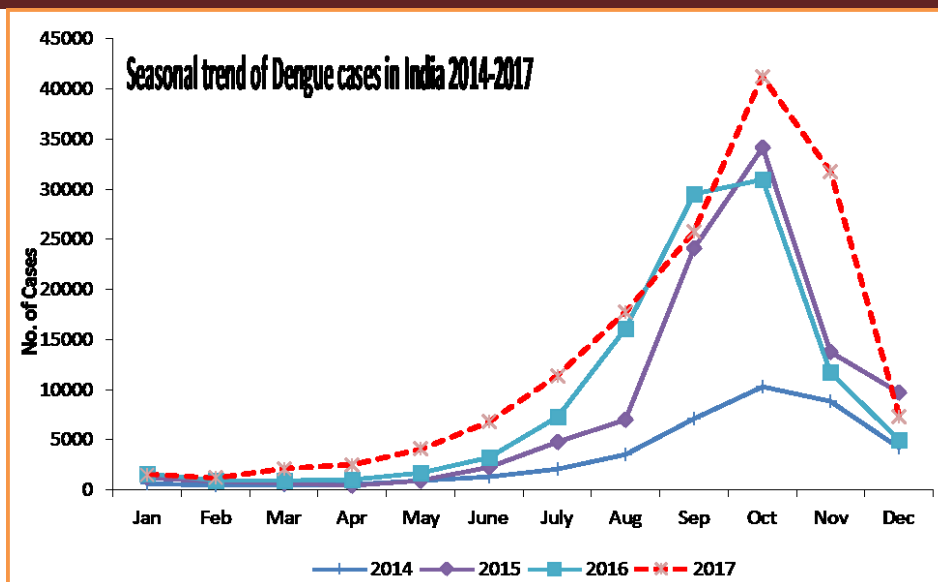


The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines or maps represent approximate border lines for which there may not yet be full agreement.

Data Source: World Health Organization Map
Production: Public Health Information and Geographic Information Systems (GIS) World Health Organization

 **World Health Organization**
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State – Month wise Dengue Trend

Underlining causes for rising spatial spread of Dengue

- Variable community owned sustained behavioral change in
 - reducing domestic and peridomestic artificial water collections
 - Observing weekly dry days
 - House screens, bed nets for infants, personal protection measures
 - Case reporting and seeking medical advise
- Inadequate Coping up mechanism by Civic bodies
 - Solid waste management
 - Erratic Water supply and deficient water management
 - Sub optimal Integrated vector control

4. Absence of inter-departmental convergence
 5. Inadequate entomological infrastructure as well as
3. Newer breeding places
 1. Demographic and societal changes- Rural people imitate urbanites
 2. Booming automobile industry & Increased pop movement
 3. Significant increase in plantations
 4. Climatic changes
 1. Perennial intermittent rain
 2. Temperature and humidity rise favouring reduction in extrinsic incubation period and vector biting frequency (dehydration)
 5. Inadequate protection of susceptible host population as well as affected
 1. No effective vaccine till date
 2. Serotypes 1 and 2 are widespread in India
 3. Attack rate in sporadic serosurveillance reveals 40 to 80 % infection in our community.
 4. Serotype specific immunity short-lived for cross-protection from other serotypes and hence the secondary infection with another serotypes elicit immunological catastrophe – DHF and / or DSS
 5. Herd immunity declines over time concomitant with effective vector control but lower vector density can still maintain the infection cycle. Caucasians' and Africans more susceptible for DHF
 6. Poor legislative measures

Surveillance is through

- 618 Sentinel Surveillance Hospitals with lab facility
- 16 Apex Referral Labs

Free diagnosis across the country

- ELISA based NS1 -1st five days of illness (Dengue)
- IgM Capture ELISA - after 5 days of illness (Den & CHK)

Two types of cases : Probable and Confirmed

Probable Dengue Fever

- A case compatible with clinical description.
- *A positive test by RDT will be considered as probable due to poor sensitivity and specificity of currently available RDTs.*

Confirmed Dengue Fever

A case compatible with the clinical description of Dengue Fever with at least one of the following :

- Demonstration of IgM antibody titre by ELISA positive in single serum sample.
- Demonstration of Dengue virus antigen in serum sample by NS1ELISA.

Isolation of the Dengue virus, Detection of virus by PCR

Updates on Chikungunya virus – Diagnosis, molecular epidemiology & treatment

Deepti Parashar

ICMR - National Institute of Virology,
Pune, India

Chikungunya virus (CHIKV) is a mosquito-transmitted alphavirus that causes chikungunya fever. As a highly contagious re-emerging virus it has infected millions of people in the recent past mainly in developing countries. The virus is transmitted to humans by several species of mosquitoes, with *Aedes aegypti* and *A. albopictus* being the two main vectors in urban settings. The associated disease is characterized by rash, high fever, and severe arthritis that can persist for years. Despite the high morbidity rate, no approved antiviral drug is available.

The CHIKV has a positive-stranded RNA genome approximately 11.8 kb in size, coding for structural (capsid and envelope) and non-structural proteins (nsP1-nsP4). The viral envelope proteins play a major role by binding and fusion with the infected cell surfaces. The non-structural proteins along with performing a variety of intracellular functions are known to be the primary mediators of viral replication. Several diagnostic tests have been developed so far to detect CHIKV infections in the acute or later stage of the disease. Although nucleic acid amplification or antigen detection can only be used during the viremic phase of the disease, serological tests are necessary to identify previous infections or to determine the immune status of a patient. CHIKV associated febrile disease has prevailed in tropical countries for the past half century and more recently it has been found to be emerging into temperate areas such as Europe and the Americas. Since the resurgence of CHIKV in India in 2005, waves of epidemic outbreaks have followed in subsequent years in diverse parts of India. Phylogenetic analysis classifies all the CHIKV strains into the three distinct genotypes with the resurged epidemic isolates from 2005 onwards falling in the Indian Ocean Lineage (IOL) of the East, Central and South African (ECSA) genotype. The phylogenetic investigations from different parts of India have contributed to understanding of the molecular evolution and epidemiology of CHIKV. To counteract this viral pathogen, a number of molecules have been tested to inhibit CHIKV replication, either as direct-acting antivirals, host-targeting drugs or those that act via a yet unknown mechanism.

Despite the numerous diagnostic assays already described, continuous efforts are being made to expand and improve CHIKV diagnostics. Regular surveillance for the Chikungunya viral infection should be carried out in endemic regions like India. The data generated from the surveillance will assist in formulation of control measures to prevent the outbreaks in endemic regions. Further, though a number of studies are available which focus on drug candidates that are effective at multiple stages of the CHIKV life cycle; these prospective candidate antiviral compounds require validation in preclinical and clinical models. The presentation will elaborate on CHIKV in the light of the above three perspectives.

Molecular Epidemiology of Dengue Viruses Circulating in India

Alagarasu Kalichamy

Dengue/Chikungunya Group,
ICMR-National Institute of Virology,
Pune, India

Dengue caused by the four serotypes of dengue viruses (DENV) have been a persistent problem in tropical and sub tropical countries. DENV belongs to the family of *Flaviviridae*, genus *Flavivirus* and is a positive single stranded RNA virus. The four serotypes of DENV are distinct but antigenically related with 30-35% amino acid diversity between the serotypes. There are two types of DENV transmission cycles. Sylvatic cycle occurs in forest habitats between non human primates and canopy dwelling mosquitoes belonging to the *Aedes* species. The endemic/epidemic transmission cycle occurs in periurban and urban settings where humans are the reservoir and amplification hosts and the vector involved is *Aedes aegyptii*, a peridomestic mosquito with African origin. *Aedes albopictus* also act as secondary vector. Four independent spill over events from sylvatic cycle facilitated by vector switching mediated through humans from canopy dwelling *Aedes* mosquitoes to *Aedes albopictus* led to the emergence of different serotypes of DENV involving series of divergence events after establishment of optimal population level to support human transmission cycles [Vasilakis and Weaver, 2008].

Though dengue like illnesses has been described since 6th century, the ecological and demographic changes brought out by World War II led to a major shift in DENV epidemiology. The geographical distribution of *Aedes aegyptii* expanded due to the movement of troops to long distances and the uncontrolled urbanizations enhanced the density of mosquito and this led to series of epidemics during 1941-1945. In India, the first record of dengue like illness was reported in 1780 at Chennai, Tamil Nadu. The first virologically confirmed outbreak of dengue fever (DF) was reported from Calcutta during 1963-1964. This was followed by series of DF outbreaks throughout India. Since 1988, dengue hemorrhagic fever (DHF) cases are being reported from India. The major outbreak of DHF was reported in Delhi during 1996 which was then reported from all over India [Gupta et al., 2012].

Dengue epidemics/outbreaks are often characterized by changes in the predominant DENV serotypes/genotypes that are circulating. Currently, though all the four serotypes are circulating in India, each outbreak was dominated by a particular serotype of DENV which was being replaced by the other serotypes in the subsequent years. Our studies in Pune revealed the dominance of DENV-1 in 2005 and 2007, followed by DENV-2 in 2008 and DENV-3 in 2009. DENV-4 was poorly represented with just one case each in 2009 and 2010 [Cecilia. 2014]. DENV-2 dominated 2009-2016 while DENV-3 was co-dominant with DENV-2 from 2009 to 2013. DENV-4 co-dominated with DENV-2 in 2014. DENV-1 and DENV-3 emerged as dominant serotype in 2017 [Unpublished Data].

Each serotype is again divided into multiple genotypes and viruses within a genotype will not have nucleotide divergence greater than 6% in the genome. For all four serotypes, the viruses circulating in India in the 1950s were either replaced with a different genotype or evolved into new lineages. Genotype shifts for DENV-2 (American to Cosmopolitan) and DENV-4 (genotype V to I) have been reported. Lineage changes for DENV-1 and DENV-3 were observed [Cecilia. 2014]. The genetic changes detected in the viruses correlated with changes in the disease profile with detection of DHF outbreaks in India. Our study in Tamil Nadu and Kerala during 2012-2015 revealed the detection of Asian genotype of DENV-1, which has been reported to be associated with large outbreaks in different Asian countries. Detecting the changes in the predominant serotypes/genotypes during early phase of dengue season through molecular surveillance of vectors and humans might help in predicting dengue outbreaks. The presentation will elaborate on these aspects.

Overview of Arboviruses & Nipah Virus

Dr.V.R.Wagh

Professor,
Department of Medicine,
D.Y.Patil Medical College,
Kolhapur, India.

Definition

Arthropod-borne viruses (Arboviruses) are viruses that can be transmitted to man by arthropod vectors. Over 130 Arboviruses known to cause disease in humans Virus families: Togaviridae, Flaviviridae. Bunyaviridae, Rhabdoviridae

Classification of Arboviruses prevalent in India

Family	Genus	Virus properties	Arthropod	Arboviruses in India
Togaviridae	Alphavirus	Spherical, 70 nm in diameter- Genome: positive-sense, ssRNA, enveloped. All viruses serologically related	Mosquitoes	Chikungunya, Sindbis viruses
Flaviviridae	Flavivirus	Spherical, 40-60 nm in diameter Genome: positive-sense, ssRNA, enveloped. All viruses serologically related	Mosquitoes, ticks	Dengue, Japanese encephalitis, Kyasanur Forest disease, West Nile fever viruses
Bunyaviridae	Phlebovirus Nairovirus Ortho-bunya virus	Spherical, 80-120 nm in diameter Genome: triple segmented, negative-sense or ambisense, ssRNA, Enveloped	Sandflies, Mosquitoes, ticks	Sandfly fever virus Ganjam, Crimean Congo hemorrhagic viruses Chittoor virus
Rhabdoviridae	Vesiculovirus	Rod- or bullet shaped, 75 X 180 nm Genome: negative-sense, ssRNA, enveloped with protruding spikes	Sandflies	Chandipura virus

Examples of Arthropod Vectors:



Aedes aegypti



Ixodid Ticks



Phlebotomine sand-fly

Mosquitoes

Japanese encephalitis, dengue, Yellow fever, Rift valley fever St. Louis encépalites, EEE, WEE, VEE

Sandflies: Sicilian sandfly fever

Ticks: Crimean-Congo haemorrhagic fever, Kyasnur forest disease and various tick-borne encephalitis etc.

Animal Reservoirs; In many cases, the actual reservoir is not known. Following animals are implicated as reservoirs. Birds, Pigs, Monkeys, Rodents

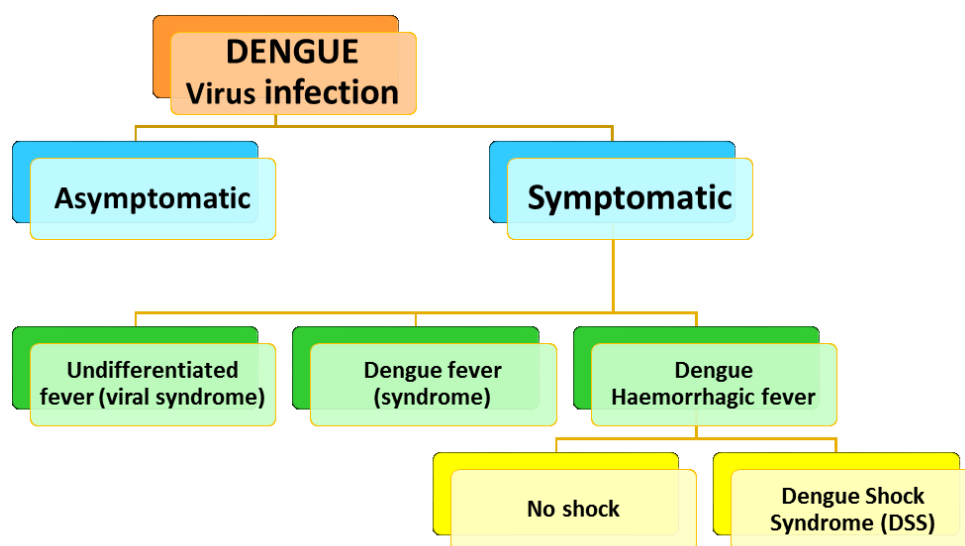
Arboviruses Transmission: Transmission intensity coincides with activity of vector.

Late spring through early fall (for mosquitoes). Incubation period: usually 3 to 18 days

Humans are dead-end hosts (i.e. do not become viremic). There are exceptions Blood transfusions, Organ transplants, and perinatal exposure. Certain viruses (e.g., Chikungunya virus, dengue, etc.)

Arboviruses clinical syndromes: Diseases produced by the arboviruses may be divided into 3 clinical syndromes: Fever with rash, Encephalitis, Hemorrhagic fever. These categories are somewhat arbitrary, and some arboviruses may be associated with more than one syndrome, eg, dengue.

Dengue



Dengue Haemorrhagic fever

Dengue clinical manifestations: Acute febrile illness with headache, retro-orbital pain, myalgia, arthralgia. “Break--bone fever”, High fever 5--7 days, Second fever for 1--2 days in 5% patients (Camel back), Followed by marked fatigue days to weeks. Classic dengue 15--60% of infections. Nausea, vomiting, diarrhoea (30%). Macular or maculopapular rash (50%). Respiratory symptoms: cough, sore throat (30%)

Dengue hemorrhagic fever: Fever 2-7 days. Usually occurs in secondary infections Thrombocytopenia (platelet count <100,000). Plasma leakage may progress to dengue shock syndrome. Mortality is 10-20% if untreated

Chikungunya: High grade fever (40°C or 104°F), Flu-like symptoms, Severe headache and chills Arthralgia or arthritis – lasting several weeks. Conjunctival suffusion and mild photophobia. Nausea, vomiting, abd. Pain, severe weakness

Chronic stage of Chikungunya: Distal poly or monoarthritis mildly improved with NSAIDs. Frequent tenosynovitis in the hands, wrists, or ankles, (carpal or tarsal tunnel syndromes) Highly sensitive to short-term systemic corticotherapy Exacerbation of pain in previously injured joints and bones requiring painkillers.

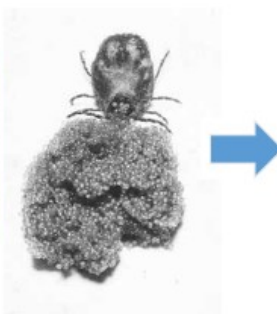
Japanese encephalitis: High Incubation Period - 5 to 15 days only 1 in 300 to 1 in 1000 infections develop into encephalitis, rest asymptomatic Course of disease- 3 stages:

a) Prodromal stage: Fever, headache and malaise. Duration- 1 to 6 days. b) Acute encephalitic stage: Fever, 38 to 40.7°C, nuchal rigidity, focal CNS signs, convulsion & altered sensorium progressing in many cases to coma. c) Late stage and sequelae: Temperature & ESR touch normal level, neurological signs become stationary. Fatality Rate (CFR): Varies between 20-40% but it may reach 58% & over, higher in children 30-50% that survive infection develop paralysis, brain damage, or other serious permanent sequelae. Parkinsonism, Seizures, Paralysis, Mental retardation, Psychiatric disorders

Kyasanur Forest Disease: Acute phase with sudden onset of fever, headache, severe myalgia with prostration lasting for 2 weeks. GI disturbances and hemorrhagic manifestations in severe cases Second phase characterized by mild eningoencephalitis after an afebrile period of 7-21 days. Case fatality varies between 4-16%



Female Ixodid tick laying eggs

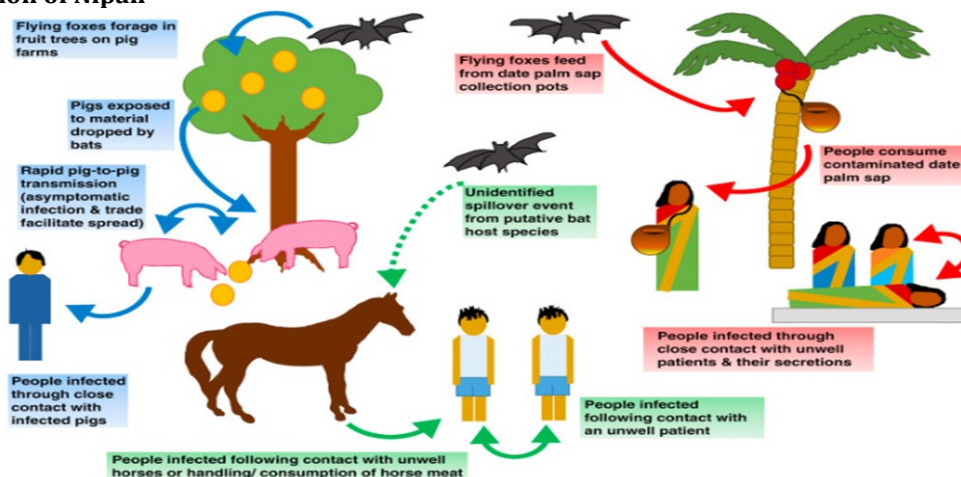


- **Crimean Congo Haemorrhagic Fever**: Incubation period – 3-7 days Haemorrhagic Fever - 3-6 days following clinical signs Fever, fatigue, dizziness, myalgia's, and prostration Signs of bleeding range from only Conjunctival haemorrhage, mild hypotension, flushing, and petechiae to shock and generalized mucous membrane haemorrhage and evidence of pulmonary, hematopoietic, and neurologic dysfunction
- **MODS in CCH Fever**: Renal insufficiency due to shock or part of disease 'Fever and Renal Syndrome' The liver becomes swollen and painful. Disseminated intravascular coagulation may occur. Acute kidney injury due to shock sometimes acute respiratory distress syndrome. Mortality rate: 10-80% Recovery usually complete but slow. May take up to one year

What is Nipah virus (NiV): Nipah virus (NiV) is a member of the family Paramyxoviridae, genus Henipavirus. NiV was initially isolated and identified in 1999 during an outbreak of encephalitis and respiratory illness among pig farmers and people with close contact with pigs in Malaysia and Singapore.

Origin of Name: Its name originated from Sungai Nipah, a village in the Malaysian Peninsula where pig farmers became ill with encephalitis.

Transmission of Nipah



NIPHA VIRUS: Given the relatedness of NiV to Hendra virus, bat species were quickly singled out for investigation. Flying foxes of the genus *Pteropus* were subsequently identified as the reservoir for NiV.

Out Break of NIPAH Virus Infection in 1999: In the 1999 outbreak, Nipah virus caused a relatively mild disease in pigs, but nearly 300 human cases with over 100 deaths were reported. In order to stop the outbreak, more than a million pigs were euthanized, causing tremendous trade loss for Malaysia. Since this outbreak, no subsequent cases (in neither swine nor human) have been reported in either Malaysia or Singapore.

Outbreak in Bangladesh and Siliguri: In 2001, NiV was again identified as the causative agent in an outbreak of human disease occurring in Bangladesh. Genetic sequencing confirmed this virus as Nipah, but a strain different from the one identified in 1999. In the same year, another outbreak was identified retrospectively in Siliguri.

Theories of Spread of NIPAH Virus: Deforestation. Bats shifted close to humans. Pigs exposed to bat excreta. Human infection. The reservoir fruit bats live in these caves and feed on the fruit trees that are in close proximity to the hog confinement barns. Deforestation - These are several of the hog confinement barns that were affected during the Malaysia Nipah virus outbreak. Transmission of Nipah virus to humans may occur after direct contact with infected bats, infected pigs or NiV infected people.

Spread by Nosocomial route: Unlike the Malaysian NiV outbreak, outbreaks occur almost annually in Bangladesh and have been reported several times in India. Person-to-person transmission reported in hospital settings from India (nosocomial transmission).

Transmission of NIPAH: Transmission also occurs from direct exposure to infected bats. A common example is consumption of raw date palm sap contaminated with infectious bat excretions.

Signs and Symptoms: Infection with Nipah virus is associated with encephalitis. After incubation period of 5 to 14 days, illness presents with 3-14 days of fever and headache, followed by drowsiness, disorientation and mental confusion. These signs and symptoms can progress to coma within 24-48 hours. Some patients have a respiratory illness during the early part of their infections. Half of the patients showing severe neurological signs showed also pulmonary signs.

Human Infections with NIPAH: Typically the human infection presents as an encephalitic syndrome marked by fever, headache, drowsiness, disorientation, mental confusion, coma, and potentially death. During the outbreak in Malaysia, up to 50% of clinically apparent human cases died.

LONG TERM SEQUELAE: Septicaemia (24%) GI bleeding (5%) Renal impairment (4%) Persistent convulsions and personality changes. Latent infections with late reactivation & death.

DIFFERENTIAL DIAGNOSIS:

- I. Classical swine fever,
- II. Porcine Reproductive & Respiratory Syndrome (PRRS),
- III. Pseudorabies,
- IV. Swine enzootic pneumonia,
- V. Porcine pleuropneumonia

Diagnosis with Real Time PCR: Virus isolation attempts and real time polymerase chain reaction (RT-PCR) should be performed from Throat and nasal swabs, Cerebrospinal fluid, Urine, and Blood.

Yet No Specific treatment for NIPAH: There is no specific treatment for Nipah Virus.

Supportive care is the general treatment.

Undertrials with Antiviral Drug: Ribavirin: The drug ribavirin has been shown to be effective against the viruses in vitro But human investigations to date have been inconclusive and the clinical usefulness of ribavirin remains uncertain

Preventing Nipah virus infection: Nipah virus infection can be prevented by avoiding exposure to sick pigs and bats in endemic areas and not drinking raw date palm sap.

Subunit vaccine on trail: A subunit vaccine, using the Hendra G protein, produces cross-protective antibodies against HENV and NIPV has been recently used in Australia to protect horses against Hendra virus. This vaccine offers great potential for henipavirus protection in humans as well

Prevent or control NIPAH: Mass culling of infected and in-contact pigs Avoid contact with the excretions or secretions of infected pigs. Burial sites are disinfected with chlorinated lime. Avoid drinking raw palm sap contaminated with bat excrement, or climbing trees coated in bat excrement.

Personal protective equipment: PPE when contamination or splashing with blood or body fluid is anticipated Disposable gloves, Plastic aprons, Face masks ,Safety glasses, goggles, Head protection, Foot protection, Fluid repellent gowns



Radiographic study of anatomical basis of Coracoacromial arch impingement due to varied morphology of acromion process

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ABSTRACT: The purpose of this study to know the different shapes of acromion process according to Beglani and Edelson and Traitz on radiographs. For this study 260 radiographs were taken - 200 radiographs of patients having shoulder pain due to impingement and 60 Radiograph of normal patients without shoulder pain. In this study according to Beglani, there were 33 radiographs of Type I, 137 radiographs of type II and 30 radiographs of Type III, Type II radiographs being more in number than Type I and Type III in case of symptomatic patients. In case of normal patients, there were 7 radiographs of Type I, 52 radiographs of type II and 1 radiographs of Type III, Type II radiographs are more in number than Type I and Type III and anatomically Type III acromion process is responsible for impingement. According to Edelson and Traitz classification, 56 radiographs of Cobra shape, 109 radiographs of Intermediate shape and 35 radiographs of Square tip were found in case of shoulder pain patients and in case of normal patients, 4 radiographs of Cobra shape, 47 radiographs of Intermediate shape and 9 radiographs of Square tip were found. In majority of cases Cobra shape acromion is responsible for impingement, also Cobra shape acromion gets rapidly converted into Type III acromion than Square tip and intermediate Shape acromion. Also, we see the Coracoacromial arch distance is very less in Type III acromion than other Type of acromion. According to Beglani, P value is 0.000. According to Edelson and Traitz also P value for coracoacromial arch is 0.001 which is highly significant. In normal radiographs of patients without shoulder pain p value is not significant.

Iridocorneal Endothelial Syndrome with secondary glaucoma

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Introduction: Iridocorneal endothelial syndrome comprises of three clinical variants: Chandler syndrome, progressive iris atrophy and Cogan Reese syndrome/ iris nevus syndrome. ICE syndrome is usually unilateral, non familiar, commonly seen in middle aged females (M: F - 1:2 to 1:5).^[1]

Pathogenesis – ICE syndrome comprises of an abnormal clone of endothelial cells which develop epithelium like characteristics.^[2]

Case details: A 47 year old female presented with blurring of vision in right eye since six months. This was associated with watering, photophobia, intermittent redness and eye pain.

Ocular examination: **1)Visual acuity:** Right eye – finger counting close to face, Left eye – 6/12 with pinhole improving to 6/6
2)Slit lamp examination: Right eye – lids – normal, Conjunctiva- hyperemia. Cornea- diffuse corneal edema, pupil- pseudopolycoria, Iris- peripheral anterior synechiae and iris atrophy patches. Left eye -Anterior segment –WNL. **3)Intraocular tension:** Right eye – 30mm Hg; Left eye – 20mmHg
4)Ophthalmoscopy: Right eye - Total glaucomatous disc; C:D – 0.8; nasalization of vessels.
5)Gonioscopy: Angles appear closed.

Treatment: Timolol maleate eye drop twice a day in right eye (following standard protocol for glaucoma treatment)

Conclusion: This case is complicated by glaucoma and has been managed so as to prevent permanent vision loss due to optic nerve involvement.

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Isolation and Antibiotic Susceptibility Pattern of Coagulase Positive *Staphylococcus Aureus* from Various Clinical Specimens at Tertiary Care Hospital,Jaipur

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ABSTRACT: Background: The present study was conducted in SMS MEDICAL COLLEGE AND ATTACHED GROUP OF HOSPITALS in year 2018 (1st april to 30st june). The various clinical samples were collected from Plastic surgery, ENT, Medicine, Trauma ,Generalsurgery, Gasroenterology, Wards and ICUs to isolate COPS.

Objectives: The present study was undertaken to asses the antibiotic susceptibility patterns of COPS species. Due to significant changes of microbial genetic ecology, as a result of indiscriminate use of antimicrobials, the spread of resistance is now a global problem.

Methods: Out of total collected samples, 100 isolates were reported as COPS. Antimicrobial sensitivity testing was done using disc diffusion (Kirby – bauer method) method with antibiotics ciprofloxacin,clindamycin, Gentamicin, cefoxitin ans doxyciline, and linezolid.

Vancomycin sensitivity was done by Vancomycin screen agar.

Result: Out of 100 isolates, maximum were sensitive to linezolid(93), doxyciline(83), gentamycin(78), and clindamycin(56). Maximum resistance was seen in ciprofloxacin(74) and cefoxitin(58)

Sensitivity of vancomycin was tested by Vancomycin screen agar(6µg/ml), all of the 100 were sensitive.

Conclusion: The increased prevalence and dissemination of multidrug resistant COPS worldwide has resulted in a major decrease in tharepeutic options because the majority are now resistant to most antibiotics. Newer antibiotics such as vancomycin and Linezolid have good in therapeutic role. Altghough their clinical use may be limited due to adverse effects.

Variation In Branching Pattern of Coeliac Trunk and Its Clinical Significance

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Tutor

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ABSTRACT:

Introduction:

In the current case, the coeliac trunk arising from abdominal aorta at the level of the T12 vertebra and runs horizontally to the right margin of lesser omentum and divided into 3 branches; common hepatic, splenic and accessory left hepatic artery. The common hepatic artery bifurcated into proper hepatic and gastroduodenal. Hepatic artery proper divided into right and left hepatic arteries. The left accessory hepatic artery is large, and it is giving origin to the left gastric artery then entered left lobe of the liver.

Materials and Methods:

The formalin-fixed cadavers dissection was performed according to standard techniques: Cunningham's Manual of Practical Anatomy method. According to the Panagouli classification Coeliac trunk patterns were measured and documented.

Result: *The hepato-splenic trunk was 4cm long and it divided into splenic and common hepatic arteries. Accessory left hepatic artery arising from left gastric artery.*

Conclusion: *Presence of arterial variations may result in misinterpretation of angiograms. Thus, knowledge of such variations is important for proper pre-operative diagnosis and planning of surgical and radiological procedures and for interventional radiologists performing arteriography.*

Keywords: *coeliac trunk, common hepatic artery, splenic artery, accessory hepatic artery, left gastric artery.*

Intra Abdominal Hypertension After Emergency Laparotomy

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ABSTRACT:

Here we have studied the outcome of Intra Abdominal Hypertension in 100 number of patients who underwent emergency laparotomy in Dr. D. Y. Patil hospital, Kolhapur from November 2016 to November 2017. The effect of Intra Abdominal Hypertension on fascial wound dehiscence and gradual development of Abdominal Compartment Syndrome.

AIM

To evaluate the effect of Intra Abdominal Hypertension in patients undergoing Emergency Laparotomy.

OBJECTIVES

- *To identify cases of Abdominal Compartment Syndrome.*
- *To evaluate association of Intra Abdominal Hypertension with Fascial wound dehiscence.*

MATERIAL & METHODOLOGY

This is a Prospective Observational study conducted among 100 number of patients who underwent Emergency laparotomy at Dr. D. Y. Patil Hospital, Kolhapur from Nov 2016 to Nov 2017.

RESULTS

100 patients who underwent emergency laparotomy and meeting the inclusion criteria were included in this study. 49 patients were diagnosed with high grade intra abdominal pressure pre-operatively, out of which 18 patients suffered fascial wound dehiscence, 2 burst abdomen, 1 laparostomy and 1 death.

CONCLUSION

It is observed that Intra Abdominal Hypertension has significant association with fascial wound dehiscence.

Rule Of 500

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ABSTRACT: Here we studied the incidence rate of RTA from December 2016 to December 2017 due to influence of alcohol in Kolhapur, d.y patil hospital & research centre. With India reporting as many as 1.34 lakh fatalities in road accidents every year, a vast 70 per cent of them being due to drunken driving, questions are now being raised on whether the mushrooming growth of liquor vends along the highways is responsible for costing precious lives in an untimely manner The Bench said its December 15, 2016, order banning liquor vends within 500 metres of highways shall remain operative for areas other than those specified in Friday's clarification order.

Introduction

Noting that deaths and injuries due to road mishaps are a national problem that must be addressed immediately, he said the damage caused by them is enormous in terms of lives and injuries as also the national cost involved in treating over 5 lakh people who get injured every year.

Aim and Objective

Aim-To evaluate the effect of RULE OF 500 implicated by the government of India on RTA patient admitting in DYPH Kolhapur.

Objectives-To measure the outcome in respective to its,

1. Impact on patient health in terms of ,a)Permeant disability, b)grievous hurt, c)operative procedure, d)death
2. Impact on patient family
3. Hospital expenses

Material and Methods

This study is retrospective which is carried out from December 2016 to December2017. This is carried out for 1 year in two different phases with respective to rule of 500

1st phase –December 2016 to June2017(active rule of 500)

2nd phase- July 2017to December 2017(non active rule of 500)

Conclusion and Result

Age group 21-27 yr has significant rate of RTA under alcohol influence. There is significant rise if death rate in phase

2ndRate of RTA with influence of alcohol is slightly rise in phase 2nd.

Utility of Haematological parameters and C-Reactive Protein levels in early diagnosis of Neonatal sepsis

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² Professor Department of Microbiology J.N.Medical College, Belagavi, Karnataka, India.

ABSTRACT:

- **Objective:** To determine the utility of Haematological parameters and C-Reactive Protein levels as diagnostic markers in early diagnosis of Neonatal Sepsis in Neonatal Intensive Care Unit at KLE's Dr. Prabhakar Kore Hospital, Belagavi, Karnataka.
- **Materials and Methods:** A Cross-sectional study was conducted on Neonates with signs suggestive of probable sepsis (according to IMCI criteria) or with suspected maternal risk factors admitted at KLE's Dr. Prabhakar Kore Hospital, Belagavi, Karnataka from period of November 2017 to July 2018. Under aseptic precautions Blood specimen was obtained from each neonate prior to commencement of antibiotic for sepsis work-up which included Blood culture, Haemoglobin levels, Total WBC counts and Platelet counts and C-Reactive Protein levels.

Results :

	Decreased Haemoglobin %	Decreased WBC Counts	Decreased Platelet counts	CRP
Sensitivity	35.2%	47.6%	38%	57.1%
Specificity	57.6%	53.8%	69.2%	92.3%
Ppv	50%	62.5%	66.6%	92.3%
Npv	57.6%	38.8%	59%	57.1%
P value	0.659	0.933	0.664	0.0039*

Conclusion: In this study determination of serum C-Reactive-Protein level was superior compared to the Haematological parameters in detecting Neonatal Sepsis. The concentration of C-Reactive-Protein was elevated in culture positive neonates. In some cases of the culture positive neonates, haematological parameters were negative but the C-Reactive-Protein level was elevated. These findings support the usefulness of C-reactive protein as diagnostic marker in early diagnosis of neonatal sepsis.

Key words: C-Reactive Protein, Neonatal sepsis, Blood culture.

Index Finger Pollicization For Traumatic Thumb Amputation

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²M.S,MCH, DY Patil Hospital, Kadamwadi, Kolhapur, India.

ABSTRACT:

Pollicization is a hand surgery technique which substitutes a functioning finger for a deficient thumb. The most common indication is thumb hypoplasia with absence or instability of the carpometacarpal joint. However, there are additional causes that may negate thumb function, such as trauma, macrodactyly, multi-fingered hand and a mirror hand.

We present a rare case of 14 year old male with a traumatic loss of left thumb and loss of index finger extensors, 4 years back in a crush injury. Index finger was pollicized & extensor tendons were reconstructed improving the function and aesthetics of the hand.

Steatocystoma simplex on the scalp

Dr. Rajat Kumar Singh Dr. Mayuresh Deshpande

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ABSTRACT:

Steatocystoma simplex (SS) is a benign dermal cyst that is believed to originate from the pilosebaceous duct junction. SS usually locates in head and neck region, as well as axillae, chest, back, and limbs.

We present a case of 67yr old female with complaint of swelling over scalp since 4yrs with headache and restricted head movements. Swelling was tender with purulent discharge. After taking S shaped incision entire swelling was removed and skin sutured. Patient improved completely after surgery.

Role of Sympathetic Block and Ozone Therapy as A Combination Therapy in Treatment of Diabetic Foot Ulcers

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Department of Surgery,
D.Y.Patil Medical College, Hospital and Research Institute, Kadamwadi, Kolhapur.

ABSTRACT:

Objectives

Diabetes mellitus is a metabolic disease causing more than 5 million deaths annually. Many complications are associated with DM, arising mainly due to the disruption of the vascular system resulting in inadequate circulation to the peripheral body thus placing the foot at higher risk of ulceration and infection. The management of diabetic foot ulcers needs a multidisciplinary approach. Conventional treatment modalities are often of limited success in promoting complete wound closure. The study is carried out to evaluate role of Combination of Ozone Therapy and sympathetic nerve blocks in treatment of diabetic foot ulcers with aiming to reduce pain associated with an ulcer & to fasten wound healing by improving vascularity and oxygen delivery to tissues thus reducing hospital stay and economic burden on patient.

Material and Methods

Following patients were included in study,

- 1. Patients with informed and valid consent, willing to participate in study*
- 2. Diabetic foot ulcer of Wegner's Classification of ulcers 1, 2 and 3.*
- 3. Ulcers of size less than 10x10 cm*

Along with conventional wound debridement, patients were treated with sympathetic nerve blocks and ozone therapy. Measurement of wound area was done at the beginning of the study and on days 10, 20 and 30 of follow-up. Healing of the wound is considered as the end-point of study for the patient.

Results

60 patients were included in study out of which 33 patients showed closure of wound, 19 patients showed significant reduction wound size compared to traditional treatment modalities, 8 patients needed surgical intervention.

Conclusion

After analysis of data it is safe to conclude that ozone therapy and sympathetic block in combination is more effective compared to regular treatment modalities and can be included in practice.

Septicemia Due to *Propionibacterium Propionicum* In Three Month Old Female Child-A Case Report

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²Assistant Professor Microbiology RCSMGMC Kolhapur, India.

³Tutor Microbiology RCSMGMC Kolhapur, India

ABSTRACT: *Propionibacterium* species are a rare cause of infection. Due to its commensal nature its presence in clinical sample needs to have strong clinical and microbiological correlation. Till now isolation had been reported from pus, post-op wound and various device related infection and lacrimal apparatus so far.

We present a case of Propionibacterium propionicum septicaemia in infant a 3 month old female child was brought by her parents for complaints of breathlessness, fever, an excessive oral secretion. X ray finding s/o bronchiolitis standard microbiological examination was perform including culture, staining and biochemical test. The isolate was identified as a Propionibacterium propionicum. 7 days course of amoxicillin resolved the infection and patient made a full recovery.

Keywords: *Propionibacterium propionicum, septicaemia, bronchiolitis*

1. Introduction

Septicemia generally refers to a systemic disease associated with the presence of pathological organisms or toxins in the blood. *Propionibacterium* is an anaerobic/microaerophilic Gram-positive non-sporing, non-acid fast. Non-motile bacteria belonging to family Propionibacteriaceae. Important members are

- *Propionibacterium acnes*
- *Propionibacterium granulosum*
- *Propionibacterium propionicum*

P. acnes is associated with acne. They have been isolated in infective endocarditis and in infections associated with implanted prostheses. *Propionibacterium propionicum* is part of normal flora of the mouth. Occasionally causing human infections.

Case History

A three month old female child was brought by her parents with chief complaints of

- ☐ Fever
- ☐ difficulty in breathing since 1-2 days
- ☐ excessive oral secretion

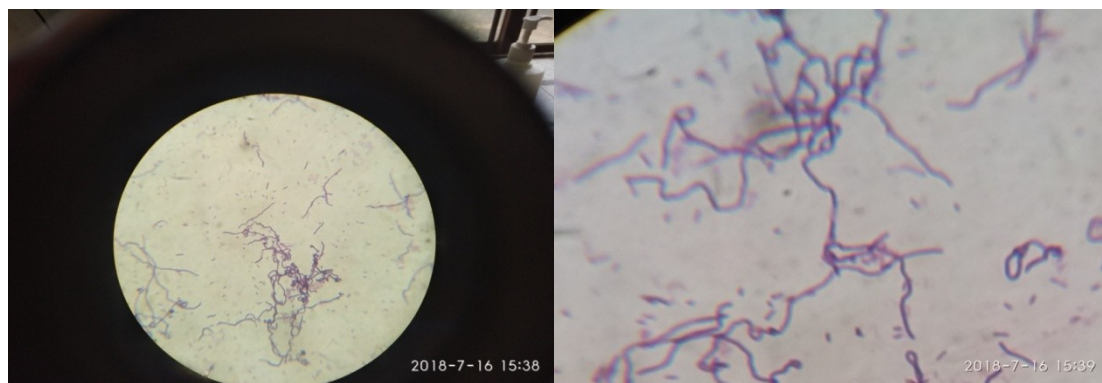
similar episode was observed in past and managed by local physician with antibiotics and nebulization.

Immunization history- Immunized for all required vaccine. Developmental-WNL Dietary history- Breast feeding Positive points- mother was Tobacco chewer. having retracted nipples. On admission patient was having fever 102°Fahrenheit. Child was in respiratory distress.

There was copious amount of salivary secretion around mouth. Child was irritable during examination. On x-ray findings- suggestive of bronchiolitis.



Blood investigation - showed leucocytosis. C-reactive protein positivity. Patient was put on antibiotics. Blood culture send to the microbiology department for evaluation ... Culture was done on blood agar, chocolate agar, Mac Conkey agar and incubated at 37°C. Mac Conkey there was no growth. Blood agar showed predominant growth of moist glistening colonies after 48 hrs of incubation. Gram-staining of the colonies showed gram positive filamentous bacilli. (pic no 2 and 3) Blood was inoculated on Sabouraud's dextrose agar and incubated at 37°C and at room temperature.(no fungal growth)



1. The isolate was non-motile. Biochemical reactions of the isolate were as follows:
 2. Catalase negative
 3. Urease negative
 4. Bile esculine hydrolysis negative
 5. TSI -glucose fermented with acid only. Antibiotic sensitivity pattern of the isolate on Mueller-Hinton agar by Kirby-Bauer disc diffusion method showed---
-
1. Sensitivity to Penicillin, Gentamicin, Amikacin, Cotrimoxazole, Clindamycin, Imipenem, Doxycycline .
 2. Resistance to Erythromycin, Cephalosporins.
 3. Blood culture was repeated after 2 days, and the same isolate was obtained with same sensitivity pattern. The isolate was identified as *Propionibacterium propionicum* based on microscopy, culture and biochemical features. The patient was treated with parenteral Amoxicillin for seven days patient improved symptomatically.

Discussion

Propionibacterium species are a rare cause of infection. Due to commensal nature, its presence in clinical sample needs to have strong clinical and microbiological correlation. Isolation has been reported from pus, post-op wound and various device related infection and lacrimal apparatus so far. To the best of our knowledge, this is the first case reported of septicemia due to *P. propionicum*. Our patient showed clinical symptom of septicemia along with respiratory involvement, fever and excessive oral secretion. This aided us to form a differential diagnosis and to confirm a microbial etiology. This case highlight the fact that though *Propionibacterium* is commonly isolated as a contaminant, on certain occasions, significant isolates can cause serious infection. Appropriate treatment can save the patient. This report also highlights the importance of culture and the usefulness of Penicillin group of antibiotic even in this era of newer higher antibiotics.

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A Study on Outcome of Surgical Management of Tympanic Membrane Retractions

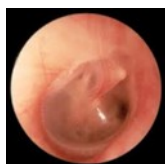
Dr. Deepal Kundu

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ABSTRACT: Tympanic membrane retraction pockets are pathological invagination of tympanic membrane into middle ear space. They are a sequel to chronic otitis media with effusion. Pars flaccida and posterosuperior region of pars tensa are more prone to retraction. Retractions may remain asymptomatic or present as recurrent ear discharge and conductive hearing loss. Sade's classification is used for grading of pars tensa retractions. Tos's classification is used for pars flaccida retractions. Regular observation is necessary to monitor disease progression. Tympanic membrane retractions if untreated may progress & cause increase in hearing loss & develop life threatening disease known as cholesteatoma. There are controversies in surgical management regarding whether to operate or not & when to operate. Earlier intervention is easy to perform but may lead to deterioration of hearing. Wait and Watch policy may increase extent of adhesions, will make surgery more difficult and may progress to life threatening disease called cholesteatoma.

Keywords: Surgical Management, Flaccida, Retractions

1. Introduction



Tympanic membrane retraction pockets are pathological invagination of tympanic membrane into middle ear space. It happens as a sequel to chronic otitis media with effusion. Tympanic membrane retraction is a clinical diagnosis

Sade's classification for pars tensa retractions:

- 1) retracted tympanic membrane.
- 2) retracted tympanic membrane with contact onto incus.
- 3) middle ear atelectasis - tympanic membrane onto promontory but mobile.
- 4) adhesive otitis media - tympanic membrane onto promontory but fixed.

s



Tos's classification for pars flaccida:

- 1) Pars flaccida- not in contact with head of malleus.
- 2) Pars flaccida- in contact with head of malleus.
- 3) Limited outer attic wall retraction.
- 4) Severe outer attic wall retraction.



Management strategy depend on 3 main parameters which are as follows :

- 1) Type or grade of retraction pocket.
- 2) Functional status of middle ear.
- 3) Behavior of retraction pocket over time (regressing pocket, stationery pocket, progressive pocket)

AIM

To analyse the result of tympanoplasty in tympanic membrane retractions (with respect to dry ear and hearing improvement).

Objectives

- 1) To analyse the intactness of neotympanum.
- 2) To analyse the stability of neotympanum.
- 3) To analyse hearing improvement.
- 4) To analyse complications of tympanoplasty surgery in these cases

Methodology

Place of Study: DR D Y Patil Medical College , Hospital and Research Institute, Kolhapur, Maharashtra.

Type of Study: Prospective Cohort Observational Clinical Study

Period of Study: Sept 2017- Sept 2019.

Sample Size: 50

Inclusion Criteria

- 1) All patients Between age group 15-50 years.
- 2) Patients complaining of ear discharge.
- 3) Patients complaining of hearing loss.
- 4) Patients with retractions in tympanic membrane.

Exclusion Criteria

- 1) Patients <15yrs and >50yrs.
- 2) Patients with sensorineural hearing loss.
- 3) Patients with mixed hearing loss.
- 4) Cases of revision tympanoplasty.

Methods

Surgical technique- Tympanoplasty

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Study Of Bacterial And/Or Fungal Infections In Clinically Diagnosed Cases Of Acute Conjunctivitis

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ABSTRACT:

AIM

Study of bacterial and/or fungal infections in clinically diagnosed cases of acute conjunctivitis

Objectives

1. To study the demographic factors in acute conjunctivitis.
2. To study the associated co-morbid conditions in acute conjunctivitis.
3. To determine the common bacteria and/or fungus in acute conjunctivitis.

Methodology

1. All patients will be examined in OPD.
2. A detail history will be asked.
3. Torch examination of both eyes.
4. Vision of both eyes will be tested.
5. Corneal sensations of both eyes.
6. Slit lamp biomicroscopic examination.
7. Corneal staining will be done with the help of sterile fluorescein sodium ophthalmic strips after using 0.5 % proparacaine eye drops.
8. Anti-virals, lubricating, ointment will be started according to standard of care.
9. Sample of conjunctival swab or corneal scrapping will be send for gram staining for bacteria and KOH staining for fungus and culture sensitivity to detect bacterial and/or fungal infection in non responsive cases.

Observations

- The following observation based on total 23 patients included in study. There is 52% male predominancy.
 - Study shows that 13% patients are paediatric age group, 52% are elders and 35% older page group.
 - Out of 23 patients 54% of patients are from low socioeconomic status, 32% from middle class, 9% from high class and only 2% from upper class.
 - 67% patients were diabetic ,16 % were hypertensive, 8% were asthmatic and 8% were patient of rheumatoid arthritis. The percentage of involvement of RE and BE is 21.%. Percentage of LE involvement is 56.5%.
 - Out of 23 patients 47.8 % had lid edema, 56.5 % patients had petechial hemorrhages and only 3.4% patients had conjunctival follicles.
-

Clinical Study of Mucormycosis –A Deadly Disease on Rise

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ABSTRACT: *Rhino cerebral Mucormycosis is an opportunistic fungal infection frequently seen in diabetic and immunocompromised patients. After Aspergillus and Candida, it is the most common invasive fungal infection. Mucormycosis has a very high mortality. Rhino-orbital-cerebral Mucormycosis is known to exist in two forms, the well-known acute form affecting orbit, nose, sinuses, cranial structures and the well-recognised chronic form. The most common features of the chronic form are ophthalmologic including ptosis, proptosis, visual loss and ophthalmoplegia. Early diagnosis and treatment with Amphotericin –B is the key to combat this disease successfully.*

1. Introduction

Mucormycosis is the term used to describe fungal infections caused by fungi in the family Mucoracea, order Mucorales, and class Zygomycetes. Through inhalation or ingestion of spores, the organism can enter into the body and can lead to Mucormycosis in immunocompromised or uncontrolled diabetic patients. Depending upon the site of the body affected, the disease may manifest in six different forms-rhino cerebral, pulmonary, cutaneous, gastrointestinal, central nervous system, or disseminated forms. Of these, rhino cerebral form is the most common disease manifestation. Mucor spores settle onto the mucosa of nose and PNS and may progress to the brain. Typical finding is the presence of black necrotic mass filling the nasal cavity. Extension of the disease into maxillary and ethmoid sinus can lead to orbital involvement. Intracranial spread through ophthalmic artery, superior orbital fissure and cribriform plate.

Materials and Methods

Recently we came across 4 new cases of chronic indolent variety of mucormycosis. Investigations included KOH staining, fungal culture and histopathology and HRCT

Sr No	Age/Sex	Underlying Conditions	Affected sites
1	52/M	Uncontrolled DM with DKA	Nose,Maxillary sinus,orbit
2	48/F	Uncontrolled DM	Nose,Maxillary sinus,orbit
3	27/M	Uncontrolled DM with DKA	Nose,maxillary sinus
4	61/M	Controlled DM	

All were treated with iv Amphotericin B and endoscopic debridement and underwent orbital exenteration Our self modified management protocol.All findings are confirmed by diagnostic nasal endoscopy -Biopsy is sent for HPR.Once HPR is reported as mucormycosis patient is subjected to surgery Nasal endoscopic debridement followed by regular DNE every week and placement of amphotericin soaked pack after every debridement is done. Regular dosage of Inj.Amphoteracin is given with strict charting of RFT's and I/O charting

Regular follow up

Sr.No	Age/Sex	Underlying conditions	Surgical Management	Medical management Dosage of Amphotericin (in gms)
1	52/M	Uncontrolled DM with DKA	Repeated endoscopic debridement with local sino-nasal amphotericin packs and left eye orbital exenteration	2.1
2	48/F	Uncontrolled DM	Repeated endoscopic debridement with local sino-nasal amphotericin packs	1.4

3	27/M	Uncontrolled DM with DKA	Repeated endoscopic debridement with local sino-nasal amphotericin packs	2.6
4	61/M	Controlled DM	Repeated endoscopic debridement with local sino-nasal amphotericin packs	2.4

Discussion

The World Health Organisation predicts the number of diabetics in India would go up to 75 million by 2025. Initially all our patients were admitted with physicians for the control of the diabetes and later noticed symptoms like nasal obstruction and facial oedema. Changes of "sinusitis" in an uncontrolled diabetic should be considered as mucormycosis. ENT surgeon can confirm the diagnosis at the earliest by nasal endoscopy and biopsy. The treatment outcome is better if diagnosed at an early stage.

Conclusion

- We mainly focussed on repeated debridement rather than completing the optimal dosage of IV Amphotericin. Dosage of Amphotericin B ranged from 1.4 to 2.6gms
- With good control of Diabetes and adequate debridement with simultaneous amphotericin therapy till HPR was negative for the disease
- No recurrence till date

Status of Ossicular Chain in Chronic Otitis Media

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Objectives

1. To study the incidence of ossicular chain pathology in chronic otitis media.
2. To study the frequency of involvement of each ossicle.
3. To compare the ossicular chain involvement in safe and unsafe type of chronic otitis media
4. To compare the frequency of ossicle involvement in safe and unsafe type of chronic otitis media

Materials and Methods

- Duration- July 2016 - July 2018.
- Consent - Informed written consent.
- Detail clinical history & complete ENT examination, investigations and necessary operative procedure.
- Follow up – 1st& 3rd (post op graft takeup and hearing assessment)
- Data collected and analysed

Result

- Total number of cases - 200
- No. of patients with ossicular pathology – 49
- No. of patients without ossicular pathology – 151
- Incidence of ossicular chain pathology in COM – 24%
- Commonest ossicle involved - Incus

Conclusion

Ossicular chain involvement is more commonly seen in unsafe type of chronic otitis media and incus is the most commonly affected ossicle in both types and to be more specific, the lenticular process.

Isolation and Antibiotic Susceptibility Pattern of Coagulase Positive from Various Clinical Specimens At Tertiary Care Hospital, Jaipur

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ABSTRACT: Multidrug resistance of *Staphylococcus aureus* to macrolides (Erythromycin, Clarithromycin) and Lincosamides (Clindamycin, Lincomycin), is on the rise, leaving very few therapeutic options. Newer antibiotics like Vancomycin, Linezolid and Quinupristin-Dalfopristin have also been advocated in the management of such isolates, but recent reports of resistance to these agents raise real concern.

Keywords: xyz, word.

1. Introduction

To isolate and identify *Staphylococcus aureus* from clinical specimens.

To determine antibiotic resistance pattern of *Staphylococcus aureus*.

To detect VRSA from *Staphylococcus aureus* isolates.

Material and Methods

Techniques: Vancomycin The present study was conducted in SMS MEDICAL COLLEGE AND ATTACHED

Group of Hospitals in year 2018 (1st april to 30st june). The various isolates received from IPD and OPD patients. : Gram's staining, catalase test, slide and tube coagulase test, colonies on mannitol salt agar (MSA) and Oxidative fermentation test.

Hinton agar (MHA) by Kirby Bauer disc diffusion method with the antimicrobial agents.

ATCC 25923 was used as control and results interpreted as per Clinical Laboratory Standards Institute (CLSI) 2017 guidelines. In house prepared BHI agar screen plates containing 6µg/ml vancomycin were prepared. Inoculum suspensions were prepared by selecting colonies from overnight growth on nutrient agar plates. The colonies were transferred to sterile saline to produce a suspension that matches the turbidity of a 0.5 McFarland standard. The final inoculum concentration of 10⁵-10⁶ CFU per spot was prepared by adding the sterile saline to the bacterial suspension. These suspensions were inoculated into BHI screen agar plates and were incubated at 35°C for 24-48 hour.

Investigation Techniques

Antimicrobial Susceptibility Testing was done on Mueller-

Inoculation on BHI Vancomycin screen agar V

Staphylococcus aureus

Out of 100 isolates, maximum were sensitive to Linezolid (93%), Doxycycline (83%), Gentamycin (78%), and Clindamycin (56%). Maximum resistance was seen in Ciprofloxacin (74%) and Cefoxitin (58%). Sensitivity of Vancomycin was tested by Vancomycin screen agar (6µg/ml), all of the 100% were sensitive.

Result & Discussion

Figure No. 2 Showing Antibiotic Susceptibility

Pattern of Coagulase Positive *Staphylococcus Aureus*

Figure No. 1 Showing Various Clinical samples Figure No. 3 Showing Sensitivity plate of coagulase Positive *Staphylococcus Aureus*

Conclusion

The increased prevalence and dissemination of multidrug resistant COPS worldwide has resulted in a major decrease in therapeutic options because the majority are now resistant to most antibiotics. Newer antibiotics such as vancomycin and Linezolid have good therapeutic role. Although their clinical use may be limited due to adverse effects.

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Wet Mount Microscopy and Gram Staining in The Diagnosis of Urinary Tract Infection

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ABSTRACT:

Background :- urinary tract infection (UTI) is one of the most important causes of morbidity and mortality in the developing countries like INDIA. UTIs are among the most common bacterial infections and account for significant part of the work load in clinical laboratories. This study assessed the standard urine analysis technique and sediment stain techniques as predictors of bacterial culture results for urine.

Materials and methods :- The present retrospective study was conducted at Dr D Y Patil Hospital and Research Center, Kolhapur, India by analysing the record of urine samples collected for routine microscopic examination as well as for culture and sensitivity test over a period of three months (Feb 2018 – April 2018).

Results :- 450 patient were enrolled in the study out of which 173 showed significant wet mount findings like pyuria. Out of 173 samples sent for urine culture and sensitivity, 112 showed no growth and 61 were culture positive. Gram negative bacilli caused 65.57% of UTI. Ecoli were most commonly found bacteria causing UTI (44.26%).

Conclusion :- urine culture is the gold standard test in a diagnosing UTI. Microscopic urine analysis is the strong tool and is helps in diagnosing UTI.

Keywords: UTI (urinary tract infection), E coli ,Wet mount microscopy, Urine culture, Gram positive cocci, Gram negative bacilli.

Craniofacial Measurements in Maharashtra Population: A Cephalometric Study

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ABSTRACT: *The aim of the study was to establish cephalometric standards in adult Maharashtra population. The present study included 100 males (age between 18-25 years) and 100 females (age between 18-25 years). All individuals included in the study were residing in Maharashtra since their three to four generations. All subjects had class I malocclusion and normal growth and development. Maxillary and Mandibular arches were well aligned in all participants. Bony landmark used were Nasion(N), Sella(S), Gonion(Go), Pogonion(Po), Gnathion(Gn), Articulare(Ar) and Menton(Me). With the help of these landmarks, Anterior facial height(AFH), Posterior facial height(PFH), Anterior cranial base(ACB), Posterior Cranial Base(PCB), Facial angle, Saddle angle and Facial height ratio(FHR) were studied. The present study showed significant difference in gender dimorphism.*

Keywords: Craniofacial, Cephalometry, Maharashtra Population, Gonion, Nasion, Sella

Reliability of Stature Estimation From Facial Parameters Amongst Sangli District Population

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ABSTRACT:

Introduction: Stature is an vital indicator of identity of an individual. Anthropometric parameters vary from age, gender, region, shape of individual. Anthropometric data is objective and hence is very useful for forensic experts. It is affirmed that there is need of establishment of own findings for stature estimation due to ethnic, dietary and climatic variations.

Aim and Objectives: The measure accuracy of the stature with facial height and bizygomatic width in males and females of Sangli district.

Material and Methods: 259 males and 259 females of Sangli district population, in age group 18-60 yrs were studied. Height of the subject was measured by measuring tape in standing position. Facial height and Bizygomatic facial width were measured using a Vernier caliper. Statistical analysis of measured data was done, regression equations was derived from regression coefficient.

Observation & Results: In males, mean stature was 166.80 ± 9.19 cms, facial height 11.02 ± 0.65 cms and mean Bizygomatic width 11.81 ± 0.69 cms. In females, mean stature was 152.91 ± 6.84 cms, mean Facial height 10.05 ± 0.67 cms and mean Bizygomatic width 11.50 ± 0.89 cms.

Conclusion: Stature, facial height and bizygomatic width were more in males than females in Sangli district population. Statistically significant absolute correlation is seen between facial height and stature as well as between stature and bizygomatic width. Of the facial parameters, facial height is the most predictable parameter for prediction of stature using regression equation in both males and females..

Keywords: stature, facial height, Bizygomatic width, correlation

“Epidemiology and sero-positivity of dengue fever cases in tertiary care hospital of western Maharashtra, India“

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Introduction

Dengue – A Flavi virus is endemic since last two centuries ^[1]. There is rapid increase in number and severity of disease in recent past. It is undergoing geographical expansion from urban to rural area as well ^[2]. WHO (2005) declared, dengue as notifiable disease ^[3,4]. Large morbidity and mortality associated with dengue infectivity necessitates the early diagnosis and demonstration of IgM or IgG or viral antigen NS1 (non structural protein 1). Goup & type specific determinant appears from day one. Such as IgM on 7th day & IgG on 14th days in primary. Targeting NS1 therefore should be the approach for early diagnosis and timely management^[5].

Aims and objective

This study was planned by keeping all this in mind with following aims and objectives.

1. To determine sero-positivity of clinically suspected dengue fever cases
2. To study demographic profile of sero-positive cases
3. To study seasonal prevalence of dengue fever

Material and methods

Study design:-It is retrospect study, carried out in the department of Microbiology, Bharati Vidyapeeth Deemed to be University, Medical College, Sangli.

Study population:-Clinically suspected DF/DHF cases, which includes all ages and both the sexes

Study Period:-Three years (April 2015-March 2018)

Results

From total 3150 patients, blood samples were collected and serum was separated. This was tested for Dengue NS1 antigen and Dengue specific IgG and IgM using Immunocromatography (ICT) test (Dengue day 1 kit, mfd by J. Mitra & Co. Pvt. Ltd). Results were considered valid after checking control bands for both antigen and antibodies. This kit is having sensitivity of 98.9% and Specificity of 99%

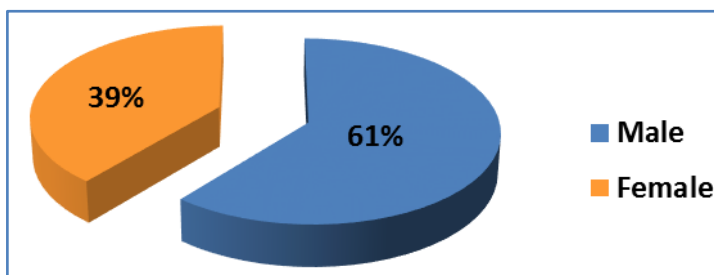
Table No. 1:Distribution of dengue fever cases

Total no. of cases	Positive	Negative
3150	916	2234
Percentage	29.08	70.92

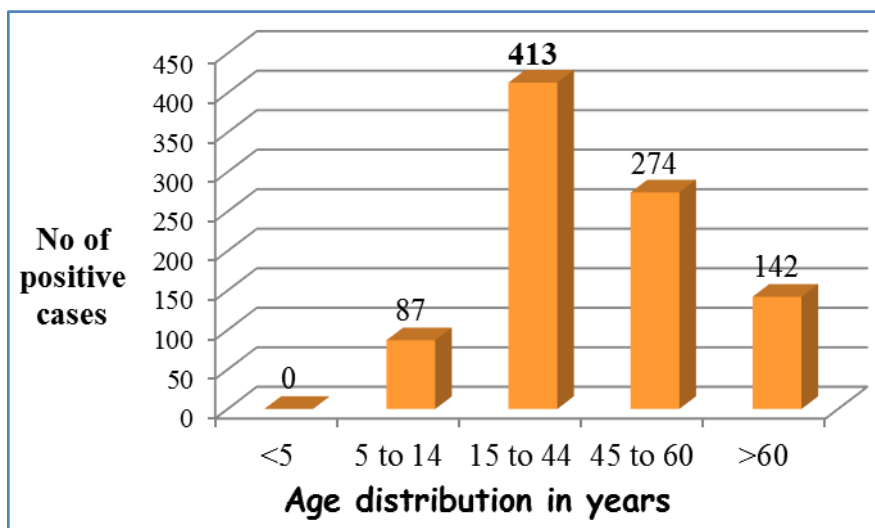
Table No. 2:Year wise distribution of dengue fever cases

Year	Positive	Percentage
April 2015-March16	216	23.5
April 2016-March17	306	33.4
April 2017-March18	394	43.1
Total	916	100

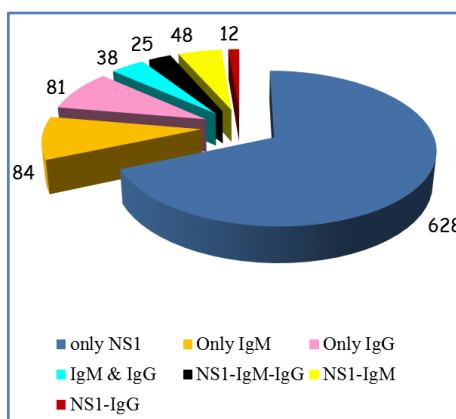
● **Graph No.1:-** Sex wise distribution of dengue fever cases



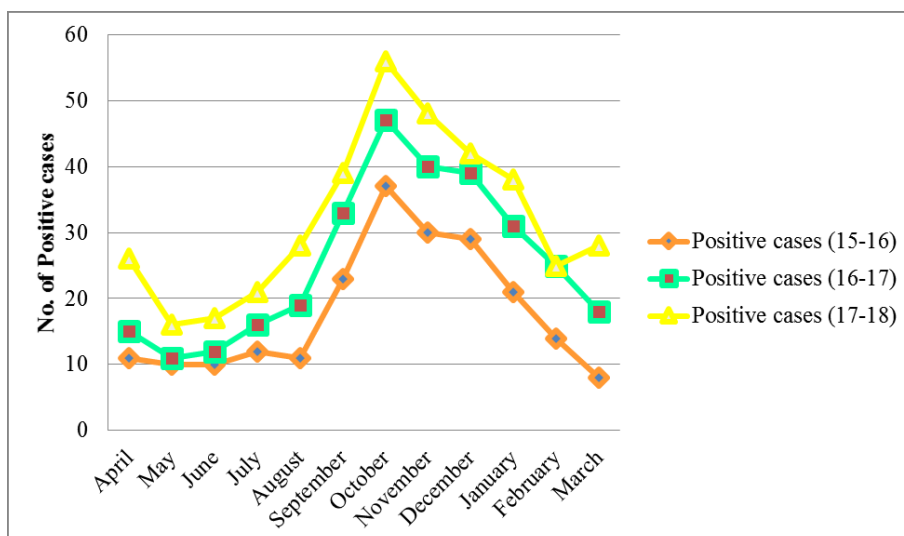
● **Graph No.2:-** Age wise distribution of dengue fever



● **Graph No.3:-** antigen and antibodies wise distribution of dengue fever cases



Total 713/916 (77.8%) were positive for NS1 alone or in combinations. Primary infection (Positive for NS1, IgM, NS1+IgM) was seen in 760/916 (83%). However, secondary Infection (Positive for IgG, IgM+IgG, NS1+IgG, NS1+IgM+IgG) was seen in 156/916 (17%)

Graph No.4:- Month and year wise distribution of dengue fever cases

In the present study Male to female Ratio was 1.5:1. Prevalence of dengue seropositive among clinically suspected cases was 29.08%. Prevalence of vector and silent circulation of virus may be the cause of rise in dengue cases in our hospital. Dengue was common during monsoon and post monsoon period. Transmission increases post monsoon. Presence of stagnant water after rain fall favors the mosquito breeding. Such type of data generations will give information of hotspot which will be helpful to enforce and strengthen vector control measures. Dengue is a serious illness where no specific symptoms necessitate lab confirmation. In our study, there was male preponderance in all age groups was noted. Young adults (15-44) most affected population. This study is carried out in a hospital situated near a rural area draining most of the villages around. This is indicating expansion in rural part and which is a worrisome trend shown by spread of virus. Combination of antigen and antibodies detection tests with ICT increases diagnostic efficiency. Primary infections are significantly higher. Results call attention to the need for continuous surveillance for timely formulation and implementation of effective dengue control programs.

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To study whether our medical colleges inculcate health promoting life style among Medical students – A cross sectional study from a medical institute from western Maharashtra

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ABSTRACT:

Background: The overall development of a student is completely dependent on the ability of the student to cope up with the growing pressure of college and modern life mentally as well as socially. It is seen that this new setting brings about certain changes in the lives of students which either may have a positive or negative impact on their overall well-being. Many students get involved in unhealthy and risky lifestyle behaviours like alcohol abuse, tobacco use, physical inactivity and unhealthy dietary practices which may adversely affect their health in the long-term. In India, research with regard to health and lifestyle patterns amongst students remains limited^{1,2}.

Cost effective and feasible instrument is required to assess the health promoting behaviours of Medical students from the stressful and hectic schedule of their training in the Medical College. Hence with the help of HPLP-II questionnaire, lifestyles of second year MBBS students were assessed. The primary objective of this cross-sectional study was to assess the health promoting behaviors of 300 medical students from II M.B.B.S. students using HPLP II questionnaire³.

Aims & Objectives: 1) To know the life style of students from second year

2) To evaluate health promoting life style among II M.B.B.S. students

3) To study health promoting behaviour among II M.B.B.S. students using HPLP II questionnaire.

4) To find out bad habits & addictions among II MBBS students.

Materials & Methods: This cross-sectional self-administered health Promotion Lifestyle Profile II (HPLP-II) questionnaire study was undertaken among the First MBBS students & Second M.B.B.S. students aged 19-22 year, in D.Y.Patil Medical College, Kolhapur to find out the health promoting behaviors among them.

Results and Discussion: Out of 300 medical students 284 students completed the HPLP-II questionnaire. 67.5% students were Hostel students and 58.9% were Male. 20.8% students consume alcohol and 4.6% smoke cigarette. 58.4% students were in moderate and severe category of stress. Males experienced more stress also scored less marks compared to females. Day scholars living healthy lifestyle and were also good in academics. Stress was invariably present among medical students. Alcohol consumption found to have a vicious cycle with stress.

Conclusion: By adopting a healthier lifestyle, Health status of an individual can be significantly improved. Since this study shows low health promoting behaviors in medicos, there is need to develop guidelines, interventions and periodic investigation for the students for their good health.

Keywords: Health promoting lifestyles, Medical students

1. Introduction

Medical students undergo a period of transition and major life changes when they enter medical colleges for the first time. More than 60000 students pursuing MBBS course from almost 450 Medical colleges in India which are mostly located in urban areas^{1,2}. A positive lifestyle can bring health and happiness, while a negative 2 lifestyle can lead to illness and depression. Health risk behaviors such as smoking, alcohol abuse, unhealthy dietary patterns, sedentary habits, and unsafe and aggressive behaviors have been 3- found to have an important influence on morbidity and mortality 5. Health risk behaviors, which develop over time, can also 6-7 contribute to an unhealthy lifestyle. Medical students experience a relatively high level of personal distress, with adverse consequences on academic performance, competency, professionalism, and health. Studies show that prevalence of alcohol consumption and smoking ranges from 20%-50% and 10-35% respectively among medical students. Psychological morbidities among medical undergraduates are quite common at various stages of their training, which vary depending on academic pressures, different sociodemographic 13 factors and the scale of measurement^{3,4}.

Most of the Students (Male and female) coming to Medical college are at the age group of 16-21 year of age, and are not able to anticipate and cope with the sudden increase of professional studies on one hand and at

the same time on other hand, physiological changes in the body that is in the process of making a impact in the body.¹ Medical students also suffer in term of Health and very little is being said about the empowerment of health to these students in their vigorous study timetable. Health is taught but health promotion is not done. There is no place of physical activity in the Medical curriculum

Aim – Study the lifestyle of I MBBS & II MBBS students at D.Y.Patil Medical College, Kolhapur and to promote healthy lifestyle among them.

Objectives

1. To study different lifestyle factors among Medical Undergraduates and its impact on academic outcomes.
2. To study lifestyle of II MBBS students through HPLP – II questionnaire.
- 3 To assess stress level by using College Student's Stressful Event Checklist of HPLP –II

Materials and Methods

The Questionnaires HPLP II survey was done on 284male and female from II MBBS students, aged 19-22 in D.Y.Patil Medical College Kolhapur. The survey of medical students was done in two parts. The first part included demographic questions (i.e., gender, age,) and second part contained questionnaires related to Health-Promoting Lifestyle Profile (HPLPII). The revised HPLP II questionnaire developed by Walker measure behaviours in the theorized dimensions of health promoting lifestyle The items were subjectively attempt by the medical students and each item has a 4point likert scale scoring range of 1 to 4 for never, sometimes, often, and always respectively. For each subscale, the scores for each item were added and were divided by the number of item in the subscale for obtaining the subscale scores. The final total score was obtained for this scale was by adding the scores for all the items and dividing by the total number of items. The HPLP-II has been used by many researchers for health promotion and is reported to have high validity and reliability for use in different population^{5,6}.

This study was carried out after consent from the students and approval from ethical committee.

HPLP – II Profile Questionnaire -

LIFESTYLE PROFILE II^{3,4,5,6}

Directions: This questionnaire contains about your present way of life or personal habits. Please respond to each item as accurately as possible and try not to skip any item. Indicate the frequency with which you engage in each behaviour by circling:

N –for never, **S** – for sometimes, **O** – for often, **R** – for routine

Sr.No.	Items	Never	Sometimes	Often	Routinely
1	Discuss the problem with close ones				
2	Opt for low fat, low cholesterol diet				
3	Eat junk foods				
4	If unusual signs & symptoms appear then consult a health professional				
5	Go for exercise everyday				
6	Smokes				
7	Has negative style of thinking				
8	Always think in positive way				
9	Practice meditation and yoga				
10	Read books on how to maintain good health				
11	Watch T.V. programmes about improving health				
12	Consume drugs				
13	Take some time for relaxation				
14	Believe that life has a purpose				
15	Maintain good healthy relations with friends & others				
16	Accept the things in life that cannot be changed				
17	Feel content and at peace with oneself				

18	Consume balanced diet				
19	Discuss health problems with health professional				
20	Use specific method to control stress				
21	Work towards achieving long term goals in life				
22	Inspect my body at least monthly for physical changes / danger signs				
23	Am aware of what is important to me in life				
24	Check my pulse rate when exercising				
25	Get support from a network of caring people				
26	Have faith in god				
27	Accept challenges to achieve goals				
28	Attend educational programmes on personal health care				
29	Place myself to prevent tiredness				
30	Balance time between work and play				

Results

284 out of 300 (94.66%) undergraduate students of II MBBS completed this survey. Out of 284 students 168 (59.15%) were females and 118(41.85%) were males. Around 58 female students (20.42%) had health promotional life styles whereas 46(16.19%) males had health promotional lifestyle. Out of 300 medical students 284 students completed the HPLP-II questionnaire. 67.5% students were Hostel students and 58.9% were Male. 20.8% students consume alcohol and 4.6% smoke cigarette. 58.4% students were in moderate and severe category of stress. Males experienced more stress also scored less marks compared to females. Day scholars living healthy lifestyle and were also good in academics. Stress was invariably present among medical students. Alcohol consumption found to have a vicious cycle with stress.

Discussion

Medical colleges are generally situated at district level places and many of them are in metro cities. The process of admission in colleges gives opportunity to all students from different parts to come and pursue their education in this urban areas. But this transition exposes many students to unhealthy lifestyle and different kind of stress. Many students stick to cigarette smoking and alcohol consumption.

Young adults transitioning between high school and college find themselves in an environment with increased opportunities to make personal and lifestyle decisions without supervision or input from their parents. Coupling this newfound freedom with growing academic pressure and an expanding social network can lead to stress and unhealthy lifestyle. For many students, the college years represent a time of new experiences and increased opportunities to make personal health decisions. Some of these decisions encompass the areas of nutrition and physical activity. Students are on their own; free to eat what they want, when they want. Busy academic and social schedules can take priority over eating well and exercising regularly. Class and work schedules vary from day to day and change every semester. Lifestyle changes, peer pressure, and limited^{7,8,9}

Finances may lead to an increase in stress, triggering overeating that result in weight gain In addition, the steady availability of a wide variety of food, both nutritious and not so nutritious, can make wise food choices difficult. Unhealthy lifestyles during youth are strongly linked to unhealthy habits in adolescents. Health-related behaviours in early stages of life affect the disease risks related to lifestyle in later periods of life. Although it is difficult to change unhealthy habits that adults have adopted in their youth, many effects of health risk factors among adults are avoidable if these behaviours are identified and changed at an early stage¹⁰.

Therefore, it is important to increase healthy lifestyle behaviours among young people.

Conclusion

We observed by this study that more health promotion should be done among students at regular interval for improving self care of a individuals. At regular interval of time basic investigation should be done as a routine procedure.

By adopting a healthier lifestyle, Health status of an individual can be significantly improved. Since this study shows low health promoting behaviors in medicos, there is need to develop guidelines, interventions and periodic investigation for the students for their good health.

Issue Of Conflict If Any

No issue of conflicts as the analysis of Life style questionnaire was carried out after prior consent from the students and prior approval from the ethical committee of the institution.

Acknowledgement

We the authors are highly obliged to Mr.Sanjay D.Patil Chancellor & President D.Y.Patil Education Society, Institution Deemed to be University, Kolhapur for his constant support & inspiration. The authors are also thankful to the students for their cooperation while carrying out this study

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Study of Bloodstream Infections in Paediatric ward in a Teaching Hospital, Kolhapur, Maharashtra

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ABSTRACT:

A bloodstream infection in paediatric patients is one of the important cause of morbidity and mortality. Blood culture technique is the gold standard for the diagnosis of such infections. Early diagnosis and appropriate treatment is essential to reduce mortality rate. Multidrug resistant bacterial strains are very difficult to treat. Present study was undertaken at Dr D Y Patil Hospital & Research Centre, Kadamwadi, Kolhapur to identify the bacteria associated with bloodstream infections and detect their antibiotic sensitivity pattern.

Materials and Methods: Blood cultures from 94 paediatric patients were screened for bloodstream infections by automated systems BC-32 Render and the positive blood culture bottles were subcultured and were run on Vitek II for antibiotic sensitivity with their MIC and ESBL, MRSA was noted.

Results: Out of 94 bloodstream infection suspected cases, 24 (25.5%) were culture positive, 41% isolates were gram negative bacilli, 37% isolates were CONS, 12.5% isolates were coagulase positive staphylococci. 30% strains were ESBL producers, 33% were MRS (CONS), 22% were MRSA.

Conclusion: Proper diagnosis of bloodstream infections in paediatric patients is lifesaving. Using automated systems like BC-32 and Vitek II reduces the time for diagnosis and appropriate treatment of bloodstream infections

Keywords: Automation, Blood Culture, Bloodstream infections, BC-32, Vitek II

1. Introduction

Bloodstream infection is one of the common causes of morbidity and mortality in paediatric patients. Various organisms are associated with bloodstream infections such as *E.coli*, *Coagulase negative Staphylococci*, *Haemophilus influenza*, *Listeria monocytogen*, *Pseudomonas spp*, *Acinetobacter spp* etc.

In neonates the risk factors for septicaemia may be due to premature rupture of membrane, prolong labour, premature birth, low birth weight, congenital anomalies, urinary tract infection of the mother⁽¹⁾. The signs of blood stream infections include bradycardia, high grad fever, vomiting, diarrhoea and jaundice. Serious complications such as shock, multiorgan failure, disseminated intravascular coagulation and death⁽¹⁾.

Early diagnosis is essential and the gold standard for detection of bloodstream infections is isolation of bacterial agents by blood culture⁽²⁾. Automated systems like BacTech, BC- 32 blood culture system, Vitek etc are useful for correct and faster detection of bacteria causing bloodstream infections and also help for management of such infections . Antibiotic sensitivity testing with MIC of the antibiotics plays a significant role in treatment of multidrug resistant bacterial strains .

Present study was undertaken to identify the bacteria associated with bloodstream infections in paediatric patients and to study the antibiotic resistance in those pathogens.

Materials & Methods

The study was conducted between April 2017 to March 2018 at Dr D Y Patil Hospital & Research Centre, Kadamwadi, Kolhapur. The samples were collected from paediatric ward and PICU patients of suspected blood stream infection.

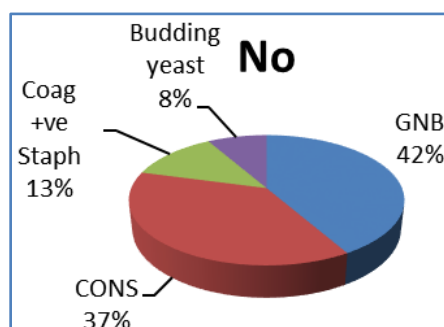
Blood samples were collected under strict aseptic precautions and 3-4 ml blood was inoculated in 30 ml Aerobic blood culture bottles for BC-32 machine. These bottles were incubated in BC-32 Render automated system. The bottles which showed growth were removed and subcultures were done on Blood agar and MacConkey agar (primary isolation)⁽³⁾, smear from the colony was prepared and stained with Gram stain to identify gram positive or gram negative bacteria. Then colonies were run on VITEK II automated system for identification of organism and Antibiotic Sensitivity with their Minimum Inhibitory Concentration (MIC) as per CLSI ⁽⁴⁾ and detection of Extended Spectrum Beta Lactamases (ESBL) in GNB, *Methicillin Resistant Staphylococci* (MRS), *Methicillin Resistant Staphylococcus aureus* (MRSA) in GPC. After seven days of incubation with no growth in BC-32 bottle, negative report was given.

Results

In the present study out of 94 paediatric blood culture samples screened for bloodstream infections, there were 24 (25.5%) blood culture were positive.

Out of the se 24 positive blood cultures 10 isolates were Gram Negative Bacilli (GNB), 9 were Coagulase Negative Staphylococci (CONS), 3 were Coagulase positive Staphylococci, 2 were budding yeast as shown in diagram 1.

Diagram 1: isolation of various organisms from blood culture



The frequency of isolation of GNB & GPC is shown in Table 1 & 2

Table 1: Distribution of GNB

GNB	No & percentage
<i>Salmonella typhi</i>	5 (50%)
<i>Escherichia coli</i>	3 (30%)
<i>Acinetobacter baumannii</i>	1 (10%)
<i>Serratia marcescense</i>	1 (10%)

Table 2: Distribution of CONS

CONS spp	No & percentage
<i>Staphylococcus hemolyticus</i>	5 (55%)
<i>Staphylococcus hominis</i>	3 (33%)
<i>Staphylococcus gallinarum</i>	1 (11%)

Antibiogram of GNB were 100% sensitive to Amikacin, Imipenem, Meropenem, Tigecycline similar findings were reported by Chandra Madhur et al⁽⁵⁾, Piperacillin-Tzobactam, Tobramycin & Tigecycline & 90% sensitivity to Amikacin, Ampicillin-Sulbactam, Gentamicin etc. *Acinetobacter baumannii* & *Serratia marcescense* showed sensitivity to all antibiotics

In GNB 2 strains of *E.coli* & 1 strain of *S.typhi* were ESBL producers (30%).

Antibiogram of GPC: (*CONS* & *S.aureus*) : 100% sensitivity to Linezolid & Teicoplanin, 90% sensitivity to Amikacin, Gentamicin, piperacillin-Tazobactam.

In GPC 1 strain was MRSA(22%), 2 strains were MRS (33%)

Discussion

Blood stream infections are common cause of neonatal death in developing countries. Proper isolation of causative agents and their antibiotic susceptibility is essential. In present study total no of 94 paediatric blood samples were studied and septicaemia was detected in 25.5% of patients. Bhat et al (47%)⁽¹⁾, Chandra Madhur et al (37%)⁽⁵⁾, Bhattachacharya et al (32%)⁽⁶⁾ in their study. *Salmonella typhi* strains were isolated from PICU patients age group between 2-15 years. Other organisms were isolated from neonates. In present study 41% isolates were GNB and 37% were CONS, 12% were *Staphylococcus aureus*. Higher incidence of

neonatal sepsis was reported Baby et al reported (78%) in GPC & 20 % in GNB their study⁽⁷⁾, Lee CY et al reported 30% in GPC and 56% in GNB in their study⁽⁸⁾

In our study 30% strains of GNB isolates were ESBL producers. Bhat et al reported 35% ESBL producers in their study⁽¹⁾, Lee C Y et al reported 20% ESBL producers in their study⁽⁸⁾. In our study staphylococcal isolates 33% were MRS (CONS) & 22% were MRSA. Baby et al reported 14% MRSA in their study⁽⁷⁾. In our study we used automated systems as they are more sensitive than conventional methods.

Conclusion

Proper diagnosis of blood stream infections in paediatric patients is lifesaving. The antibiotic resistance is increasing day by day which leads to treatment failure and mortality in patients. Using automated systems like BacT/Alert & VITEK II reduces the time for diagnosis and appropriate treatment is life saving in blood stream infections.

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Prevalence of Amp C β -lactamase production in gram negative uropathogens

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ABSTRACT:

Introduction – Urinary tract infection (UTI) is faced by millions of people every year. Gram negative bacilli are among most prevalent bacteria detected in the patients with UTI. β -lactam antibiotics are most widely used group of antimicrobials. Resistance to these antibiotics is increasing through production of β -lactamases. Amp C β -lactamases are important cephalosporinases encoded on chromosomes of many Enterobacteriaceae which mediate resistance to most of penicillin and β -lactamase inhibitor combination.

Materials & methods- Present study was undertaken to find out prevalence of Amp C β -lactamase producing gram negative uropathogens. The study was carried out in Dr. D. Y. Patil Medical College, Hospital, Research Centre, Kolhapur, over a period from 1/1/2015 to 30/6/2015. Isolation & identification of gram negative bacilli (GNB) was done by conventional methods. Isolates showing resistance to 3rd generation cephalosporins (3rd GC) were subjected to screening, Disk Antagonism Test (DAT). Amp C producers are confirmed by Amp C Disk Test. Minimum Inhibitory Concentration (MIC) of these isolates for Ceftazidime was determined.

Results-Total 180 urine samples were processed. Out of which 138 samples were positive for significant bacteriuria (76.66%). Gram negative bacilli (GNB) isolated were 105 (76%). *E. coli* (53.62%) was most common organism. Screening test for Amp C β -lactamase production in these isolates was positive in 29 isolates (27.61%). 22 isolates out of these were confirmatory for Amp C production by Amp C Disk Test. (20.95%).

Conclusion- It is concluded that prevalence of Amp C β -lactamase producing gram negative bacilli in urinary tract infection (UTI) was 20.95%. Regular reporting of these resistant strains will help the clinician to select proper antibiotic at the earliest.

Keywords: Urinary tract infection (UTI), gram negative bacilli (GNB), Amp C β -lactamase, Disk Antagonism Test (DAT), Amp C Disk Test.

1. Introduction

Urinary tract infections (UTI) are most common bacterial infections affecting human being throughout their life time. They are the frequent cause of morbidity in out-patients as well most frequently involved in the cause of nosocomial infection in many hospitals.¹ Urinary tract infections are the leading cause of gram negative bacteremia in patients of all ages & is associated with high risk of morbidity & mortality especially elderly which account for significant health care cost.^{2,3} Wide spectrum of organisms are implicated in its etiology, most common being *E. coli* & other GNB followed by gram positive organisms.^{4,5,6} Several antibiotic resistance mechanisms have been emerged among GNB especially related to β -lactam antibiotics is through production of β -lactamase enzyme that breaks down the β -lactam ring of penicillin and other antimicrobial of similar structure. β -lactamase are classified on the basis of primary structure into four molecular classes (A-D). Class C (Amp C) enzymes though having broad spectrum of activity are always encoded by chromosomes & are carried by plasmids.⁷

The increased resistance of uropathogens to antibiotics is due to empirical administration of antibacterial therapy even before availability of urine culture. So the present study was carried out in the Dept. Of Microbiology, Dr. D. Y. Patil Medical college, Hospital & Research Centre, Kadamwadi, Kolhapur to find prevalence of Amp C β -lactamase producing gram negative uropathogens.

Aim- To find prevalence of Amp C β -lactamase producing gram negative bacilli (GNB) in urinary tract infections. (UTI)

Objectives-

- 1) To isolate & identify gram negative bacilli (GNB) in urine samples obtained from patients of UTI
- 2) To detect prevalence of Amp C β -lactamase producing gram negative bacilli by screening and confirmatory test.

Materials & Methods

The present study was carried out in Dr. D. Y. Patil Medical College, Hospital & Research Institute, Kolhapur over a period from 1/1/2015 to 30/6/2015. Institutional ethics committee clearance was taken. Informed consent was obtained from all the patients before collection of urine samples.

Inclusion criteria- 1) All patients with suspected UTI were included in the study.

2) Gram negative bacilli isolated from urine samples with significant bacteriuria.

Exclusion criteria- 1) Urine samples of insignificant bacteriuria.

2) Gram positive bacteria isolated from urine samples of significant bacteriuria.

Sample collection- Midstream urine samples were collected in wide mouth sterile bottle by clean catch technique.

Processing of samples- It was done without delay. Samples were plated on Blood agar (BA), MacConkey Agar (MA) and plates were incubated at 37°C for 24 hrs. Isolation & identification of GNB was done by study of colony characteristics, gram staining, biochemical tests. After that all the isolates were subjected to Antimicrobial Susceptibility Testing (AST) by Kirby-Bauer Disk Diffusion method. Results were recorded as per CLSI guidelines.⁹

Antibiotic disks used-

(Hi-Media, Mumbai) Ceftriaxone (30 µgm), Ceftazidime (30 µgm), Cefotaxime (30 µgm), Ceftazidime-clavulanic acid (30/10 µgm), Piperacillin-tazobactam (30/10 µgm), Amikacin (30 µgm), Gentamicin (10 µgm), Ciprofloxacin (30 µgm), Imipenem (10 µgm), Meropenem, Nitrofurantoin (300 µgm)

Screening was done to identify probable of Amp C β-lactamase producers-

1) Isolates which showed resistance to 3rd generation cephalosporins and decreased susceptibility to β-lactamase inhibitors like clavulanic acid.

2) Disk Antagonism Test¹⁰- A lawn culture of test isolate (0.5 McFarland std.) was done on Muller-Hinton agar plate. Ceftazidime (30 µgm) & ceftoxitin (30 µgm) were placed 15mm apart. Plates were incubated at 37°C overnight. Isolates which showed blunting of zone of inhibition adjacent to ceftoxitin disk were considered as 'screen positive'.

Confirmation of Amp C β-lactamase producing isolates was done by Amp C disk test.^{10,11}

Amp C Disk Test - A lawn culture of std. strain *E.coli* ATCC 25922 was done on Mueller-Hinton agar plate. A Ceftoxitin disk (30 µgm) was placed on the inoculated media. Sterile filter paper disk (6mm) was moistened with sterile distilled water (20 µl). This disk was placed beside ceftoxitin disk (almost touching). Several colonies of test organism were inoculated on filter paper. Plates were inoculated at 37°C overnight.

Results were interpreted as

1) Positive - if flattening or indentation of ceftoxitin inhibition zone in the vicinity of test disk.

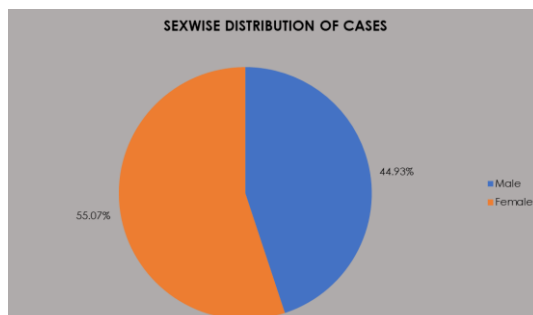
(3) Undistorted zone was taken as negative.

Minimum Inhibitory Concentration (MIC) was determined in all the isolates positive for Amp C β-lactamase production by Hi-Comb MIC stripes.

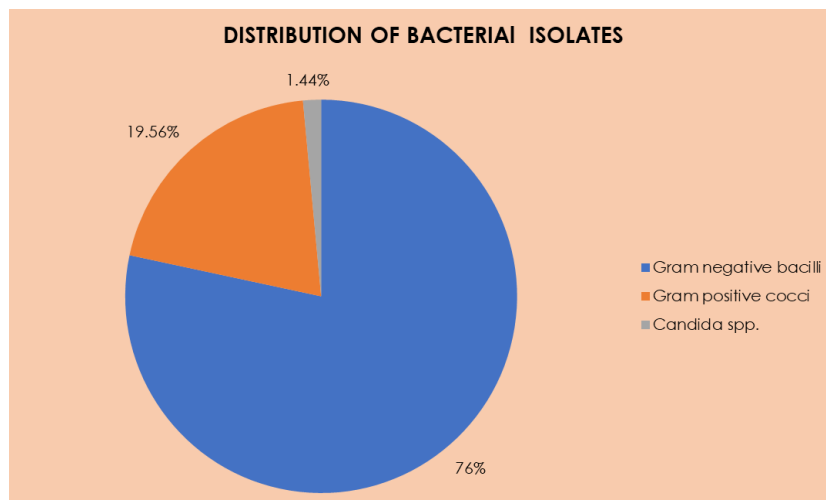
Results

Total 180 urine samples were processed. Out of which 138 samples gave significant growth of pathogens (76.66%). Prevalence of UTI was high among female 76/138 (55.07%) & in male was 62/138 (44.93%).

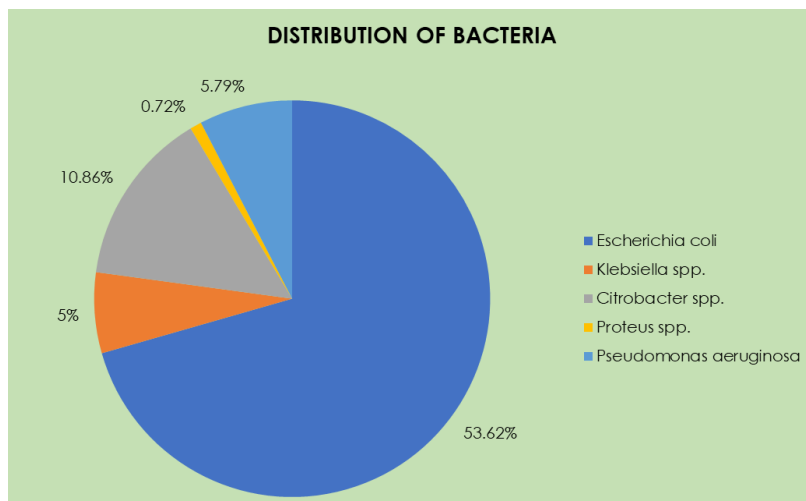
1) Sex wise distribution of cases



2) Distribution of bacterial isolates from Urine samples



3) Distribution of gram negative bacilli in urine samples

**Table 1:** Shows gram negative isolates positive for probable Amp C β -lactamase production(screening test)

Name of bacteria	Number of isolates	Percentage
<i>Escherichia coli</i> (n=74)	19	25.67%
<i>Citrobacterfreundii</i> (n=8)	3	37.5%
<i>Citrobacterkoseri</i> (n=7)	3	42.8%
<i>Klebsiella Pneumoniae</i> (n=7)	3	42.8%
<i>Pseudomonas aeruginosa</i> (n=8)	1	12.5%
<i>Proteus spp.</i> (n=1)	0	0%
Total(n=105)	29	27.61%

Data source- Dr.D.Y.Patil Medical College,Hospital& Research Institute,Kolhapur.

Total no. of Probable Amp C producers – 27.61%

Table 2: Shows gram negative isolates positive for Amp C β -lactamase production by confirmatory test

Name of the bacteria	Number of isolates	Percentage
<i>Escherichia coli</i> (n=74)	16	21.62%
<i>Citrobacterfreundii</i> (n=8)	2	25%
<i>Citrobacterkoseri</i> (n=7)	2	28.5%
<i>Klebsiella pneumonia</i> (n=3)	2	66.6%
<i>Pseudomonas aeruginosa</i> (n=8)	--	0%
<i>Proteus spp.</i> (n=1)	--	0%
Total (n=105)	22	20.95%

Data source- Dr.D.Y.Patil Medical College,Hospital& Research Institute,Kolhapur.

Total no. of Amp C producers by confirmatory test – 20.95%

Table 3: Minimum inhibitory concentration (MIC) of Ceftazidime in Amp C producers

MIC of Ceftazidime(μ gm/ml)	Isolates of <i>E.coli</i>	Isolates of <i>Klebsiella spp.</i>	Isolates of <i>Citrobacter spp.</i>
2			
4	3		1
8	-		
16	2		
32	2		2
64	6	1	
128	2	1	1
256	1		

Data source- Dr.D.Y.Patil Medical College,Hospital& Research Institute,Kolhapur.

Table 4: shows sensitivity of Amp C β -lactamase producing organisms to different antibiotics

Name of organism	(Ca)	Ci/CTR	(Ce)	Ak	PIT	Ca _c	IPM	MR	NIT	Cf/CIP	Gen
<i>E.coli</i> (n=16)	(3) 18.75 %	(1) 6.25%	(3) 18.75 %	(12) 75%	(6) 37.5 %	--	(16) 100 %	(13) 81.25 %	(14) 87.5 %	(11)68.75 %	(10) 62.5 %
<i>Citrobacter spp.</i> (n=4)	(1) 25%	(1) 25%	--	(3) 75%	(2) 50%	-	(3) 75%	(4) 100%	(3) 75%	(3) 75%	(3) 75%
<i>Klebsiella spp.</i> (n=2)	--	--	(1) 50%	(2) 100 %	(1) 50%	-	(2) 100 %	(1) 50%	(2) 100%	(1) 50%	(1) 50%

Data source- Dr.D.Y.Patil Medical College,Hospital& Research Institute,Kolhapur.

Antibiotics- Ceftazidime (Ca), Ceftriaxone (Ci/CTR),Cefotaxime (Ce),Amikacin (Ak), Piperacillin-tazobactam (PIT),Ceftazidime + clavulanic acid (Cac),Imipenem (IPM),Meropenem(MR),Nitrofurantoin(NIT),Ciprofloxacin(CF/CIP),Gentamicin(Gen).

Discussion

In India prevalence of Extended spectrum β - lactamase (ESBL) and Amp C β - lactamase is increasing.In the present study, processing of 180 urine samples from cases of suspected UTI was done.Out of these 138 samples were positive for significant bacteriuria (76.6%).Prevalence of UTI was high among female 76/138(55.07%) as compared to male which was 62/138(44.93%).

Ref.no.	Authors	Prevalence
12	Pramod Kumar Za,RatnaBaral et al.B.P.Koirala Institute of health sciences,Nepal.	Male-44.37% Female-55.63%
13	Syed Mustaq,RamkrishnaPai et al A Tertiary Care Centre in North Kerala.	Male- 32.36% Female-67.12%

Total 105 samples were positive for GNB (76%) , 27 samples showed GPC (19.56%) & 2 samples showed Candida (1.44%).Most isolated organism was *E.coli* (53.62%), followed by *Citrobacter spp.*(10.86%),*Pseudomonas* (5.79%),*klebsiella spp.*(5%),&*Proteus spp.*(1%).

Ref.no.	Authors	Prevalence
17	K.D.Deshapande,A.P.Pichare et al.MIMSR Medical college,Latur.	<i>E.coli</i> 50.02%, <i>Klebsiella</i> 24.6%, <i>Pseudomonas</i> 9.8%, <i>Citrobacterspp.</i> 5.4% <i>Proteus</i> 8.4%
18	Prakash Modi,Awadeshkumar et al,Gov. Medical College,Jamnagar,India.	<i>E.coli</i> 48.23%, <i>Klebsiella</i> 18.44%, <i>Pseudomonas</i> 14.19%, <i>Proteus</i> 1.42%

In the screening test for Amp C β -lactamase producers,cefotixin resistance (Disk Antagonism Test) was noted in 29 /105 isolates of GNB (27.61%).Amp C producers were confirmed by Amp C Disk Test. Out of screened isolates, 19 isolates were found to be amp C producers.

Ref.	Authors	Results
14	Ephraim, Ehidiamen et al.Study in Nigeria.(2018)	Amp C producers in UTI - 18.3%
15	AnithaMadhavan, V. Jaylaxmi et al.Gov. Medical college,Alappuza,Kerala,India (2016)	Amp C producers in UTI – 41.42%

In the present study, all these Amp C producers were sensitive to Imipenem (96%),meropenem(88%).

Conclusion

To conclude ,in the current study prevalence of Amp C β -lactamase producing gram negative uropathogens is 20.95%.Simple tests like Disk antagonism Test & Amp C Disk Test are useful in identifying Amp C Producers.Increasing drug resistance to third generation cephalosporins is observed in uropathogens. There is a need of reporting multidrug resistant organisms like Amp C producing GNB to avoid treatment failure & morbidity.

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Evaluate the Awareness of Hand washing by using Semi- Structured Questionnaire from a tertiary care hospital, Kolhapur

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ABSTRACT: Hand-hygiene is very importance in hospital setting to prevent the transfer of cross infection and antimicrobial resistance. Aim of the present study is to evaluate the awareness of hand washing by using Semi-Structured Questionnaire from a tertiary care hospital. Junior and senior resident doctors, nurses were included in this study. Knowledge of hand hygiene was by semi-structure WHO questionnaire. Scores of each corrected answers were calculated and compared. Of these, overall scores on hand hygiene was moderate level (80.64%), nurses had better knowledge when compared to others.

Keywords: Hand hygiene, Nurses, Infection

1. Introduction

A health care worker (HCW) harbors commensal flora as well as pathogenic microorganisms on hands during patient care. It acts as vector for the transmission of nosocomial infection ¹. Hospital acquired infections (HAIs) is one of the most preventable and challenging in modern medicine. ² In 2007 International Nosocomial Control Consortium (INCC) had reported the incidence rate of HAI is 4.4% i.e., 9.06 infections per 1000 intensive care units (ICUs). ³ Hand hygiene is one of the leading measure to prevent the cross transmission of nosocomial infection. ^{4,5} It can be performed by using simple soap and water, sanitizer (alcohol based) hand rubs.

Hand washing with soap and water is a 15 seconds procedure which can reduce the microbial load on skin by 0.6 to 1.1 log₁₀, Whereas 30 seconds reduces 1.2 to 2.8 log 10⁶; but it can remove only transient loosely adherent bacteria⁷. WHO recommends alcohol-based hand rubs to effective removal of pathogens and prevents the transmission of microbial resistance⁸

However, the basic hand washing with soap and water saves millions of lives to prevent the HAIs globally, especially in developing countries and it is too difficult to implement the hand washing protocol with alcohol based hand rubs in underdeveloped countries. WHO recommends five “My five moments for hand washing”. The concept is mostly used to improve the understanding, training and creating awareness of the hand hygiene among health care workers.⁹

The aim of the present study is to evaluate the awareness of the hand hygiene among nurses, resident doctors and post graduate medical students from a tertiary care teaching institute, Kolhapur by using a semi- structured questionnaire

Materials and Methods

Cross –Sectional, Observational and Questionnaire- based survey was conducted among Nurses, Junior resident doctors, and Senior resident doctors in a tertiary care teaching institute, Kolhapur, Maharashtra, India. The study protocol was approved by the institutional ethical committee.

Exclusion Criteria

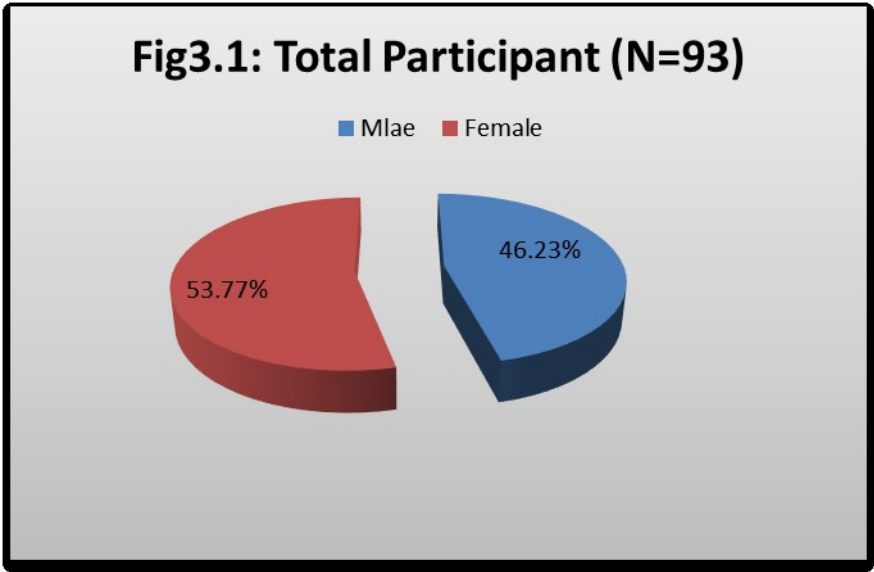
Those who on leave and who were not available for duties were excluded from this study.

Semi structured WHO hand hygiene questionnaire was used to access the knowledge of hand washing and it was distributed to respective wards. It consists of 15 questions, each correct answer was given 1 marks and incorrect answer zero mark.

The scores were calculated and given overall scores by using percentage, < 50% considered poor, 50-74% moderate, 75% was taken as good.

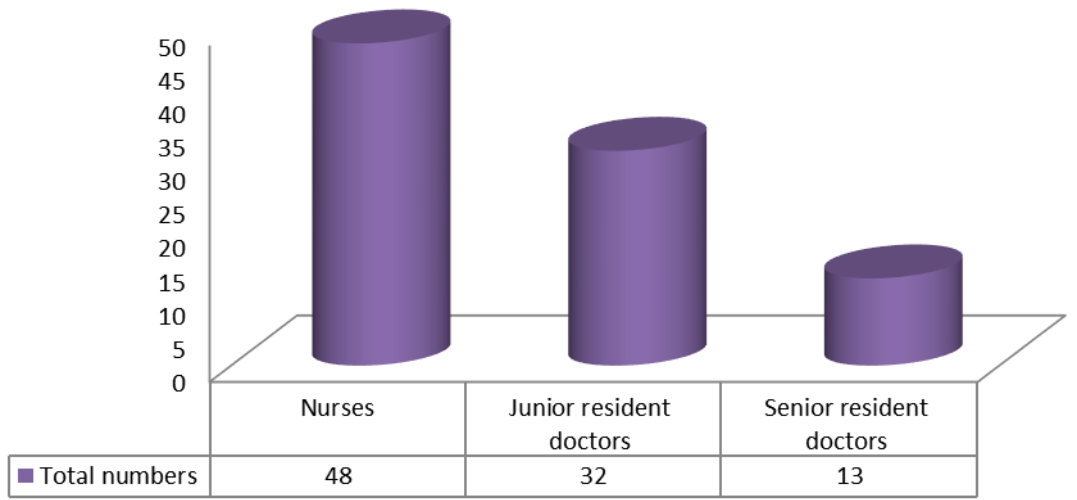
Results

Out of 93 participants, 43 (46.23%) were male and remaining 50(53.77%) were female [Figure3.1]



Of these 93 participants, 48 were staff nurses, 32 were Junior resident doctors and remaining 13 were Senior resident doctors [Fig 3.2]

Fig 3.2: Questionnnarie Distribution



Participant hand hygiene Questionnaire and Knowledge has been summarized in Table 3.1

Table 3.1: Questionnaire and distribution of correct answers

Sr. No	Questions	Nurses (n=48)	Junior Resident Doctors (n=32)	Senior Resident Doctors(n=13)
1	Do you routinely use an alcohol-based hand rub for hand hygiene?			
	Yes	43(89.58%)	30(93.75%)	12(92.30%)
	No	5(10.42%)	2(6.25%)	1 (7.70%)
2	In your opinion, What is the percentage of hospitalized patients who will develop a health care associated infection			
	20-40%	36(75%)	27(84.37%)	10(76.92%)

	Above 50%	8(16.67%)	3 (9.38%)	1 (7.70%)
	Don't Know	4(8.33%)	2(6.25%)	2(15.39%)
3	What is the effectiveness of hand hygiene in preventing HAI?			
	High	39(81.25%)	27(84.38%)	11(84.61%)
	Low	9(18.75%)	5(15.62%)	2(15.39%)
4	Hand hygiene reduces chances of spreading infections			
	Agree	40(83.33%)	29(90.62%)	12(92.30%)
	Not agree	8(16.67%)	3 (9.38%)	1 (7.70%)
5	Hand Hygiene is regularly talked about at staff meetings			
	Yes	30(62.5%)	18(56.25%)	08(61.53%)
	No	18(37.5%)	14 (43.75%)	5(38.47%)
6	In this organization I feel hand hygiene is an important part of my job			
	Yes	35(72.92%)	17(53.12%)	09(69.23%)
	No	13(27.08%)	15(46.88%)	4(30.77%)
7	I can't wash my hands at times because			
	Too busy	3(6.26%)	2(6.25%)	2(15.39%)
	Forget or don't think about it	-	-	-
	Products or practices damage my skin	5(10.41%)	4 (12.5%)	1 (7.69%)
	Others	40(83.33%)	26(81.25%)	10(76.92%)
8	Among all patient safety issues, how important is hand hygiene at your institution			
	High priority	41(85.41%)	27(84.38%)	11(84.61%)
	Low priority	7(14.59%)	5(15.62%)	2(15.39%)
9	The best way to be reminded to wash my hands is by my			
	Co-worker	-	-	-
	Leader	29(60.41%)	24(75%)	07(53.84%)
	Patient	19(39.59%)	8(25%)	06(46.16%)
10	In your opinion, how effective would the following actions to be improving hand hygiene permanently in your institutions? [Effective/Not effective]			
11	The health care facility makes alcohol-based handrub always available at each point of care	42(87.5%)	29(90.62%)	13(100%)
12	Hand hygiene posters are displayed at point of care as reminders	40(83.33%)	31(96.87%)	13(100%)
13	Each health care worker receives education on hand hygiene	34(70.83%)	25(78.12%)	09(75%)
14	Clear and simple instructions for hand hygiene are made visible for every health care worker	38(79.16%)	30(93.75%)	13(100%)
15	Health care worker regularly receive feedback on their hand hygiene performance	37(77.08%)	25(78.12%)	07(58.33%)
16	Patients are invited to remind health care workers to perform Hand hygiene	35(72.91%)	23(71.87%)	06(50%)

Table 3.2:Distribution of score level

Score Level	Nursing (48)	Junior Resident doctors (32)	Senior Resident Doctors(13)	Percentage(N=93)
Moderate	42(87.5)	26(81.25)	7(53.84)	75(80.64%)
Good	4(8.33)	4(12.5)	2(15.39)	10(10.76%)
Low	2(4.17)	2(6.25)	4(30.77)	8(8.60%)

Knowledge of hand hygiene among all participant (N=93) was moderate (80.64%), followed by Good (10.76%) and low level were only 8.60%

Nursing staff had better knowledge about hand hygiene compared to junior resident doctors and senior resident doctors [Table 3.2]

Discussion

Hand washing is the least expensive and simplest procedure to reduce the prevalence of nosocomial infection and the transmission of antimicrobial resistance¹¹. In modern health care institutions, adherence to hand hygiene is very low and were risk for developing multi drug resistance¹².

In our study, to evaluated the awareness of the hand hygiene in health care workers; awareness of the hand washing was found to be moderate level (80.64%). Of these, nursing staff had better awareness (87.5%), followed by Senior resident doctors (81.25%) and Junior resident doctors (53.84%). This data was similar to the finding of Nair et al, *Van de mortel et al*, *Ariyaratne et al*.

In the present study, we conclude that awareness of the hand hygiene was found to be moderate. The knowledge about hand hygiene is lacking in some of the nurses and resident doctors. To change this attitude is a challenging task. While studying we must include and give practice about hand hygiene, conduct regular poster presentation and quiz competitions among the health care workers and medical graduates to create the awareness about hand hygiene.

Limitation

This study was conducted at a single private teaching institute and it is a cross sectional study with small sample size. Semi-structured questionnaire was used for self-assessment. Further multicentric and qualitative studies are required to identify the potential gaps in hand hygiene among medical graduates and health care workers

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Incidence of *Pseudomonas Aeruginosa* in Post – Operative Wound Infections in A Teaching Hospital in Western Maharashtra

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ABSTRACT:

Background: Until the middle of the 19th century, when Ignaz Semmelweis and Joseph Lister became the pioneers of infection control by introducing antiseptic surgery, most wounds became infected. In cases of deep or extensive infection this resulted in a mortality rate of 70-80% ¹. Since then a number of significant developments, particularly in the field of microbiology, have made surgery safer. However, the overall incidence of healthcare associated infections (HAIs) remains high and represents a substantial burden of disease. Surgical site infections are the third most commonly reported nosocomial infection & they account for approximately a quarter of all nosocomial infections ² They have been responsible for the increasing cost, morbidity & mortality related to surgical operations & continued to be a major problem even in hospitals with most modern facilities & standard protocol of pre-operative preparation & antibiotic prophylaxis ³

Aims and Objective: The main aim of this study was to know the incidence of *Pseudomonas aeruginosa* in post-operative wound infection and its sensitivity pattern. In cases of deep or extensive infections the mortality rate was 70-80% ¹. Surgical site infections are the third most commonly reported nosocomial infection & they account for approximately a quarter of all nosocomial infections ²

Materials and Methods: Clinical Isolates were obtained from various samples like pus, discharge etc. from the surgical wounds of 100 patients undergone surgery at D.Y.Patil Hospital, Kolhapur and processed in our clinical microbiology lab. The microscopic characters of these isolates were studied. Isolation of organisms from the said samples using suitable media like Nutrient agar, Blood agar, MacConkey agar & Robertson Cooked Meat media for anaerobic organisms was carried out. Organisms were identified by using specific biochemical tests.

Conclusion: Out of the 100 bacterial isolates from post-operative wound infection, 24 (24%) were *Pseudomonas aeruginosa* followed by *Staph. aureus* (22%), *CONS* (20%); *Escherichia coli* (15%); *Klebsiella pneumoniae* (15%); *Proteus vulgaris* (2%); & *Citrobacter freundii* (2%). *Pseudomonas aeruginosa* isolates were sensitive to Imepenem (87.5%), Ceftazidime – Clavulanic acid (87.5%) & Aztreonam (83.33%)^{4,5,6}.

This study showed an increased rate of incidence of *Pseudomonas aeruginosa* in post - operative wound infections.

Keywords: Post-operative wound infection, *Pseudomonas aeruginosa*, nosocomial infection, antibiotic.

1. Introduction

Post-operative wound infections are surgical site infections & they are the main culprits for nosocomial infections. Because the consequences of surgical site infections are so significant to patients, surgeons and other care providers as well as to local and national healthcare organizations, it is worth reviewing the most common sources of surgical wound contamination and steps that can be taken to minimize the pathogen dose and virulence^{3,4}. The virulence of the organism also depends on immunological status of host as well as the condition of tissue infected^{1,2,3}.

The leading cause of hospital associated infection is *Pseudomonas aeruginosa* and is the second most common infections by gram negative bacteria contributing to wound related morbidity and mortality all over the world including India. *Pseudomonas aeruginosa* spills over into the blood from the site of entry and causes septicemia that may reach the skin causing a black Coloured necrotic lesion called ecthyma gangrenosum. Lipopolysaccharides (LPSS), exotoxin A, leukocidin, extracellular slime, proteases, phospholipase, and several other enzymes secreted by *P. aeruginosa* makes it the most clinically significant pathogen among non-fermenting bacteria. These substances increase the virulence of *P.aeruginosa*. It also carries plasmids that code for resistance to many drugs. The virulence and the invasive capacity of the organism determines the rate of nosocomial infection^{4,5,6}

Routine surveillance for hospital-acquired infections is recommended by both the Centers for Disease Control & Prevention & the Surgical Infection Society. Surgical wound infection was defined as one in which there was any skin eruption or drainage at the surgical site that was positive for bacteria by culture within 60 days of a surgical procedure ^{5,6} Surgical site infections are the third most commonly reported nosocomial

infection & they account for approximately a quarter of all nosocomial infections. They have been responsible for the increasing cost, morbidity & mortality related to surgical operations & continued to be a major problem even in hospitals with most modern facilities & standard protocol of pre-operative preparation & antibiotic prophylaxis. The surgical site infection due to *Pseudomonas aeruginosa* is becoming more serious in developing countries⁷.

Objective of this study is to know the incidence of *Pseudomonas aeruginosa* in post – operative wound infection and its susceptibility pattern.

Materials & Methods

The study was conducted at Clinical Microbiology Laboratory at D.Y.Patil Hospital & Research Institute, Kolhapur.

Study Sample: 100

Study Design: randomized cross-sectional Study, conducted in D.Y.Patil Medical College, & Hospital Kolhapur after approval From IEC (Institutional Ethical Committee) over a period of 1 years.

Clinical Isolates- The clinical samples like pus; discharge oozing from surgical wound and wound swabs were collected from the patients who had undergone surgeries and developed post – operative wound infection.

Inclusion criteria: All the patients who had undergone surgery, having post-operative wound infection and admitted to different surgical wards were included in this present study.

Exclusion criteria: Patients with wound Infections without surgery were excluded.

Sample collection: The pus and the serous fluid samples from the patients with post – operative wound infection were collected by two sterile & moist swab sticks under all aseptic precautions.

Transportation & Storage: The swab sticks were transported to the clinical microbiology laboratory of D.Y.Patil Hospital & research center Kolhapur as early as possible embedded in normal saline & Brain Heart Infusion Broth (HBI). In case of inevitable delay they were refrigerated.

Processing of samples: One swab stick dipped in normal saline was used for Gram staining & incubated at 37°C for 24 hours. The second swab stick dipped in BHI broth was inoculated on Blood agar & McConkey's agar & were incubated at 37°C for 24 – 48 hours. The isolates were identified on the basis of cultural characters, & the biochemical tests such as Indol production test, motility test, Methyl red test, Voges Proskauer test, catalase test, coagulase test, urease test and oxidase test. Subsequently the isolates were cultured on Nutrient agar, Mannitol salt agar.

All the above culture media and reagents were procured from Hi Media Mumbai. The culture plates were incubated at 37°C for 18 – 24 hours. The plates were read the following day and extended upto 48 hours if no growth within 24 hours. Gram staining and biochemical tests were carried out for the isolated colonies for identification. Identification was done with the help of standard biochemical tests^{8,9}.

Antibiotic Susceptibility testing: Antibiotic susceptibility testing of isolated organism was carried out on Mueller Hinton Agar by Kirby Bauer disc diffusion method, according to CLSI guidelines. The antibiotic susceptibility testing was not done for the other organisms that were isolated from the infected surgical wounds, since this study focused on prevalence of *Pseudomonas aeruginosa* causing surgical site infection. The standard strain of *Pseudomonas aeruginosa* (ATCC27853) was used as control. Antibiotics used in the study included Piperacillin (100 µg), Ceftazidime (30 µg), Cefepime (30 µg), Imipenem (10 µg), Meropenem (10 µg), Ampicillin/ Sulbactam (10/10 µg), Piperacillin/Tazobactam(100/10 µg), Ticarcillin/Clavulanate(75/10 µg), Gentamycin(10 µg), Tobramycin(10 µg), Amikacin(30 µg), & Ciprofloxacin(5 µg).

Results and Observations

Statistical analysis by percentile method

Table 1:Prevalence of Post – Operative wound infection

Department	No. of cases	%
Surgery	38	54.28
OB / GY	18	25.71
Ortho	4	5.71
Minor OT	4	5.71
ENT	3	4.28
Ophthalm	3	4.28

Data source: D.Y.Patil Hospital &Research Institute Kadamwadi Kolhapur

Table 2:Organisms isolated from post – operative wound infection Wound Infection

No. of cases	Organism isolated	%
Pseudomonas	24	24
Staph. Aureus	22	22
CONS	20	20
E.coli	15	15
Klebsiella pneumoniae	15	15
Proteus vulgaris	02	02
Citrobacter frundii	02	02

Data source: D.Y.Patil Hospital &Research Institute Kadamwadi Kolhapur

Results and Observations

Total 100 samples were collected from the patients with post – operative wound infections admitted to surgery, Orthopedic, Obstetric wards. Among these 100 (100%) samples revealed growth. *Pseudomonas aeruginosa* 24(24%) was the most common isolate followed by *Staphylococcus aureus* 22(22%), CONS 20 (20%), *E.coli* 15 (15%), *Klebsiella pneumonia* 15 (15%), *Proteus vulgaris* 02 (2%) & *Citrobacter frundii* 02 (2%). *Pseudomonas aeruginosa* isolates were sensitive to Imipenem (87.5%), Ceftazidime – Clavulanic acid (87.5%) & Aztreonam (83.33%). Out of 24 isolates of *Pseudomonas aeruginosa* 4 isolates (16.67%) were resistant to three or more class of antibiotics. Frequency of isolation of *P. aeruginosa* was maximum in patients who underwent caesarean section followed by abscess drainage and Diabetic foot. The infection with *Pseudomonas aeruginosa* was common amongst surgical patients. The post-operative wound infection with *Pseudomonas aeruginosa* was common in males (83.83.33%) than in females (16.67%)^{10, 11, 12}.

Discussion

Surgical site infection is one of the most important cause of hospital associated infection and occurs in patients with longer hospital stay. Narayan Kamat, Dr.Swaminathan et al from Padmashree Dr.D.Y.Patil Medical College & Hospital, Nerul, Navi Mumbai 2008 – 2009, determined the bacteriological profile of SSI in their hospital. They found *E.coli* (18.7%), *Klebsiella* (14.7%), *Pseudomonas* (15.37%), *Staph.aureus* (29.26%).All these isolates showed 100% sensitivity to Linezolid, Imipenem, Piperacillin – Tazobactam^{12, 13, 14}.

A study on bacteriological profile & antibiotic study pattern of post-operative wound infection, B.M.Patil Medical College, Bijapur by Laxmi Kakhandi et al found *Staph aureus* 31%, *Klebsiella* 7%, *E.coli* 4%, *Proteus* 3%. These isolates were sensitive to Linezolid, Imipenem, Amikacin, & Amoxi – Clav^{13,14}.

In present study organisms isolated in post – operative wounds are as follows. –

Staph aureus 22%, *Pseudomonas* 24%, *E coli* 15%, *Proteus vulgaris* 2%, *Klebsiella pneumoniae* 15% & *Citrobacter frundii* 2%. *Pseudomonas aeruginosa* isolates were sensitive to Imepenem (87.5%), Ceftazidime – Clavulanic acid (87.5%) & Aztreonam (83.33%), while maximum resistance was seen to Amikacin (58.33%) and Ceftriaxone (54.16%) and Cefotaxime (54.16%).Cefotaxime resistant strains were probably ESBL producers¹⁵.

Conclusion

To conclude, the prevalence of *Pseudomonas aeruginosa* was 24% So it is rising as one of the cause for post – operative wounds infection. Most were sensitive to Imepenem (87.5%), Cefazidime – Clavulanic acid (87.5%) & Aztreonam (83.33%)

According to WHO, the low rate of post – operative wound infection indicates good health of treating Hospital. To minimize post – operative wound infection & development of drug resistance, the hospital must have –

1. Rational Hospital Drug Policy & rational antibiotic policy
2. A functioning Hospital Infection Control Committee comprising Clinicians, Microbiologists & Nursing Staff.

Consent

A verbal and written consent was obtained from the patients to continue with this study.

Institutional Ethical Committee Approval

As this study was a part of my thesis work it was duly approved by the institutional ethical committee.

Source of Conflict

None to be declared

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Knowledge, attitude and practices regarding prevention of dengue and malaria among residents of urban area of Kolhapur.

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ABSTRACT:

Background-

Vector-borne diseases account for more than 17% of all infectious diseases, causing more than 1 million deaths every year globally. Since we got epidemic of dengue this year in Kolhapur District, it was very much necessary to plan health education programs in our field practice area. Vector control methods can be successful, only if there is community participation and for success of a community-based program, it's important to assess community's perception regarding the disease, its mode of transmission and breeding sites. Knowledge, attitude and practice studies serve as an educational diagnosis of population.

Objective-

- 1) *To assess knowledge, attitude and practices regarding dengue and malaria among residents of Kolhapur city.*

Methodology

Community-based cross sectional study was conducted from 1stJuly to 15thAug 2018 in the field practice area of UHTC under jurisdiction of Department of Community Medicine. Data was collected by face to face interview by using pretested, semi-structured questionnaire by trained interviewers. The houses for data collection were selected by simple random sampling. One adult respondent from each selected households were selected randomly & verbal consent was obtained before collecting the information. Details were collected regarding causative agents for dengue and malaria, their breeding places, signs and symptoms, treatment seeking behaviour & prevention & control measures.

Comparative study of NAFLD fibrosis score and APRI score in ultrasonographically diagnosed NAFLD with type2 diabetes patient.

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Introduction

The prevalence of NAFLD is highest in populations with pre-existing metabolic conditions such as obesity and type II diabetes. Many studies investigating the natural history of NAFLD verify the progression from NASH to advanced fibrosis and hepatocellular carcinoma. (1) Dyslipidemia, diabetes and NAFLD are present in asymptomatic general population. Males and persons with high BMI, atherogenic dyslipidemia and diabetes or prediabetes have greater odds of having hepatic steatosis. Association of atherogenic dyslipidemia with NAFLD provides both a clinical marker and therapeutic target for NAFLD. Type 2 diabetes mellitus is seen in 25% of NAFLD patients, whilst about 75% of the diabetic population have NAFLD. (2)

The presence of NAFLD in patients with T2D is related with an increase in all-cause mortality, whereas the presence of Type-2DM triples the risk of fibrosis, doubles the risk of hepatocellular cancer and correlates independently with the overall mortality in patients with NAFLD. (3) Because of the proven and strong bidirectional correlation between T2D and NAFLD, it is now recommended that the patients diagnosed with NAFLD should be also tested for the presence of T2D and vice versa regardless of the levels of the liver enzymes. (4)

The diagnosis of NAFLD is based on clinical findings, blood tests (classic and newer biomarkers), imaging studies and liver biopsy. Though hepatic ultrasonography (USG) is usually the index investigation, its sensitivity is low (30%) when only mild steatosis is present. (5,6) USG does not help to differentiate between fatty liver and NASH and nor is it a reliable to assess the extent of fibrosis. (7) Non-invasive scores to better determine the grade of steatosis and fibrosis in NAFLD, which would be not only inexpensive, but also easy to perform & would be ideal. (8,9,10) Many non-invasive scores have been proposed for NAFLD detection and staging. (11, 12)

USG has interobserver variability for grading of fatty liver but the fibrosis scoring systems are specific predictors of fibrosis. The objective of this study was to compare NFS, APRI score in relation to the USG grading of NAFLD patients.

Materials and method

This cross sectional observational study was conducted over 2 years at a tertiary level teaching hospital in Western Maharashtra, India. Approval of the Institutional Ethics Committee was taken. Type 2 diabetic patients diagnosed with nonalcoholic fatty liver disease on ultrasonography were selected with exclusion of critically ill patients, patients with multi-system disorders or diagnosed viral hepatitis, those on hepatotoxic drugs and history of hepatobiliary surgery. Informed consent was taken from all patients for use of their data. Total of 150 patients were included in the study.

History (including family corroboration of alcohol intake where required), detailed examination, anthropometric measurements, Complete Blood Count, Liver Function Tests were carried out in the study participants. NAFLD Fibrosis Score (NFS) & AST to Platelet Ratio Index (APRI) score were calculated and compared with each other in relation to the USG grading of NAFLD patients. USG grading was done by an independent, single radiologist to reduce inter-observer variability.

Statistical analysis

Software STRATA version 22 was used to analyze the data. Percentages were calculated. Chi square test was used as the test of significance. Pearson correlation coefficient was calculated to see the correlation between scoring system. P value less than 0.05 was considered for significance. Results are presented in form of tables and graphs.

Results

Table 1: Gender distribution of study subjects according to USG Grading

Gender	USG grading		Total (%)	Chi Square test
	I (%)	II (%)		
Male	42(80.76)	74 (75.51)	116 (77.33)	X ² =0.53, P=0.46
Female	10 (19.24)	24 (24.49)	34 (23.64)	
Total	52 (100)	98 (100)	150 (100)	

Significantly larger number of subjects had Grade II fatty liver on USG. 34.67 % (n=52) having grade I and 65.33 % (n=98) having grade II fatty liver on USG. (p=.000).

Table 2:Sensitivity and specificity of NFS score in diagnosis of fibrosis

Type of score	Fibrosis	Fibrosis on USG	
		Present	Absent
NFS	Present	95	45
	Absent	3	7

Sensitivity and specificity of NFS score in diagnosis of fibrosis was 96.94% and 13.46 % respectively

Table 3:Sensitivity and specificity of APRI score in diagnosis of fibrosis

Type of score	Fibrosis	Fibrosis on USG	
		Present	Absent
APRI	Present	88	24
	Absent	10	28

Sensitivity and specificity of APRI score in diagnosis of fibrosis was 89.8 % and 53.85 % respectively

Discussion

The issue of detecting liver disease in diabetics is relevant since cirrhosis is a significant cause of morbidity and mortality in this patient population and this end-organ damage is frequently overlooked in the primary care setting. (13) In the present study we used a non-invasive surveillance approach for detecting hepatic fibrosis in NAFLD patients with type 2 diabetes. Here we compared various scoring systems with Ultrasonographic diagnosis of hepatic fibrosis

In present study total of 98 subjects (65.33%) showed Grade II fatty liver. Female subjects had a higher proportion of Grade II fatty liver (70.59%, n=24) compared to the male subjects (63.79%, n=74) (p>0.05).

APRI score has maximum specificity (53.85%) in detection of fibrosis. There are variation in results shown by other studies. KadriAtay et al (2017)(14) in their study mentioned that APRI score was 35% sensitive respectively in diagnosis of advance fibrosis. Suzanne E. Mahady et al (2017)(15) found that NFS and APRI scores 7% and 13% sensitive in diagnosis of advanced fibrosis. Jessica K Dyson (2013) (16) in their review mentioned that using NFS score advanced fibrosis can be reliably excluded (NPV 93%) using the low cut-off score (<-1.455) and diagnosed with high accuracy (PPV 90%) using the high cut-off score (>0.676) which reliably excludes advanced fibrosis. Singh A (2018)(17) in their retrospective chart analysis found 20% and 22.1% sensitivity for NFS score and APRI score respectively in diagnosis of advanced fibrosis. A. M. Angelidi et al (2017) (18) in their study found that APRI scores 49% sensitive in diagnosis of hepatic fibrosis.

In our study sensitivity of scores in diagnosis of fibrosis was very high. This may be because of number of patients with advanced fibrosis were less in our sample. Also we were unable to carry out liver biopsy which is gold slandered test for diagnosis.

Conclusion

NAFLD fibrosis score was more sensitive than APRI score in diagnosing fibrosis in NAFLD with type 2 diabetes

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