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TELANGANA AND ANDHRA PRADESH COMBINE CHAPTER

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NEWS BULLETIN - 2022

Editor: **Dr. A. Kishore,** Professor, Apollo Institute of Medical Scinces & Research, Chittoor

PRESEIDENT'S MESSAGE

It is my great pleasure to welcome you all for the 25th IAMM TAPC Chapter Silver Jubilee Annual State Conference 2022. I extend my best wishes to the organizing team, Department of Microbiology, GITAM Institute of Medical Sciences & Research, Visakhapatnam. I wish the conference a great success which transitioned from hybrid to physical bringing all together on one platform. **I** sincerely thank all the dynamic executive members who conducted membership drive and were successful in reaching the target. I congratulate the organising committee members for coming up with Post Graduate write ups with knowledgeable topics.

ICMR has established Viral Research Diagnostic Laboratories national wide and trained all the laboratories which helped to establish RTPCR facility at every corner of the country which facilitated in the early diagnosis of infections, leading to quarantine and containment of the infection.

Now RTPCR testing has become a common tool which is now available in all health care facilities.

Under the guidance and support of ICMR, INDIA could do testing of **88,80,68,681** samples till September 06,2022.

Whole genome Sequencing (WGS) has played a key role in identifying the novel SARS-CoV-2 in Wuhan in 2019. Despite seminal advances towards understanding its infection mechanism, SARSCoV-2 continues to cause significant morbidity and mortality worldwide. Though mass immunization programs have been implemented in several countries, the viral transmission cycle has shown a continuous progression in the form of multiple waves. A constant change in the frequencies of dominant viral lineages, arising from the accumulation of nucleotide variations (NVs) through favourable selection, is understandably expected to be a major determinant of disease severity and possible vaccine escape. Indeed, worldwide efforts have been initiated to identify specific virus lineage(s) and/or NVs that may cause a severe clinical presentation or facilitate vaccination breakthrough. Since host genetics is expected to play a major role in shaping virus evolution, it is imperative to study role of genome-wide SARS-CoV-2 NVs across various population

As the virus spread across the globe and adapted to various ethnic groups, immunological and environmental challenges, several variants emerged as numerous mutations got accumulated progressively. WGS helped in identifying the VOC in spatio-temporal context enabling various countries to formulate and implement public health measures for containment of the virus. The emergence of new variant has resulted in multiple waves of the pandemic in different countries. We have witnessed 3 waves in India, each caused by different variants, namely, first wave by B.1 (Kappa variant), second wave by Delta variant, and the third wave by Omicron dominated by BA.2 lineage. CDFD and Gandhi Medical College are part of INSACOG performing sequencing for Telangana region.

INSACOG is the forum set up under the Ministry of Health and Family Welfare by the Government of India on 30 December 2020, to study and monitor genome sequencing and virus variation of circulating strains of COVID-19 in India. The robust surveillance system was very efficient in guiding the outbreak investigation processes in the country and illustrates the future importance of molecular epidemiology in leading the national response to outbreaks and pandemics. To prepare for the next phase of the pandemic, a systematic approach is needed to link global genomic surveillance and timely assessment of the phenotypic characteristics of novel variants, which will support the development and updating of diagnostics, vaccines, therapeutics and non-pharmaceutical interventions. Gandhi medical college Secunderabad Telangana has been included as one of the IGSL site from 13 th August 2021 .ICMR and Telangana state government played a major role in establishing Whole genome sequencing platforms and Reagents which helped in the real time monitoring of SARSCoV2 variants. CDFD and Gandhi Medical College are part of INSACOG performing sequencing for Telangana region Since the sequencing facility at GMC was made operational in October 2021, GMC have sequenced **3228** samples and obtained whole-genome sequences of SARS-CoV-2 for **2677** samples using the **Illumina MiSeq next-generation sequencer**.

I appreciate your participation and hope you will find this conference stimulating and rewarding.

Best wishes to the Organizing committee – 2022

- **Dr.K.Nagamani,**

President –IAMM TAPC Chapter XXIV Professor, Vice Principal
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Principal Investigator-SLVRDL, Nodal Officer -MDRU
State Nodal Officer –Viral Genome Sequencing
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Secunderabad, Telangana.

SECRETARY'S MESSAGE

August greetings to all the IAMM TAPC Life members Its almost a year with my journey as General secretary, I have attempted to keep the rich legacy of IAMM TAPC Association, works done by previous office bearers whilst trying to introduce newness in certain spheres of association through meetings among the executive committee members regularly. We have streamlined the WhatsApp group, grown leaps and bounds with increasing life membership among faculty, including Pro-life PG membership. Awaited and long-standing registration of association almost done, simultaneously we are at the verge of completion the website update. The 25th Silver Jubilee annual conference at GITAM is a special occasion to inscribed in the history of IAMM TAPC chapter, looking forward to it and planning extensively to make it memorable for one and all.

On behalf of the executive committee, I assure you that more academics online/offline mode series for Undergraduate and Post Graduate Students.

We have lost many of love and revered members down the journey. May the departed soul rest in peace. My sincere prayers for all the family members and close ones,

Last but not the least I thank our President Dr Nagamani madam, Joint secretary Dr Vijendra. K, Treasurer Dr A. Kishore, EC members for providing me constant support and instilling in me confidence. On behalf of Executive Committee, I extend a warm welcome to all the senior faculty members, looking forward their strong support cooperation for fruitful discussions in academic meetings of the association, request also all the Senior IAMM life members come ahead and support the various academic activities thereby strengthening our association with every coming day.

It is with Immense pride and great pleasure that I take a note of 25th Silver Jubilee Annual TAPC Conference at GITAM, Vizag (9th – 11th September 2022)

I am quite happy and thrilled to note that this conference will bring in interactive academic sessions, papers and guest lectures by eminent personalities among us.

Long live IAMM TAPC ASSOCIATION

-Dr Mohammed Khaleel,
General Secretary, IAMM TAPC CHAPTER,
Professor & HOD, Microbiology,
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Vikarabad, Telangana

ANTI PARASITIC AGENTS: THE NEGLECTED ARENA

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Introduction: Parasitic diseases constitute the majority of the neglected tropical diseases. As they are neglected diseases, so consequently, development of anti-parasitic agents have also lagged behind compared to antibacterial or anti-viral agents. Thus, for example, from 2010-21 more than thirty anti-viral agents have been approved by FDA compared to only *five* anti-parasitic agents, and only one during 2010-17. The major reason is because market forces are insufficient to drive the discovery and development of new drugs for parasites. This has led to the withdrawal of many large pharmaceutical companies from direct involvement in anti-parasitic drug discovery.

Challenges in the development of anti-parasitic drugs: There are multiple roadblocks in the research and discovery of anti-parasitic drugs. Briefly, these include:

a. *Flaw in the older approach*: Historically, many anti-parasitic drugs were first developed for unrelated use. Thus, there was an opportunistic advantage before using it against parasites, because of existing knowledge about the drug. It did not favour the introduction of novel drugs and we have reached a stage of diminishing returns because of drug resistance
b. *Need for Multi-disciplinary and Multi-Organizational Collaboration*: There is an urgent need for formation of effective partnerships for 'virtual drug discovery'. Increasing the role in such partnerships of researchers, public-health and industry leaders in the disease-endemic countries remains a challenge.
c. *Optimum Product Profile*: For the 'neglected' diseases, drug discovery is principally field-driven which means an emphasis on low cost of goods, short treatment regimes and the ability to use the drug safely in the absence of close medical supervision. Thus, there is need to define desired product profiles based on what is required for use in resource-poor settings.
d. *Parasite Selection*: There is lack of good, highly predictive *in vitro* and *in vivo* assays for activity using the same parasitic organism that infects the human patient. The helminth models offer particular challenges since *in vitro* assays of anti-helminthic activity are not standardized. Moreover, although the parasitic strains used in laboratory tests are often the same or very similar to those infecting the human patient there are certain cases where important differences exist. For example, the standard animal models for malaria infection use *P. berghei, P. chabaudi, P. yoelii* or *P. vinckei* rather than the *Plasmodium* species that infect humans. Similarly, the *Onchocerca gutturosa* worms used as *in vitro* models for onchocercal infection are parasites of cattle rather than humans. These differences can be especially crucial when a molecular target-based drug discovery strategy is followed exemplified by the cysteine proteases of *P. vinckei (*vinckepains*)* which differ from those of *P. falciparum* (falcipains). Genetically modified parasites in which the pathogen target replaces the model target gene would be one solution to this problem.
e. *Animal Models*: Most of the diseases require testing in several types of animal model. Mouse and hamsters are the most common animals used and for many parasites, ideal animal model is lacking.
f. *Economic Considerations*: High cost of drug R&D and the lack of any significant commercial return from the neglected diseases have resulted in a general withdrawal of the pharmaceutical industry from R&D for tropical diseases. This level of 'market failure' exists even for major disease like malaria, and the problem is multiplied many-fold for the other neglected diseases such as leishmaniasis, African trypanosomiasis, soil transmitted helminths and others.

New Anti-Parasitic Agents: During the last ten years (2011-2021), FDA has approved only five drugs.

a. Miltefosine (2014): It is the first oral formulation for leishmaniasis which was first developed as an anti-neoplastic agent in 1980s. In 1998, Phase II oral trial for visceral leishmaniasis was conducted by Shyam Sundar and his team from Varanasi. This drug has been developed by public-private partnership and approved for all forms of leishmaniasis. It has got multiple effects on the parasite like interference with lipid metabolism, inhibition of phospatidylcholine, disruption of mitochondrial function and inhibition of Cytochrome c activity, and apoptosis like cell death.
b. Tefenoquine (2018): It is the first new drug to be approved for the treatment of relapsing *Plasmodium vivax* malaria in 70 years. Chemically, it is an 8-aminoquinoline antimalarial drug related to primaquine but it is metabolically stable and thus slowly eliminated compared to primaquine with a half-life of 14 days. It acts by hampering the detoxification of haemoglobin metabolites by the parasite and the haemozoin formation is inhibited. It is indicated for radical cure of vivax malaria as well as for prophylaxis against all *Plasmodium*

species. As per 2020 FDA recommendations. tafenoquine radical cure should be restricted to co-administration with chloroquine only.

c. Moxidectin (2018): It is an important drug in veterinary medicine for nematodes and other helminths in cattle, horses, sheep, cats, dogs etc.
It has been licensed for use in Onchocerciasis in humans, ≥ 12 yrs of age in a single oral dose. It binds to glutamate-gated chloride channels, gamma-aminobutyric acid receptors and/or ATP-binding cassette transporters. This leads to increased permeability, influx of chloride ions, hyperpolarization and muscle paralysis. This drug is active against the microfilariae of *O. volvulus*, and not against adult worms.

d. Triclabendazole (2019): It is a benzimidazole derivative used in veterinary practice since 1983 for treatment of fascioliasis in domestic livestock. The drug acts by disruption of tegument via inhibition of microtubule-based processes or adenylate cyclase activity. It is the only treatment for fascioliasis recommended by the WHO and PAHO. A single dose, per oral, is effective in curing *F.hepatica* and *F.gigantica* infection and has activity against *Paragonimus* spp as well.

e. Fexnidazole (2021): This is the first oral treatment of Human African trypanosomiasis caused by *Trypanosoma brucei gambiense*. Bacterial-like nitroreductases encoded by trypanosomes activate fexinidazole and its metabolites through reduction to form reactive intermediates capable of damaging DNA and proteins. Ten days of oral administration is effective in both stage 1 (haemo-lymphatic) and stage 2 (meningo-encephalitic) of the disease. It is also being tested against *T.cruzi.*

Drugs in Development: A number of molecules are being tried in animal models for various protozoan and helminth parasites, although only very few have entered Phase I or II human trials. The biggest group is the anti-malarials with 14 drugs in clinical development, nine of which are in Phase II. Majority of them are blood schizonticides intended for the treatment of uncomplicated *P. falciparum,* although a few have multi-stage activity. Artefenomel, Cipargamin, Fosmidomycin, Methylene blue etc are examples of agents to have entered Phase II trials. Phase II study has also been conducted for Benznidazole-fosravuconazole combination for Chagas Disease and Clofazimine (anti-leprosy agent) for cryptosporidium. Emodepside is a molecule which is effective against isolates of helminth parasites that are resistant to benzimidazole, levamisole, and ivermectin. It has efficacy against filarial nematodes and has been approved for Phase I trial. Amidantel and tribendimidine are being tried against soil transmitted helminths. In recent years, research is being directed towards 'ethnopharmacology' with scientific validation of the uses of traditional or "folk" medicine including medicinal plants used in ayurveda and other alternative medicine practices.

Conclusion: During the last two decades, a large number of parasite molecular targets have been identified from genomics programmes. In addition, proteomic studies and bioinformatics are increasingly being used in drug discovery. Potential parasite drug targets are being validated using chemical or genetic methods like gene-expression profiling following drug treatment, RNA interference or genetic knockout techniques. It is expected that target-based or high-throughput screening of chemical compound libraries can expedite effective drug development in near future.

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NEXT GENERATION SEQUENCING

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Sequencing is the process of determining the sequence of nucleotides (As, Ts, Cs, and Gs) in a piece of nucleic acid

First Generation Sequencing

The first generation of sequencing technology is based on the chain termination method developed by Sanger and Coulson in 1975 or the chemical method (chain degradation) invented by Maxam and Gulbert during 1976 and 1977.

The **Maxam-Gilbert method** is based on chemical modification of DNA and subsequent cleavage of the DNA backbone at sites adjacent to the modified nucleotides.

Sanger sequencing uses specific chain-terminating nucleotides (dideoxy nucleotides) that lack a 3′-OH group. Thus no phosphodiester bond can be formed by DNA polymerase, resulting in termination of the growing DNA chain at that position. The ddNTPs are radioactively or fluorescently labeled for detection in "sequencing" gels or automated sequencing machines, respectively. Although the chemistry of the original Maxam-Gilbert method has been modified to help eliminate toxic reagents, the Sanger sequencing by synthesis (SBS) dideoxy method has become the sequencing standard.

In the first automated fluorescent DNA sequencing equipment, a complete gene locus for the hypoxanthineguanine phosphoribosyltransferase (HPRT) gene was sequenced, using for the first time the paired-end sequencing approach (Edwards et al. 1990). In 1996, ABI introduced the first commercial DNA sequencer that utilized a slab gel electrophoresis by the ABI Prism 310. Two years later, the considerable labor of pouring slab gels was replaced with automated reloading of the capillaries with polymer matrix by ABI Prism 3700 with 96 capillaries. This automated DNA sequencer was successfully utilized in the sequencing of the first human genome in 2003 taking into account 13- years of efforts of the human genome project consortium, and with an estimated cost of $2.7 billion.In 2008, by comparison, a human genome was sequenced over a 5-month period for approximately $1.5 million. The latter accomplishment highlights the capabilities of the rapidly evolving field of "next-generation" sequencing (NGS) or "High throughput" sequencing technologies that have emerged during the past 5 years.

All in all, the first generation of sequencing technology has the read-length ability of 1000bp with the 99.999% accuracy, which are the main feature. However, its high cost, low throughput are disadvantages.

Second Generation Sequencing/Next Generation Sequencing

In 2G NGS, the genetic material (DNA or RNA) is fragmented, to which oligonucleotides of known sequences are att.ached, through a step known as adapter ligation, enabling the fragments to interact with the chosen sequencing system. The bases of each fragment are then identified by their emitted signals.As a massively parallel process, NGS generates hundreds of megabases to gigabases of nucleotide-sequence output in a single instrument run, depending on the platform.

In 2000, Jonathan Rothberg founded **454** Life Sciences, which developed the first commercially available NGS platform, the GS 20, launched in 2005, was able to amplify millions of copies of a particular DNA fragment in a massively paralleled way in contrast to Sanger sequencing combining single-molecule emulsion PCR with pyrosequencing

By 2006, the **Solexa Genome Analyzer by Illumina**, the first "short read" sequencing platform, was commercially launched. uses a flow cell consisting of an optically transparent slide with 8 individual lanes on the surfaces of which are bound oligonucleotide anchors.Template DNA is fragmented into lengths of several hundred base pairs and end-repaired to generate 5′-phosphorylated blunt ends. The polymerase activity of Klenow fragment is used to add a single A base to the 3′ end of the blunt phosphorylated DNA fragments. This addition prepares the DNA fragments for ligation to oligonucleotide adapters, which have an overhang of a single T base at their 3′ end to increase ligation efficiency. The adapter oligonucleotides are complementary to the flow-cell anchors. Under limiting-dilution conditions, adapter-modified, single-stranded template DNA is added to the flow cell and immobilized by hybridization to the anchors. In contrast to emulsion PCR, DNA templates are amplified in the flow cell by "bridge" amplification, which relies on

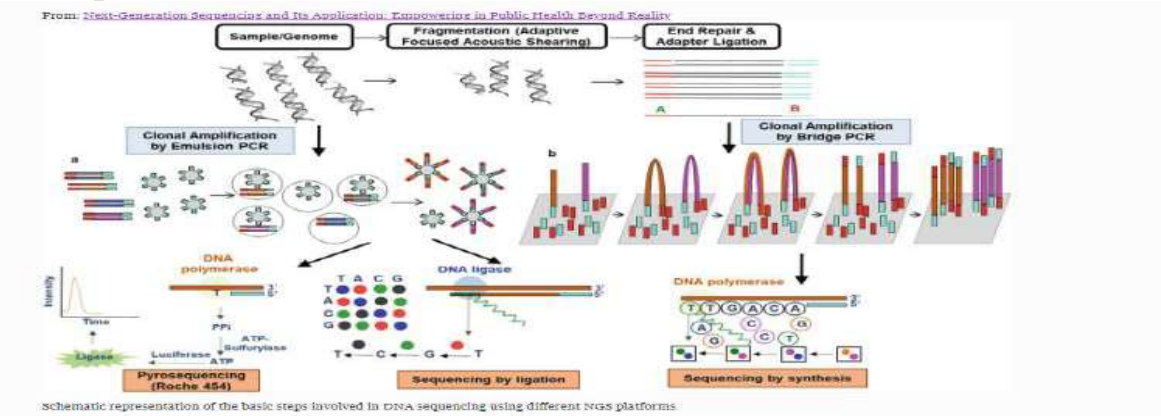
captured DNA strands "arching" over and hybridizing to an adjacent anchor oligonucleotide. Multiple amplification cycles convert the single-molecule DNA template to a clonally amplified arching "cluster," with each cluster containing approximately 1000 clonal molecules. Approximately 50 × 10^6 separate clusters can be generated per flow cell. For sequencing, the clusters are denatured, and a subsequent chemical cleavage reaction and wash leave only forward strands for single-end sequencing. Sequencing of the forward strands is initiated by hybridizing a primer complementary to the adapter sequences, which is followed by addition of polymerase and a mixture of 4 differently colored fluorescent reversible dye terminators. The terminators are incorporated according to sequence complementarity in each strand in a clonal cluster. After incorporation, excess reagents are washed away, the clusters are optically interrogated, and the fluorescence is recorded. With successive chemical steps, the reversible dye terminators are unblocked, the fluorescent labels are cleaved and washed away, and the next sequencing cycle is performed. This iterative, sequencing-by-synthesis process requires approximately 2.5 days to generate read lengths of 36 bases.

The newest platform, the Genome Analyzer II, has optical modifications enabling analysis of higher cluster densities. Coupled with ongoing improvements in sequencing chemistry and projected read lengths of 50-plus bases, further increases in output should be realized. Illumina and other NGS technologies have devised strategies to sequence both ends of template molecules. Such "paired-end" sequencing provides positional information that facilitates alignment and assembly, especially for short reads.

The **SOLiD** (Supported Oligonucleotide Ligation and Detection) System 2.0 platform, which is distributed by Applied BiosystemsDNA fragments are ligated to oligonucleotide adapters, attached to beads, and clonally amplified by emulsion PCR. Beads with clonally amplified template are immobilized onto a derivitized-glass flow-cell surface.It is a short-read sequencing technology based on ligation.

Ion Torrent™ technology by Thermo-Fisher directly converts nucleotide sequence into digital information on a semiconductor chip (Rothberg et al., 2011). To begin the process, DNA is fragmented into 200–1500 base fragments which are ligated to adapters. The DNA fragments are attached to a bead by complementary sequences on the beads and adapters and are then amplified on the bead by emulsion PCR (emPCR). This process enables millions of beads to each have multiple copies of one DNA sequence. The beads are then flowed across the chip containing the wells such that only one bead can enter an individual well. When the sequencing reagents are then flowed across the wells, when the appropriate nucleotide is incorporated, a hydrogen ion is given off .This changes the pH of the solution which can be recorded as a voltage change by an ion sensor, much like a pH meter. If no nucleotide is incorporated, no voltage spike occurs. A major advantage of the system is that no camera, light source or scanner is needed; nucleotide incorporation is directly converted to voltage which is recorded directly, greatly speeding up the process.

2G NGS technologies in general offer several advantages over alternative sequencing techniques, including the ability to generate sequencing reads in a fast, sensitive and cost-effective manner. However, there are also disadvantages, including poor interpretation of homopolymers and incorporation of incorrect dNTPs by polymerases, resulting in sequencing errors. The short read lengths also create the need for deeper sequencing coverage to enable accurate contig and final genome assembly. The major disadvantage of all 2G NGS techniques is the need for PCR amplification prior to sequencing. This is associated with PCR bias during library preparation (sequence GC-content, fragment length and false diversity) and analysis (base errors/favoring certain sequences over others).



Schematic representation of the basic steps involved in DNA sequencing using different NGS platforms.

Third Generation Sequencing

The introduction of 3G sequencing circumvents the need for PCR, sequencing single molecules without prior amplification steps

PacBio **Single molecule real time (SMRT™) sequencer**- The principle of SMRT sequencer relies on single molecule real time sequencing by synthesis method provided on the sequencing chip containing thousands of zero-mode waveguides (ZMWs). The sequencing reaction of a DNA fragment is performed by a single DNA polymerase molecule, which is attached to the bottom of each ZMW so that each DNA polymerase resides at the detection zone of ZMW. During the sequencing reaction, the DNA fragment is elongated by DNA polymerase with dNTP's that are fluorescently labeled (each nucleotide is labeled with a fluorophore of different color) at the terminal phosphate moiety. The DNA sequence is determined with CCD array on the basis of fluorescence nucleotide detection, which is performed before nucleotide incorporation, while the labeled dNTP forms a cognate association with the DNA template. The fluorescence pulse is stopped after phosphodiester bond formation, which causes the release of a fluorophore that diffuses out of ZMW. Subsequently, the labeled nucleotide incorporation and detection allow us to determine the DNA sequence

Advantages of 3G NGS include:

- Real-time monitoring of nucleotide incorporation
- Non-biased sequencing and
- Longer read lengths

Nevertheless, high costs, high error rates, large quantities of sequencing data and low read depth can be problematic.

Fourth Generation Sequencing

In 4G systems the single-molecule sequencing of 3G is combined with nanopore technology. Similar to 3G, nanopore technology requires no amplification and uses the concept of single molecule sequencing but with the integration of tiny biopores of nanoscale diameter (nanopores) through which the single molecule passes and is identified.

Nanopore DNA sequencer by Oxford Nanopore Technologies (ONT)-In contrary to all DNA sequencers mentioned above, sequencing a DNA molecule with the Nanopore DNA sequencer is free of nucleotide labeling and detection. This technique was developed from studies on translocation of DNA through various artificial nanopores. This system involves the use of electrolytic solutions and the application of a constant electric field. As the nucleic acid passes through the nanopore, the change in the current pattern and magnitude is measured.The principle of this technique is based on the modulation of the ionic current through the pore as a DNA molecule traverses it, revealing characteristics and parameters (diameter, length and conformation) of the molecule . During the sequencing process the ionic current that passes through the nanopore is blocked by the nucleotide, i.e., the previously cleaved by exonuclease from a DNA strand that interacts with cyclodextrin. The time period of current block is characteristic for each base and enables the DNA sequence to be determined .

The 4G systems currently offer the fastest whole genome sequence scan but are still quite expensive, error prone compared to 2G techniques and relatively new. Consequently, there is currently less extensive data available for the technique.

NGS is an extremely powerful tool for conducting basic biological research and it offers unmatched opportunities for improved diagnostics, prognostics and targeted therapies.

Next-generation sequencing bottlenecks

NGS has enabled us to discover and study genomes in ways that were never possible before. However, the complexity of the sample processing for NGS has exposed bottlenecks in managing, analyzing and storing the datasets. One of the main challenges is the computational resources required for the assembly, annotation, and analysis of sequencing data. The vast amount of data generated by NGS analysis is another critical challenge.

Applications of NGS

- Rapidly sequence whole genomes
- Deeply sequence target regions

- Utilize RNA sequencing (RNA-Seq) to discover novel RNA variants and splice sites, or quantify mRNAs for gene expression analysis
- Analyze epigenetic factors such as genome-wide DNA methylation and DNA-protein interactions
- Sequence cancer samples to study rare somatic variants, tumor subclones, and more
- Study the human microbiome , phylogeny, ancestry
- Identify novel pathogens
- Metagenomic studies
- Transcription studies, micro RNA studies
- Exome sequencing
- Ribosome & small RNA profiling
- Identification *of* Biomarkers of drug resistance
- For Drug and Vaccine Development , for Targeted therapies

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MUST KNOW CORE CONCEPTS: MANAGEMENT OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA)

Surveillance to be done with accuracy and accountability

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DEFINITION

This policy describes the steps needed to prevent the spread of Methicillin-Resistant Staphylococcus aureus (MRSA) to patients, staff, and visitors.

INTRODUCTION

- MRSA refers to strains of Staphylococcus aureus that are resistant to synthetic penicillin (oxacillin, nofloxacin, and methicillin).
- MRSA refers to strains of Staphylococcus aureus that are resistant to
- It is also resistant to cephalosporins, other beta lactam antibiotics and to other antibiotics (erythromycin, clindamycin, aminoglycoside, and quinolones).
- Concerns about MRSA are related to the potential for healthcare-associated infections (HAIs)transmission and the limited number of antibiotics available to treat infections caused by this microorganism.
- Screening can be initiated in the Emergency Department (ER).
- Patients being admitted from the ER who qualify for screening should not be held in the ER awaiting screening results, as this will unnecessarily delay admission.
- Initiate empiric contact isolation precautions during the screening procedure.

PROCEDURE

A. Management of Patients with Suspected MRSA Infection or Colonization

- Initiate empiric contact isolation precautions during the screening procedure, if possible.

a. Screen all patients who are:

- ✓ Admitted to the intensive care units (ICU).
- ✓ Transferred from other hospitals or have been treated in another hospital/clinic within the past six months.
- ✓ Undergoing liver or cardiac, orthopedic (including spine) surgery (pre-operatively).
- ✓ Hemodialysis patients admitted for their first dialysis treatment and for placement of any type of vascular access (i.e., A-V Fistula, permanent catheter, graft or port access device.)
- ✓ Known to be previously MRSA positive.
- ✓ Roommates of positive patients not on isolation precautions.

b. Sites to screen include:

- ✓ Anterior nares.
- ✓ Non intact skin areas (e.g., tracheostomy, pressure sores or surgical wounds).
- ✓ Neonates and pediatric patients awaiting liver or cardiac surgery should also have both groin and axilla screened.

c. Specimen collection for nares only:

- Use sterile red-top tube with double-tip dry culture swab for rapid testing.

d. Specimen collection for other sites:

- ✓ Use the packet with a sterile swab stick with transport medium.
- ✓ Clean the site with normal saline to remove debris before swabbing.
- ✓ Use the same swab for identical sites: one swab for both axilla and one swab for bothinguinal areas.
- ✓ Use separate swabs to screen other sites.

Note: The accompanying requisition should request "MRSA screen."

B. Management of MRSA-Positive Patients

1.Patients determined to be MRSA positive from surveillance screening (rapid test) or clinicalspecimens upon or after admission.

2.Readmitted patients that were MRSA positive on discharge (flag/alert).

3.Microbiology Laboratory:

- ✓ Notify the ward of MRSA-positive patients.
- ✓ Notify the Infection Preventionist (IP) of all new positive MRSA cultures.

4.Nursing:

- ✓ Request a single room for contact isolation from Admission Office. If a single room isnot readily available,

two or more MRSA-positive patients can be cohorted after consultation with infection control.
- ✓ MRSA-positive patients who are in multi-bed rooms can be managed temporarily whilewaiting to be transferred to a single room or an appropriate cohort.
- ❖ Place a sign on the cubicle or curtains of the patient's bed.
- ❖ Ensure easy access to PPE and alcohol-based hand rub.
- ❖ Practice strict standard precautions between interactions with patients in the room.
- ❖ Transfer to a single room or cohort with another patient with the same organism assoon as possible.
- ✓ Observe contact isolation precautions in addition to standard precautions with all patientcare activities.
- ❖ Place a contact isolation sign on the outside of the isolation room door.
- ❖ Ensure that staff understand and comply with the isolation precautions and handhygiene protocol.
- ❖ Cohort non-critical items such as stethoscopes and pressure cuffs along with the patient.
- ❖ Store the minimum number of supplies in the patient's room.
- ❖ Use an isolation cart for extra supplies (kept outside the room).
- ✓ Rescreening of MRSA-positive patients must occur in consultation with the IP.
- ✓ Screen exposed patients who shared a room with a known MRSA-positive patient for morethan 48 hours.
- ✓ Limit the patient's activities outside of the ward.
- ✓ Notify receiving departments/wards (e.g., Radiology, Endoscopy, Clinics, OR) of the patient's isolation status when the patient must be transported for treatment/tests.

C. Discontinuation of Contact Isolation

1.Discontinuation of isolation precautions for a MRSA-positive patient must occur in consultation with the IP team or ID.

2.Criteria for discontinuing isolation:

- ❖ Antibiotic therapy is completed at least three days prior to rescreening.
- ❖ Vancomycin levels should be zero prior to rescreening.
- ❖ Three consecutive negative culture from all previously positive sites. If the first set of samples which was taken 3 days off antibiotics is negative, repeat cultures 48 hours later.
- ❖ The patient should not be receiving antibiotic therapy at any time during the screeningprocess.

D. Rescreening MRSA-positive Patients for the Purpose of Discontinuing Contact Isolation

- Sites to screen are:
- ✓ Anterior nares
- ✓ Previously Positive sites
- ✓ Any indwelling catheter sites
- ✓ Non intact skin areas (e.g., tracheostomy, pressure sores or surgical wounds)

- Specimen Collection:
- ✓ For other sites, use the packet with blue-top sterile swab stick with gel.
- ✓ Use the same swab for identical sites (e.g., axilla and groin).
- ✓ Use separate swabs to screen other sites.
- Note : The accompanying requisition should request "MRSA screen." If your hospital is processingMRSA from other sites for rapid testing/PCR molecular, then use the red top-swab stick.

E. Screening of Healthcare Workers (HCWs) and the Environment

- ❖ Do not screen HCWs or the environment because it is not normally indicated and incursunnecessary costs.
- ❖ IP&C may initiate such measures when indicated.

Outbreak Management

- ❖ Management of outbreaks will be coordinated by the IP and will require the cooperation ofmedical, nursing, laboratory and other departments.

Cleaning of the Patient's Room

- ❖ Regular cleaning as per housekeeping protocol.
- ❖ Terminal cleaning upon patient discharge.
- ❖ The room can be used as soon as all cleaned surfaces are dry.

Linen

- ❖ Keep a linen hamper in the isolation area.

Ambulation

Patients with infected body fluids:

- If they are able to contain their body fluids (secretions, urine, stool), patients may walk in the corridors but cannot enter the visitor/patient area.
- If unable to contain their body fluids, patients must be encouraged to stay in their roomsand be reassessed frequently.

Sitters/Visitors

- Provide information about MRSA as required.
- Hand hygiene must be emphasized after patient contact.
- Sitters and visitors must be instructed to wear appropriate PPE if assisting with direct patientcare.

Decolonization Protocol

- Treat nares topically for periods not exceeding seven days with Bactroban (Mupirocin) cream(only if the organism is Mupirocin-sensitive); restrict use, as resistance to this agent is well documented.
- IP will assess patients on an individual basis to determine the need for decolonization withchlorhexidine wash (suppressive therapy) to reduce/inhibit MRSA skin colonization.
- Apply this protocol to patients awaiting liver transplants or cardiac, or orthopedic surgery, orhemodialysis patients requiring AV/fistula creation.

F. MRSA Decolonization Procedure

Assessment for decolonization will be performed by the Infection Preventionist (IP) in consultation with the attending physician and an Infectious Disease Consultant.
Maintain Contact Isolation during decolonization treatment.
Supplies: Chlorhexidine gluconate (CHG) 4%
Mupirocin/Bactroban

- Clean linens for the bed and patient personal protective equipment (PPE)
- Spread full-strength Chlorhexidine gluconate 4% solution from neck to toes, ensuringcoverage of underarms, groin, and between fingers and toes.
- Rinse with warm water and dry your skin from neck to toes with a clean towel.
- Change the bed linens and the patient's clothing completely after each bath/shower.
- Repeat this process twice a day. Shampoo hair with the Chlorhexidine solution for 3 days
- Apply Mupirocin/Bactroban ointment to anterior nares (inside nose) after Chlorhexidinetreatment, when the patient is dry and dressed
- Note: Mupirocin should not be applied to open wounds.
- These treatments must be given for 7 consecutive days.
- Take a complete set of cultures from nares and previously positive sites 72 hrs. afterdecolonization
- -If first set of samples is negative repeat cultures 48 hrs. later three negative cultures are required before the patient is cleared of MRSA and can betaken out of isolation.
- Note: These results will be assessed by the IP.

NOTES:

- ✓ The patient must not be on antibiotics at the time of screening.
- ✓ If any swab is positive, stop the screening process until further assessment.
- ✓ Please complete all documentation on this form.

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MASTERING THE BASICS OF FUNGAL CULTURES-FOR THE BUDDING MICROBIOLOGISTS

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Clinical Mycology is constantly changing. Not only the list of fungi causing human infections is expanding, new risk groups for fungal infections are emerging, new disease patterns caused by previously known fungi are being recognized and the geographical boundaries of several fungi previously known to be endemic to certain regions, are expanding[1].

Inspite of the growing impact of fungal infections across the globe, the diagnosis of systemic fungal infections remains a challenge. A combination of culture based and non-culture- based methods may be necessary to diagnose most fungal infections [2].

Asian countries including India have the largest burden of fungal infections and the problem is compounded by the lack of awareness among medical personnel regarding fungal infections, inadequate numbers of dedicated clinical mycology laboratories and trained laboratory personnel across the country [2].

The results of a Survey conducted by the Asia Fungal working group (AFWG) have highlighted the need for development of quality laboratories, accreditation and training of manpower in existing laboratories, and access to advanced non-culture-based diagnostic tests to facilitate the diagnosis of fungal infections in Asia [3]. Four tier laboratory development with different diagnostic capabilities at each level has been proposed to overcome these gaps in fungal diagnostics [2].
Inspite of several shortcomings Conventional methods i.e. direct microscopy and fungal culture still remain the backbone of Mycology laboratories at all levels from the basic to the reference laboratory. Every microbiologist should be well aware of the dos and don'ts of performing and interpreting direct microscopy and fungal cultures.

This article does not aim to discuss the laboratory diagnosis of fungal infections and fungal identification in detail, but will highlight a few suggestions from experts on how best to overcome some common challenges in performing and interpreting the conventional methods of direct microscopic examination and fungal cultures.

1. **Standard operating Procedures in mycology should be followed strictly for optimal results:** Several National and international standards are available freely on the web. ICMR has published the "**Standard Operating procedures for Fungal identification and detection of antifungal resistance"** which is freely available for download in the ICMR web site[4]

2. Biosafety in the Mycology lab[5]:
2.1. BSL-2 practices, containment equipment, and facilities- recommended for propagating and manipulating cultures known to contain the common saprophytic fungi like *Candida, Aspergillus, Mucorales* and others, which cause infections mostly in immunocompromised patients or those with debilitating underlying diseases. Any unknown fungal isolate should also be handled the same way.
2.2. BSL-3 practices, containment equipment, and facilities are recommended for propagating sporulating cultures of true pathogens such as the dimorphic fungi *Histoplasma capsulatum* in the mold form, as well as for processing soil or other environmental materials known or likely to contain infectious conidia.

3. Preanalytic and analytic factors:

- Appropriate specimen collection, Prompt transportation and storage, correct processing and inoculation of specimens onto appropriate culture media and incubation at suitable temperature are the most important factors for getting optimal results [4]

- The clinicians should be given clear, written instructions for proper sample collection (preferably before administration of antifungals) and transport to the laboratory.

4. Direct Microscopic examination [4,6]:

- **Crucial First line** procedure and the most **simple, rapid and cost-effective** method for diagnosing fungal infections and should be done as soon as possible after receipt of the specimen. Insufficiently lysed samples should be further incubated till complete lysis and re-examined without fail.
- often provide presumptive diagnosis of the fungal infection in **<1 h and** also preliminary identification based on characteristic morphology.
- Guides the mycologist in selecting the most appropriate means by which to culture the clinical material.
- Essential for **proper interpretation of fungal cultures, to differentiate pathogens from commensals/contaminants.**
- Fluorescent dyes like Calcofluor white (Sigma-Aldrich) and other special fungal stains like GMS significantly Improve the sensitivity of direct examination and should be used whenever possible.
- Positive findings in direct microscopy should be considered a **critical report and informed to the treating clinician immediately.**
- Delayed reports even if accurate may have no impact on patient management in rapidly progressing invasive fungal infections.
- Direct microscopic /Cytologic/histopathologic examination of sterile specimen obtained by needle aspiration or biopsy, showing fungal hyphae/yeast cells along with features of tissue damage, is a criteria for Proven fungal Infection[7].

5. Fungal culture media:

- **Specific Fungal media** (Sabouraud dextrose agar, Inhibitory Mould agar, Malt extract agar etc.) capable of supporting the growth of fungi likely to cause infection at that site, should be used.
  Bacterial media are less effective in isolating Fungi [8].
- **Multiple Tubes/plates** of fungal media consisting of **a combination of non-selective and selective media** should be inoculated; this will improve the yield by increasing the volume of the sample cultured, support the growth of diverse fungi and help in interpretation of any growth[9]

6. Reading of Fungal cultures :

- **A common misconception is that Fungi grow very slowly and mycology reports have no impact on patient management-**
  - Most fungi causing invasive infection, with the exception of some dimorphic pathogens, will grow within a few days, while dermatophytes from superficial infections will usually be evident after one week. Experts opine that 98 % of yeast isolates and 81 % of mould isolates are detected in the first week, and more than 96 % of moulds were detected by day 14 [10].
  - All cultures should be read **daily for the 1st week,** then **thrice a week** thereafter irrespective of whether direct examination was positive or not [4,9.10].

- The plates/tubes should be carefully examined to reveal whether any fungal isolates are associated with the area that has been inoculated. **Colonies obviously away from the inoculation streak or tissue portion** should be discounted as contaminants.

- Growth of the same fungal isolate in more than one culture tube increases the significance of the isolate.

- An assessment of maximum temperature of growth may be useful to differentiate a pathogen from a contaminant , as many environmental fungi will not be able to grow at 37 °C and so would be unlikely to cause invasive infections.

7.Processing &interpretation of a few important specimens:

7.1.Tissues:

While processing tissues for fungal culture, Inoculate a piece of the tissue directly into the media without mincing or grinding to prevent destruction of viable hyphae especially in case of sparsely septate fungi such as Mucorales. Rest of the tissue must be then homogenized and inoculated into media, to increase the chances of isolation[4]

In cases of Mycetoma, swabs taken from the surface of the sinuses should not be accepted for culture. Aspirate obtained from the depth of an active sinus/ biopsied tissue are preferred, so as to maximise the chances of obtaining granules,which are microcolonies of the fungus for Culture. Sometimes the granules may also be seen stuck to the gauze dressing over the sinuses.

These granules should be washed and then inoculated into media, to avoid superficial bacterial contaminants[4,

7.2. Respiratory specimens for suspected invasive fungal infection (J):

7.2.1.Moulds:

- Growth of dimorphic pathogens such as *Hisptoplasma capsulatum* in culture is considered significant irrespective of the results of direct microscopy.

- Growth of saprophytic moulds in culture should be interpreted carefully based on
  - the presence/absence of branching hyphal filaments on direct examaination,
  - the clinical presentation and imaging findings,
  - Host risk factors for fungal infection.
  - Results of other non culture based tests like serology and molecular tests.

- Growth of a potentially pathogenic mould from a specimen without branching hyphae being visualized on direct microscopy, should be treated with scepticism, as false positive cultures due to inhaled non-germinated fungal spores in sputum samples are quite common.

- In certain situations e.g. samples from **high-risk patients**, the recovery of a potentially pathogenic mould should always be treated seriously **even in the absence of direct positive.** Attempts should be made to repeat the direct examination and fungal cultures, **to rule out infection** with certainty.

- Branching hyphae may sometimes be seen on direct microscopic examination without corresponding growth in culture, but should be reported as significant; this could be due to loss of viability of the fungi during delay in transport or due to improper processing of the specimen or due to inadequacy of media used for culture. This is commonly seen while culturing aseptate fungi like Mucorales. Cultures should be repeated whenever possible.

7.2.2.*Candida* in the respiratory tract specimen:

Isolation of *Candida* spp. from lower respiratory tract specimens (sputum /BAL/ Tracheal aspirate etc.) is <u>not significant</u> and patients should not be treated with antifungals [10, 11]

- *Candida* is part of the human microbiota of the respiratory tract. As such, *Candida* can be cultured from the oropharynx of people with and without pneumonia, and rapidly colonizes the lower respiratory tract (LRT) in patients admitted to the ICU.
- No relationship has been found between isolation of candida from LRTI specimens and Pneumonia/Invasive candidiasis/mortality.
- Prospective study of autopsies performed on patients who died in the ICU: there was no histopathologically proven case of *Candida* pneumonia found in patients with pneumonia on autopsy and a *Candida*-positive airway culture prior to death.

- There is no benefit to treating patients with *Candida* in the airways. Several RCT shave shown that antifungal therapy did not have a significant impact on hospital/ICU length of stay and mortality in critically ill patients with *Candida*-positive airway secretions.

7.3.Blood cultures for Fungi [4,9,10]:

7.3.1.Yeasts :

- The overall sensitivity of blood cultures for yeasts is about 50–95%.This variation is mainly due to the variation in the media and methods used and the volume of blood cultured.
- Continuous monitoring blood culture systems such as BacT/Alert, Bactec show improved sensitivity compared to manual methods; 99%of fungi are isolated within 5 days.
- **20 ml-40 ml** of blood should be cultured for optimal yield;
- At least 2 sets of blood cultures should be inoculated, each set with blood collected from a different venepuncture site. If culture negative at 24 hours, another 2 sets of blood culture.
- Blood should be collected by fresh venepuncture after thorough aseptic cleaning of the puncture site.
- Blood should not be collected through a preexisting catheter, except when Catheter related BSI (CRBSI) is suspected
- In suspected CRBSI-Paired blood culture samples should be collected through the catheter and peripheral vein simultaneously.
- Isolation of candida from even one bottle is significant and should be treated, provided the blood has been collected by fresh venipuncture.
- Gram stain should be performed from the blood culture broth as soon as it is detected positive and presence of fungi/bacteria should be immediately informed to the clinician.
- All yeasts from blood cultures should be identified to the species level and Antifungal susceptibility tested as MDR candida species such as C.auris are being increasingly reported[12,13].
- Rare opportunistic yeasts (Non-*Candida,* Non-*Cryptoccus*) e.g. *Trichosporon, Saprochaete capitata, Geotrichum* etc may cause BSI in immunocompromised patients. These often occur as breakthough infections and exhibit resistance to antifungals [14].

7.3.2.Filamentous fungi[10]**:**

- Filamentous fungi are rarely isolated in blood cultures, even in patients with disseminated infection; most moulds grow as branching mycelium through the tissues and along the blood vessels, thereby causing infection by contiguous spread. Occasionally, if viable fragments break off, there may be haematogenous dissemination to remote organs, but isolation of viable fragments from blood is rare.
- Isolation of moulds including *Aspergillus* species (with the exception of *A.terreus*) from blood cultures is more likely to represent contamination either during collection of the specimen or at some point during the processing. Repeat isolation should be attempted in all cases.
- As fungemia s due to saprophytic fungi has been rarely reported, the clinical condition of the patient and the likely risk factors should be carefully considered before discounting unusual isolates.
- Of all moulds, *Fusarium* and *Lomentospora* species(earlier *Scedosporium* species), *Aspergillus terreus* and less commonly *Acremonium* spp. have the capacity to form spores in vivo (adventitious sporulation) and are more often isolated from blood stream especially in Immunocompromised patients.

7.4 . Isolation of Candida from urine[15]**:**

7.4.1.Candiduria in patients without an indwelling catheter

- Presence of pyuria does not always indicate *Candida* urinary tract infections (UTI); Several of these patients with funguria have concomitant bacteriuria.
- Quantitative urine cultures do not reliably predict infection unlike in bacterial UTI

- *Candida* colonization should be differentiated from infection-
  -A clean-catch urine sample should be repeated for culture, If the second culture is sterile, no treatment is necessary.
- *Candida* may be considered significant:
  -If the same *Candida* spp. is repeatedly isolated from a clean catch urine and the patient has symptoms of UTI
  -in critically ill or paraplegic patients with unexplained fever and candiduria.

7.4.2.Candiduria in patients with an indwelling urinary catheter

- *Candida* colonization of urinary tract is very common in catheterized patients.
- Candiduria may resolve if the urinary catheter is removed (35–40%) or replaced (20%).
- Antifungal treatment may be considered, If candiduria persists and the patient remains febrile after removal or replacement of catheter, and there is no other obvious source of infection.

7.4.3. Does colonization predict invasive candidiasis?

- Only 1–8% of colonized patients in the ICU, develop candidemia -antifungal treatment is thus not recommended.
- Empirical treatment may be needed in symptomatic patients with multiple sites of *Candida* colonization and with significant risk factors for invasive candidiasis.

8. Emergence of new population groups susceptible to Fungal Infections [16,17]**:**

- Populations at risk for fungal infections are evolving constantly.

- Microbiologists and Clinicians should be aware of the non- classical risk factors such as Liver disease, COPD, Diabetes, Tuberculosis, Influenza, COVID, ICU stay, and others, when doing risk assessment for interpretation of Fungal cultures.

To conclude, a combination of culture and non- culture-based tests is recommended for early and accurate diagnosis of fungal infections. Inspite of their low sensitivity, direct microscopy and fungal cultures can be performed at all levels of mycology laboratories and are the most direct and usually conclusive means of establishing the diagnosis of fungal infection. Microbiologists should be proficient in performing and interpreting these tests with confidence, to effectively face the growing challenge of fungal infections.

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UNRAVELLING THE ENIGMA OF MONKEY POX DISEASE – THE PAST, PRESENT AND FUTURE

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Introduction:

In view of the ongoing outbreak of Monkey pox disease, the present topic seems pertinent to be discussed in great detail. Monkeypox is a viral zoonotic disease, endemic in West and Central Africa, with symptoms similar to smallpox, although with less clinical severity. Though monkeypox virus was discovered in 1958, typical human cases were not witnessed until 1970s. Prior to this, it was assumed that infections of monkey pox were masked by smallpox, which was widely endemic then. Nonetheless, since the 1970s, reported Human Monkeypox virus infections/outbreaks have escalated due to reported human-to-human transmission. This increase is probably due to various factors, like enhanced surveillance efforts, environmental degradation and urbanization of areas where monkeypox virus is maintained among animal reservoir(s) and, consequently, serves as a nidus for human infection. In addition, genetic predisposition enables Monkeypox virus to infect many animal species, prevalent in varied geographical ranges. Monkeypox virus which was once restricted to specific regions of Africa, has now expanded, intercontinentally – suggesting that infections of this emerging viral zoonosis will continue to intensify. Two Monkey pox (MPX) clades have been identified, one in West Africa (WA) and the other in the Congo Basin (CB) . In the past, the CB clade seemed to be more virulent. Since May 2022, several non-endemic countries in four WHO regions have reported monkeypox cases. Rare cases of monkeypox in other countries are usually linked to travel to endemic countries However, most of the current cases do not have any history of travel to endemic countries. Therefore, the current outbreaks are unusual and different from previous travel-related outbreaks. On July 23, 2022, monkeypox was declared a Public Health Emergency of International Concern by WHO Director-General Tedros Adhanom Ghebreyesus. A group of global experts convened by WHO has agreed on new names for monkeypox virus variants, as part of ongoing efforts to align the names of the monkeypox disease, virus and variants – or clades – with current best practices. The experts agreed to name the clades using Roman numerals.

Epidemiology:

Monkeypox was first discovered in 1958 in colonies of monkeys kept for research, hence the name 'monkeypox.' Human case was first identified in 1970 in a 9-month-old boy in the Democratic Republic of the Congo and since then most cases have been reported across Central and West Africa. In 2003, the first monkeypox outbreak outside of Africa was in the United States of America and was linked to contact with infected pet prairie dogs. These pets had been housed with Gambian pouched rats and dormice that had been imported into the country from Ghana. This outbreak led to over 70 cases of monkeypox in the U.S. Monkeypox has also been reported in travellers from Nigeria to Israel in September 2018, to the United Kingdom in September 2018, December 2019, May 2021 and May 2022, to Singapore in May 2019, and to the United States of America in July and November 2021. In May 2022, multiple cases of monkeypox were identified in several non-endemic countries.

According to Center for Disease Control and Prevention, **till 18.08.2022**

Global Scenario: Over **40,399** Monkeypox cases across **94 locations**.

In India: **9 cases** (4 from Delhi and remaining five from Kerala) and 1 death from Kerala

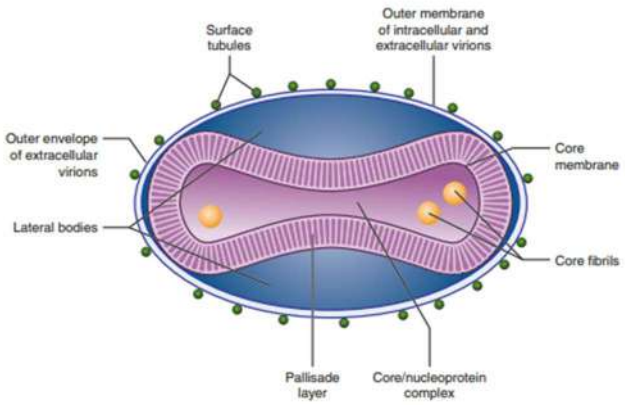
Classification :

S.No	Taxon	Name
1.	Family	*Poxviridae*
2.	Subfamily	*Chordopoxvirinae*

3.	Genus	*Orthopoxvirus*
4.	Species	*Monkeypox virus, Camelpox virus, Cowpox virus, Racoonpox virus, Skunkpox virus, Taterapox virus, Volepox virus, Vaccinia virus, Variola virus, Ectromelia virus,*

Morphology:

Monkeypox virus is an enveloped double-stranded DNA virus that belongs to the Orthopoxvirus genus of the Poxviridae family. They are brick-like particles with a size ranging from 220 nm to 450 nm in length and 140 nm to 260 nm in width therefore, Monkey pox virus is large enough to be discerned by light microscope, with its ultrastructure studied via electron microscopy. The orthopox virion consists of four major elements—core, lateral bodies, outer membrane, and the outer lipoprotein envelope. The central core contains the viral double-stranded DNA (dsDNA) and core fibrils, and it is surrounded by a tightly arranged layer of rod-shaped structures known as palisade layer. The central core, palisade layer, and the lateral bodies are enclosed together by the outer membrane that is composed of surface tubules.



Schematic representation of a poxvirus particle. Adapted from Principles of Molecular Virology, 6th Edition (p. 46), by Alan J. Cann, 2016, UK: Elsevier. Copyright 2016 by Elsevier.

There are two main strains, one more virulent and transmissible (Congo Basin clade) than the other (West African clade). The less virulent West African clade has been identified among the current cases. The clinical presentation of monkeypox resembles that of smallpox, a related orthopoxvirus infection which was declared eradicated worldwide in 1980. Monkeypox is less contagious than smallpox and causes less severe illness.

Host:

Natural reservoir is yet unknown. However, certain rodents (including rope squirrels, tree squirrels, Gambian pouched rats, dormice) and non-human primates are known to be naturally susceptible to monkeypox virus.

Incubation period:

The incubation period is usually 6 to 13 days and can range from 5 to 21 days. Although most people recover within weeks, severe complications and sequelae have been reported to be more common among those unvaccinated for smallpox compared with those vaccinated.

Period of communicability:

1-2 days before the rash to until all the scabs fall off/gets subsided.

Mode of transmission: (Reference – WHO)

- Monkeypox virus is transmitted from one person to another by close contact with lesions, body fluids and contaminated materials such as bedding, clothing or eating utensils.
- A person with monkeypox remains infectious while they have symptoms, normally for between 2 and 4 weeks.
- Ulcers, lesions or sores in the mouth can also be infectious, meaning the virus can spread through saliva.
- People who closely interact with someone who is infectious, including health workers, household members and sexual partners are at greater risk of infection.
- Transmission can also occur via the placenta from mother to fetus (which can lead to congenital monkeypox) or during close contact during and after birth. It may lead to adverse outcomes for the fetus, such as death or spontaneous abortion.

Case definitions (Reference –MoHFW Guidelines)

Suspected case:

A person of any age having history of travel to affected countries within last 21 days presenting with an unexplained acute rash AND one or more of the following signs or symptoms

- Swollen lymph nodes
- Fever
- Headache
- Body aches
- Profound weakness

Probable case:

A person meeting the case definition for a suspected case, clinically compatible illness and has an epidemiological link (face-to-face exposure, including health care workers without appropriate PPE; direct physical contact with skin or skin lesions, including sexual contact; or contact with contaminated materials such as clothing, bedding or utensils is suggestive of a strong epidemiological link)

Confirmed case:

A case which is laboratory confirmed for monkeypox virus (by detection of unique sequences of viral DNA either by polymerase chain reaction (PCR) and/or sequencing).

Signs and Symptoms:

The infection can be divided into two periods:

1) The invasion period (lasts 0–5 days) is characterized by fever, intense headache, lymphadenopathy, back pain, myalgia and intense asthenia. **Lymphadenopathy** is a distinctive feature of monkeypox compared to other diseases that may initially appear similar (chickenpox, measles, smallpox).
2) The skin eruption usually begins within 1–3 days of appearance of fever. The rash tends to be more concentrated on the face and extremities rather than on the trunk. It affects the face (in 95% of cases), and palms of the hands and soles of the feet (in 75% of cases). Oral mucous membranes (in 70% of cases), genitalia (30%), and conjunctivae (20%), and the cornea are also affected. The rash evolves sequentially from macules (lesions with a flat base) to papules (slightly raised firm lesions), vesicles (lesions filled with clear fluid), pustules (lesions filled with yellowish fluid), and crusts which dry up and fall off.
   The number of lesions varies from a few to several thousand. In severe cases, lesions can coalesce until large sections of skin slough off.

Monkeypox is usually a self-limiting disease with the symptoms lasting from 2 to 4 weeks. Severe cases occur more commonly among children and are related to the extent of virus exposure, patient health status, immune status and nature of complications.

Complications :

Complications of monkeypox can include secondary infections, bronchopneumonia, sepsis, encephalitis, and infection of the cornea with ensuing loss of vision. The extent to which asymptomatic infection may occur is unknown. The case fatality ratio of monkeypox has historically ranged from 0 to 11 % in the general population and has been higher among young children. In recent times, the case fatality ratio has been around 3–6%.

Diagnosis:

The clinical differential diagnosis that must be considered includes other rash illnesses, such as chickenpox, measles, bacterial skin infections, scabies, syphilis, and medication-associated allergies.

Lymphadenopathy during the prodromal stage of illness can be a clinical feature to distinguish monkeypox from chickenpox or smallpox. If monkeypox is suspected, health workers should collect an appropriate sample and have it transported safely to a laboratory with appropriate capability. The list of samples to be collected from the cases as laid down by the MoHFW are as follows

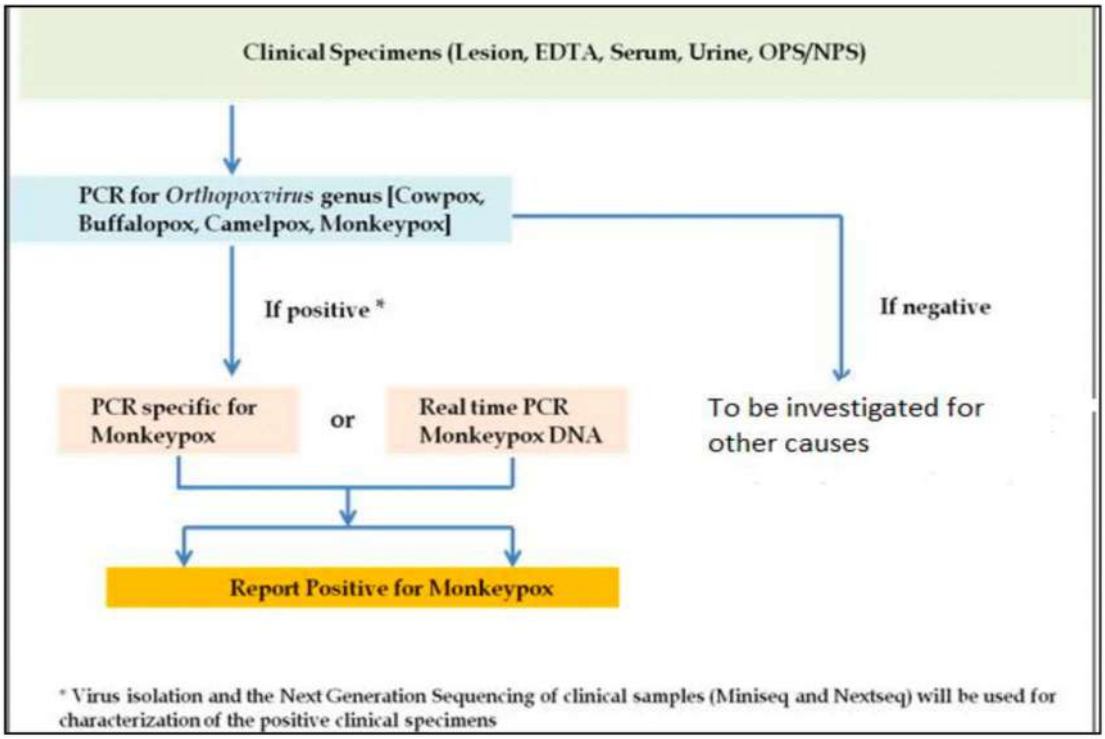
<table>
<tr><th colspan="3">Traveller from outbreak /endemic region or Community Transmission</th></tr>
<tr><td>Asymptomatic</td><td colspan="2">• Observe for the development of any signs and symptoms for 21 days' post exposure
• If signs and symptoms develop, collect specimens as per the duration of illness as mentioned below</td></tr>
<tr><td rowspan="2">Symptomatic</td><td>Rash phase**</td><td>Recovery phase</td></tr>
<tr><td>• *Lesion roof- with scalpel or plastic scrapper collected in plain tube
• *Lesion fluid with intradermal syringe
• *Lesion base scrapings with sterile polyester swab collected in plain tube
• *Lesion crust in plain tube
• NPS/OPS in dry plain tube [without any bacterial medium or VTM]
• Blood collected in SSGT (4-5 ml)
• Blood collected in EDTA (2-3ml)
• Urine in sterile urine container (3-5ml)</td><td>• Blood collected in SSGT (4-5 ml)
Urine in sterile urine container (3-5ml)</td></tr>
</table>

*** The specimens from lesion should be collected from multiple sites**

Lesion samples must be stored in a dry, sterile tube (no viral transport media) and kept cold. The samples may be kept in a cool dry room if cold chain is unavailable but it is preferred to store samples in -20°C (-80°C for long term storage).

PCR blood tests are usually inconclusive because of the short duration of viremia relative to the timing of specimen collection after symptoms begin and should not be routinely collected from patients. As orthopoxviruses are serologically cross-reactive, antigen and antibody detection methods do not provide monkeypox-specific confirmation. Additionally, recent or remote vaccination with a vaccinia-based vaccine might lead to false positive results.

Confirmation of the Monkey pox virus infection is best done by polymerase chain reaction (PCR) as it is the only method which can differentiate between the various orthopoxvirus species. Hence PCR is the preferred laboratory investigation .



According to MoHFW, the clinical specimens should be subjected to PCR for Orthopoxvirus genes and only if the sample is positive should the sample be tested for Monkeypox PCR (Real-time or conventional).

Treatment

Clinical care for monkeypox is mainly symptomatic, preventing further complications and long-term sequelae.

Component of management	Symptoms/Signs	Management
Protection of compromised skin and mucous membranes	Skin rash	• Clean with simple antiseptic • Mupironic Acid/Fucidin • Cover with light dressing if extensive lesion present • Do not touch/ scratch the lesions • In case of secondary infection relevant systematic antibiotics may be considered
	Genital ulcers	• Sitz bath
	Oral ulcers	• Warm saline gargles/ oral topical anti-inflammatory gel
	Conjunctivitis	• Usually, self-limiting • Consult Ophthalmologist if symptoms persist or there are pain/ visual disturbances
Rehydration therapy and nutritional support	Dehydration can occur in association with poor appetite, nausea, vomiting and diarrhoea	• Encourage ORS or oral fluids • Intravenous fluids if indicated • Encourage nutritious and adequate diet
Symptom alleviation	Fever	• Tepid sponging • Paracetamol as required
	Itching/Pruritus	• Topical Calamine lotion • Antihistaminics
	Nausea and vomiting	• Consider anti-emetics
	Headache/ malaise	• Paracetamol and adequate hydration

An antiviral agent known as tecovirimat that was developed for smallpox was licensed by the European Medicines Agency (EMA) for monkeypox in 2022 based on data in animal and human studies. It is not yet widely available.

If used for patient care, tecovirimat should ideally be monitored in a clinical research context with prospective data collection.

Immunoprophylaxis

Vaccination against smallpox was demonstrated through several observational studies to be about 85% effective in preventing monkeypox. Thus, prior smallpox vaccination may result in milder illness. At the present time, the original (first-generation) smallpox vaccines are no longer available to the general public.

A still newer vaccine based on a modified attenuated vaccinia virus (Ankara strain) was approved for the prevention of monkeypox in 2019. This is a two-dose vaccine for which availability remains limited. Smallpox and monkeypox vaccines are developed in formulations based on the vaccinia virus due to cross-protection afforded for the immune response to orthopoxviruses.

Prevention:

Raising awareness of risk factors and educating people about the measures they can take to reduce exposure to the virus is the main prevention strategy for monkeypox. Scientific studies are now underway to assess the feasibility and appropriateness of vaccination for the prevention and control of monkeypox.

Reducing the risk of human-to-human transmission

Surveillance and rapid identification of new cases is critical for outbreak containment. As the source of this outbreak is being investigated, it is important to look at all possible modes of transmission in order to safeguard public health. During human monkeypox outbreaks, close contact with infected persons is the most significant risk factor for monkeypox virus infection.

Health workers and household members are at a greater risk of infection. Health workers caring for patients with suspected or confirmed monkeypox virus infection, or handling specimens from them, should implement standard infection control precautions. If possible, persons previously vaccinated against smallpox should be selected to care for the patient.

Samples taken from people and animals with suspected monkeypox virus infection should be handled by trained staff working in suitably equipped laboratories. Patient specimens must be safely prepared for transport with triple packaging in accordance with WHO guidance for transport of infectious substances.

Reducing the risk of zoonotic transmission

Over time, most human infections have resulted from a primary, animal-to-human transmission. Unprotected contact with wild animals, especially those that are sick or dead, including their meat, blood and other parts must be avoided. Additionally, all foods containing animal meat or parts must be thoroughly cooked before eating.

Preventing monkeypox through restrictions on animal trade

Captive animals that are potentially infected with monkeypox should be isolated from other animals and placed into immediate quarantine. Any animals that might have come into contact with an infected animal should be quarantined, handled with standard precautions and observed for monkeypox symptoms for 30 days.

Surveillance Strategies:

The aims of the proposed surveillance strategy are to rapidly identify cases and clusters of infections and the sources of infections as soon as possible in order to:

1. Isolate cases to prevent further transmission
2. Provide optimal clinical care
3. Identify and manage contacts
4. Protect frontline health workers
5. Effective control and preventive measures based on the identified routes of transmission.

Surveillance outline

a. Use Standard Case Definitions by all District Surveillance Units (DSUs) under Integrated Disease Surveillance Programme (IDSP) and at Points of Entry (PoEs).
b. Even one case of monkeypox is to be considered as an outbreak. A detailed investigation by the Rapid Response Teams needs to be initiated through IDSP.
c. Report any suspected case immediately to the DSU/State Surveillance Units (SSUs) and CSU (Central Surveillance Unit), which shall report the same to Dte. GHS MoHFW.
d. Send the samples as per the guidelines to the designated laboratories.

Core Surveillance Strategy

a. Hospital based Surveillance: - Health facility-based surveillance & testing – in Dermatology clinics, STD clinics, medicine, paediatrics OPDs etc.
b. Targeted Surveillance: This can be achieved by:
    i. Measles surveillance by Immunization division
    ii. Targeted intervention sites identified by NACO for MSM, FSW population

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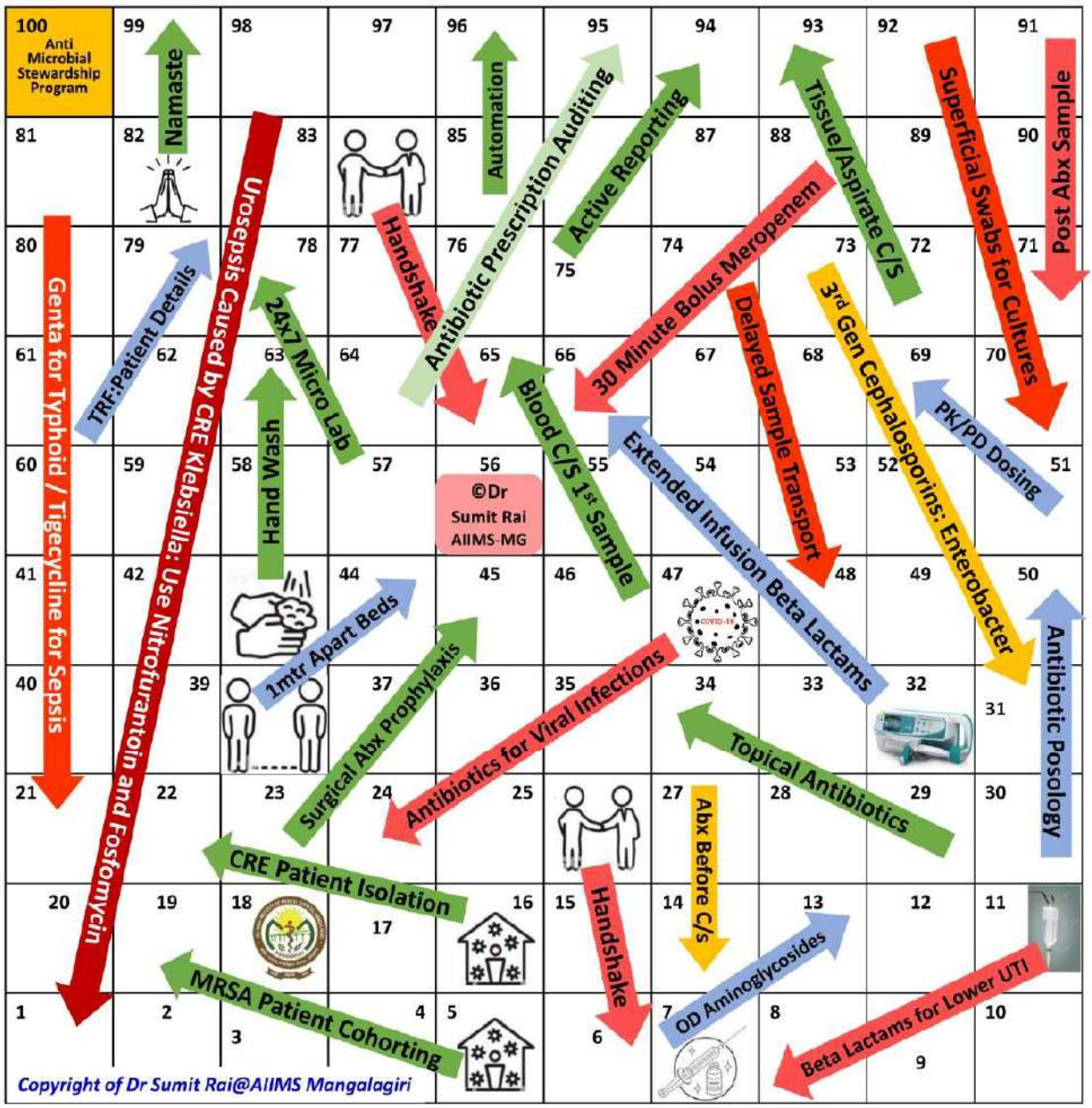
Antimicrobial Stewardship Program: Mistakes (Snakes) & Lessons (Ladders): Dr Sumit Rai



Antimicrobial Stewardship Do's and Don'ts: More Practical Pearls of Wisdom

- Always Collect Blood Culture as the FIRST Specimen from a patient with fever / sepsis.
- The AMSP Core Team makes Customized AST Reports with Clinical footnotes. Please read the footnotes carefully
- Amoxi-Clav Does NOT work in MRSA Infections and MSSA Infections can be effectively treated with Beta Lactamase Stable Penicillins [Clox, Diclox, Fluclox]. Basically Amoxi-Clav is useless in all Staph infections
- Separating a patient harbouring an MDR Pathogen and having dedicated instruments is the most effective method of infection control after Hand Hygiene: Cohort Nursing
- Topical Antibiotics should be chosen in absence of systemic symptoms, but preferably should avoid those for whom systemic formulations are available

- Avoid All Forms of Touch Greetings in the Hospital
- Avoid Use of Beta Lactams in bolus dosing of 30 minutes; These must be given as Extended Infusions of approx 3hrs
- Avoid dividing the daily dosing of Aminoglycosides as it reduces their efficacy. Total daily dose must be given OD
- Do NOT given Antibiotics for Routine Cough, Cold & COVID
- Avoid Aminoglycosides as monotherapy. Aminoglycosides do not work in Typhoid and Anaerobic Infections
- Avoid using Fosfomycin for systemic infections. Its intended use is only for Lower UTI Caused by E. coli and Enterococcus faecalis
- Avoid sending superficial swabs for culture. The isolated organisms are not true pathogens. Send biopsy or tissue

PG WRITE-UPS

AUTOMATION IN MICROBIOLOGY

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Final Year Postgraduate
Osmania Medical College, Hyderabad

DEFINITION: The use of a machine designed to follow repeatedly and automatically a predetermined sequence of individual operations

HISTORY OF AUTOMATION: Automation in microbiology first occurred in the early 1970 s with the introduction of the first semi-automated blood culture systems.This was followed by the early instrumented systems for identification and susceptibility testing of bacteria. In the 1980s an instrumented screening device for the detection of bacteriuria was introduced...

ADVANTAGES OF AUTOMATION:

- Elimination of subjective variability, Limited exposure to hazardous material ,Saving of media and reagents, Less need to find and train skilled technicians ,Reduced labour costs, Identification of category A and B potential agents of bioterrorism,Earlier production of useful information, Improved record keeping

Thus, automating process can revolutionalize the microbiology lab with more efficient, standardised practices that will improve quality, safety and cost -efficiency.

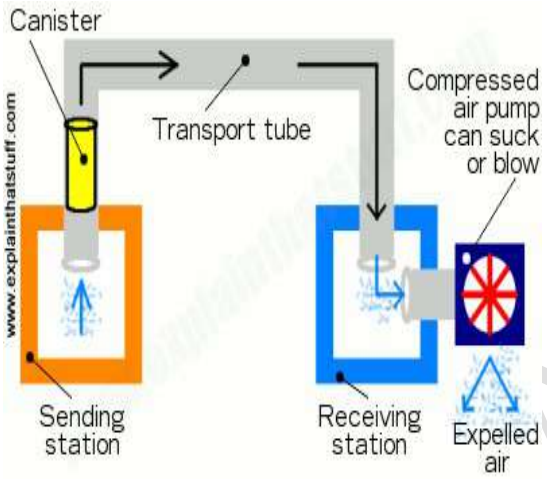
OUTLINE:

1.SPECIMEN COLLECTION	: VEEBOT
2.SPECIMEN TRANSPORT	: PNEUMATIC TUBE TRANSPORT
3.MICROSCOPY	: AUTOMATIC STAINING METHODS
	: URINE ANALYSER
4. PROCESSING	: INNOVA
	: INOQULA
	: PREVI- ISOLA & WASP
5. IDENTIFICATION	: DIGITAL READING
	: MALDI-TOF
	: VITEK
	: VIDAS
	: OMNILOG
6. AST	: ID / AST SYSTEMS
	: NOVEL TECHNOLOGIES
	: RAISUS ID / AST SYSTEM
	: DRAST
	: ABBOTT AVANTAGE (AV) DS
	: INK JET PRINTER
	: MAC CHIP

VEEBOT: It is a specimen collection Portable robotic device for automated phlebotomy, works near infra -red visualization producing stereoimages and robotic kinematics introducing needle and effecting venepuncture It has Accuracy, safety, precision which is able to locate small peripheral vein.

PNEUMATIC TUBE TRANSPORTER SYSTEM: Capsule pipelines

Capsule pipelines advantages are fast, easy,less labour, disadvantages are hemolysis of blood samples and can interfere with results and cost of installation and maintainance.



AUTOMATED LOOP STERILISATION

HiMedia Automatic Loop Sterilizer (HiLoop Auto Steriliser LA001-230 V AC, 50 Hz): Stainless steel electrical device having a rotor and a gas burner. Rotor has the capacity to hold 8 nichrome loops. A nozzle is attached to this device at one end for the supply of gas.

Electric loop sterilizer: Hi Loop Electric Steriliser Without flame (LA832), SteriMax (WLD-TEC). It is a unique double tube system, made of robust quartz glass and wear-resistant ceramic, reaches its optimal temperature of 900° C (1650° F) after only a few minutes. To sterilize the inoculation loop, simply insert into the quartz tube. After 5-7 seconds, the inoculation loop is sterilized.

AUTOMATION IN GRAM STAINING:

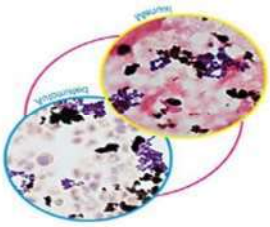
Gram staining is still a key staining protocol in bacteriology, guiding both the diagnostic processes and therapeutic management of bacterial infections. Despite its use over a century, Gram staining is still error prone, as slight variations in the (pre-/post-) analytical process

BIOMERIEUX – PREVI® COLOR GRAM

- It is a fully automated process from fixation to slide drying , slides are all stained the same way,up to 30 slides ready in 5 minutes, Can be integrated with Full Microbiology Lab Automation

POLY STAINER 5300

- It is a fully user programmable robotic stainer delivering high quality stained slides.To speed up staining time the Polystainer features slide agitation at the stain and water stations, which can be adjusted for speed and amplitude. Staining racks made of stainless steel (long life &durability) hold 20 slides.


PRE-ANALYTICAL PLATE STREAKERS: For this liquid based transport media is needed. It loads the sample on selected media and Spreads the inoculum for isolated colonies after incubation
Suppliers: WASP (Copan) Previ-Isola (BioMerieux) Innova (BD) and Inoqula (KIESTRA)

180 plates per hour

AUTOMATED URINE ANALYZER:

SYSMEX: UF 5000

Principle: Laser based flow cytometry along with impedance detection, forward light scatter and fluorescence to identify cells

- The system aspirates 0.8 mL of urine. It analyse cells [erythrocyte, leukocytes (WBC) and epithelial cells] Bacteria and casts, use electrical impedance for volume, forward light scatter for size. It uses a couple of fluorescent dyes for nuclear and cytoplasmic characteristics
- The formed elements are categorised in a two-dimensional space (scattergrams) on the basis of their size, shape, volume and staining characteristics.

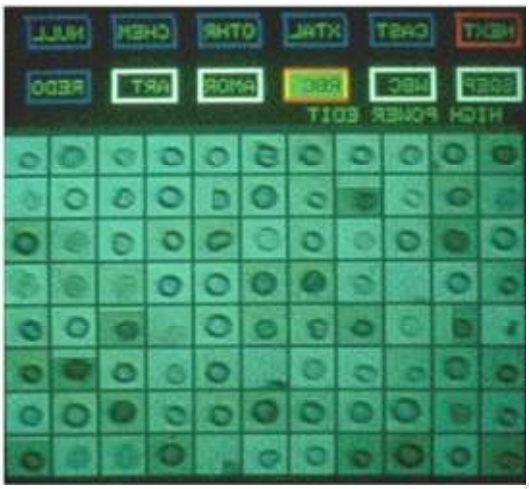
Beckman coulter: iQ200 Series Automated Urine Microscopy Analyzer

Here urine particles are isolated, identified and characterized on the screen

Eg: 1. proprietary Digital Flow Morphology technology

2.Auto-particle Recognition (APR) software

Iris flow videomicroscopy Urine is drawn through a flat chamber, video snaps are sorted by computer, technician scans images and deleted dud ones, computer then adds up #/cmm. these are RBCs



AUTOMATION IN SPECIMEN PROCESSING

- The currently available specimen processors include:

1] Innova processor -BD diagnostics

2] InoqulA full automation/manual automation [FA/MI] specimen processing device-BD Diagnostics

3] Previ Isola Automatic plate streaker-Biomerieux

4] Walk Away Specimen Processor [WASP]-Cogan diagnostics

- It process LIQUID -BASED SPECIMENS

1. INNOVA PROCESSOR

- It contains 5 Drawer x 40 tubes = 200 samples, 270 agar plates can be loaded, specimen can be added as they arrive in lab, up to 180 plates per hour processed in streak only mode.

2. INOQULA FA/MI

- Utilized for Slide preparation, autoinoculation of liquid specimen, manual plating of other specimens, inoculation is done using magnetic beads, more isolated colonies than manual

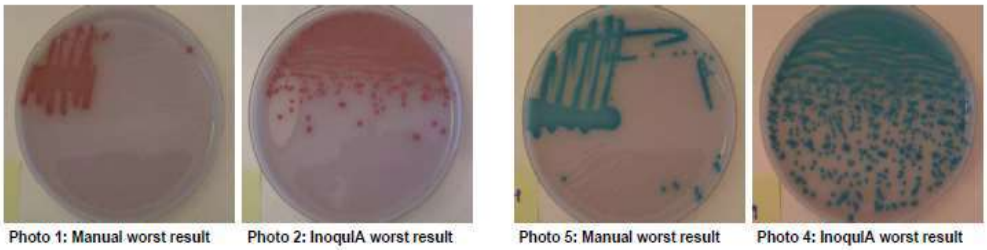


Photo 1: Manual worst result | Photo 2: InoqulA worst result | Photo 5: Manual worst result | Photo 4: InoqulA worst result

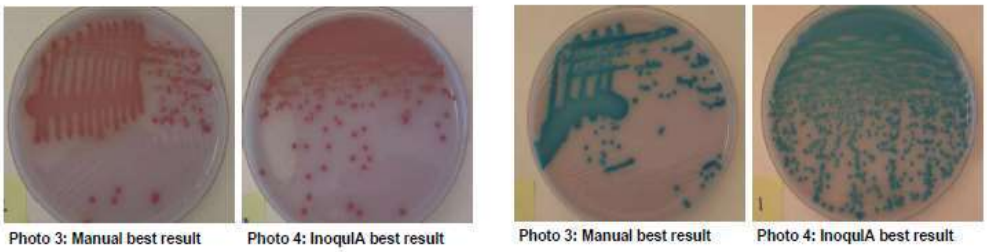


Photo 3: Manual best result | Photo 4: InoqulA best result | Photo 3: Manual best result | Photo 4: InoqulA best result

3. PREVI ISOLA AUTOMATED PLATE STREAKER

Specimens must be in uncapped bottles , uses disposable applicator and pippette for each plate and specimen, capacity is 180 plates/hr, streaked plates are ejected to output stacks,more isolated colonies

Figure 1. Streaking Comparison: Previ Isola vs Manual

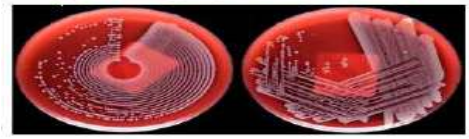


Figure 2. Previ Isola Applicator

Figure 3. Previ Isola System

4. WASP (Walk Away Specimen Processor)

- Utilizes SCARA Robot to move specimens and plates,capacity of 320-370 plates, utilizes reusable automatic loop changer -1,10,30 microL loops, includes full library of streaking pattern,streaked plates into stacks, half plates can be streaked and labelled.Gram slide prep module is available for slide preparation.

IDENTIFICATION SYSTEMS:

1. DIGITAL PLATE READING

Patient Based Reading	• View all plates from a single patient at the same time on one screen • Zoom into specific plates for more detailed view
Dynamic Plate Reading	• Digital images of plates are taken at set intervals throughput incubation process • View progression of growth over time
Vision Toolbox	• Image analysis software automatically sorts +/– plates • Provides presumptive ID for chromogenic plates

BACTERIAL RAPID DETECTION USING OPTICAL SCATTER TECHNOLOGY (BARDOT)

- Irradiation of bacterial colonies grown in a Petri dish with a red laser to generate light scattering patterns. The light scattering patterns are dependent on the three-dimensional (3D) morphology of bacterial colonies.It distinguished Listeria, Staphylococcus, Salmonella, Vibrio, and Escherichia with classification accuracy of 90–99% . Five species of Listeria, three species of Vibrio, and seven serogroups of E. coli have been discriminated with the accuracy of >91%, >96%, and >81%, respectively.

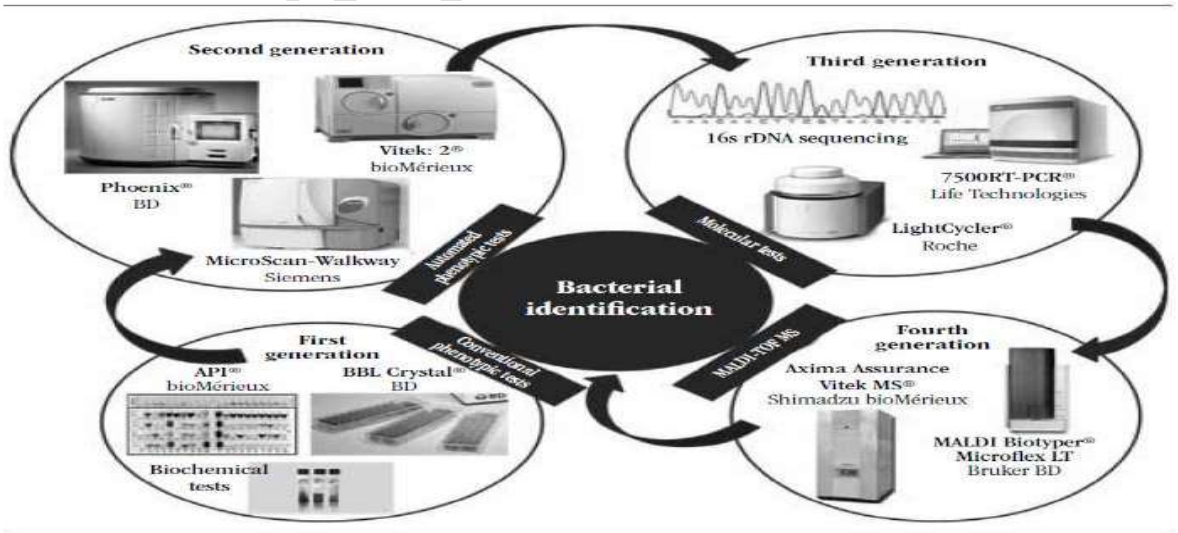


FIGURE 1 — *Evolution of bacterial identification in the microbiology laboratory*
rDNA: ribosomal deoxyribonucleic acid; MALDI-TOF MS: matrix assisted laser desorption/ionization-time of flight mass spectrometry

2. API (ANALYTICAL PROFILE INDEX) IDENTIFICATION SYSTEM

Packaged system for identification of

- Enterobacteriaceae -20 E ID
- non fermentative bacilli -20 E
  -20 NE system
- Gram positive cocci -API Staph Ident
  -API Staph
  -API ID 32 Staph

API 20 E SYSTEM:

- Used for identification of Enterobacteriaceae, and non-fermenters like P. aeruginosa, S. maltophila, Acinetobacter species. Consists of a plastic strip with 20 cupules containing dehydrated substrates and a plastic incubation chamber.
- Same day identification-5 hrs or after 24-48 hrs (more accuracy and precision).

BBL CRYSTAL ENTERIC /ON FERMENTER ID SYSTEM //GRAM POSITIVE IDS

- Miniaturised identification system, consists of fluorogenic and chromogenic substrates, reproducibility 96.3%-100%, accuracy of 96.9%

3. VITEK SYSTEM

The VITEK 2 is an automated microbiology system utilizing growth-based technology. fully automated process for card identification, organism suspension dilution, and card filling. Uses calorimetric technology with 3 wavelengths of light

4. MATRIX ASSISTED LASER DESORPTION/IONISATION -TIME OF FLIGHT MASS SPECTROMETRY.

Identification Based on PEPTIDE MASS FINGERPRINT
Commercially available systems include
-Vitek MS -[bioMerieux]
-MALDI Biotyper-[Bruker]

MALDI TOF IN BACTERIOLOGY

- Identification of bacteria from clinical specimens, culture plates, bacterial strain typing and taxonomy. Detect antimicrobial resistance-beta lactamase production, Carbapenemase activity. Detect the antibiotic mass alteration due to chemical modification, detection, identification and inactivation of biological warfare, food and water safety

APPLICATIONS IN VIROLOGY

- Diagnosis of Influenza, enterovirus, HPV, herpes, hepatitis viruses, genotyping of hepatitis B & C virus, JC polyoma virus, for typing, subtyping & tracing the lineage of human influenza virus, for detection of mutation in hepatitis B virus, for drug resistance of Gancyclovir in CMV

APPLICATION IN MYCOLOGY

- Fungi identified are Candida, Cryptococcus, Dermatophytes, Fusarium, Aspergillus, Pencillium.

5. SENSITITRE AUTO IDENTIFICATION SYSTEM:

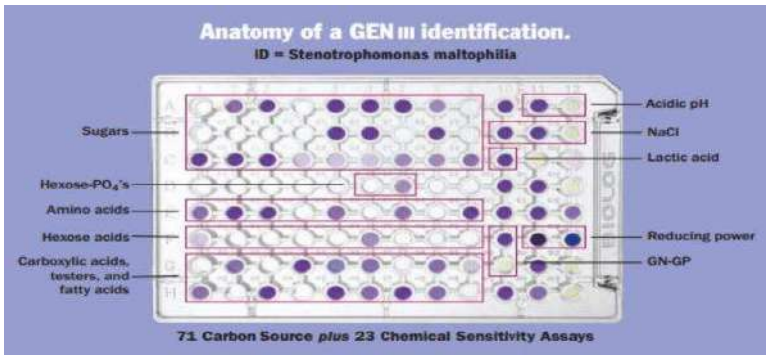
- utilizes fluorescence technology for detection of bacterial growth by monitoring the activity of specific surface enzymes produced by the test organism. Consists of 32 biochemical tests and fluorescent tests. Each biochemical test medium along with appropriate fluorescent indicator is dried into individual wells of Sensititre plate. Tests are read for the presence or absence of fluorescence after 5 hr/ overnight incubation. Results are transmitted to computer for analysis and identification.

6. THE PHOENIX SYSTEM

- A newly developed fully automated identification and antimicrobial susceptibility test system. Comprised of disposable panels that combine identification and AST. Perform automatic reading at every 20 minutes during incubation.

7. OMNILOG ID SYSTEM:

Fully automated system. Uses carbon source utilization method. Simultaneously incubates, read and interprets the microplate.



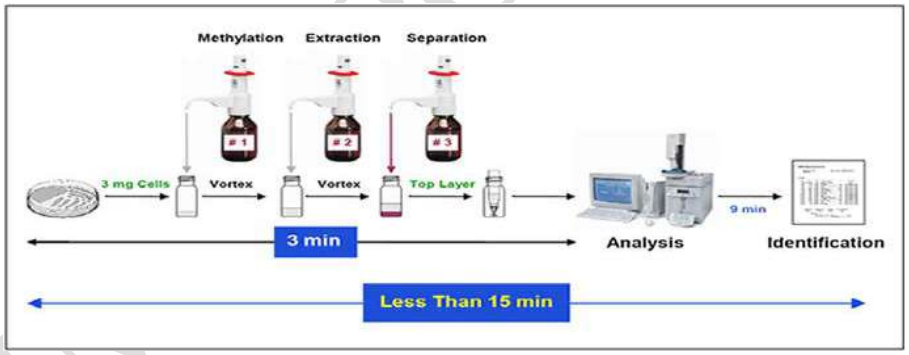
8.VIDAS AND MINIVIDAS

PRINCIPLE

- Combines an enzyme immunoassay competition method with a final fluorescent detection. The Solid Phase Receptacle (SPR®) serves as the solid phase as well as the pipetting device for the assay. Reagents for the assay are ready-to-use and pre dispensed in the sealed reagent strips. All of the assay steps are performed automatically by the instrument. The reaction medium is cycled in and out of the SPR several times.

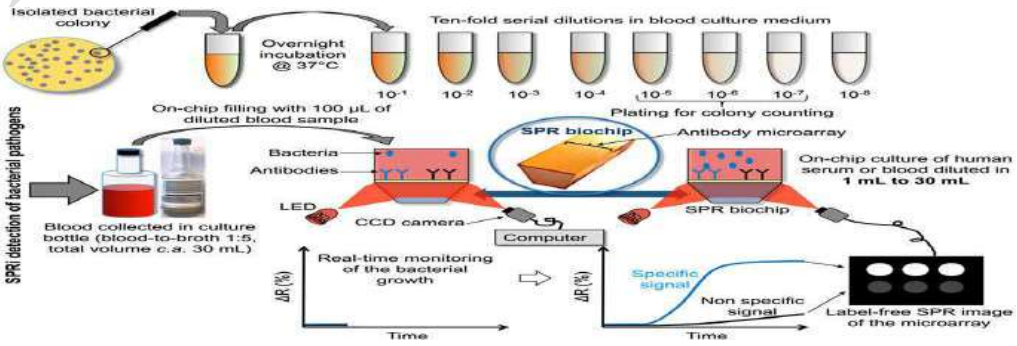
SHERLOCK MICROBIOLOGICAL IDS

- Gas chromatography (GC) system dedicated to bacteria identification by fatty acid methyl ester (FAME) analysis is the Sherlock Microbial Identification System (MIS)
- Principle: microorganisms have typical cellular fatty acid compositions, which can be compared with the mean fatty acid composition of the strains used to create the library. After comparison, the identities of unknown microorganisms are determined.



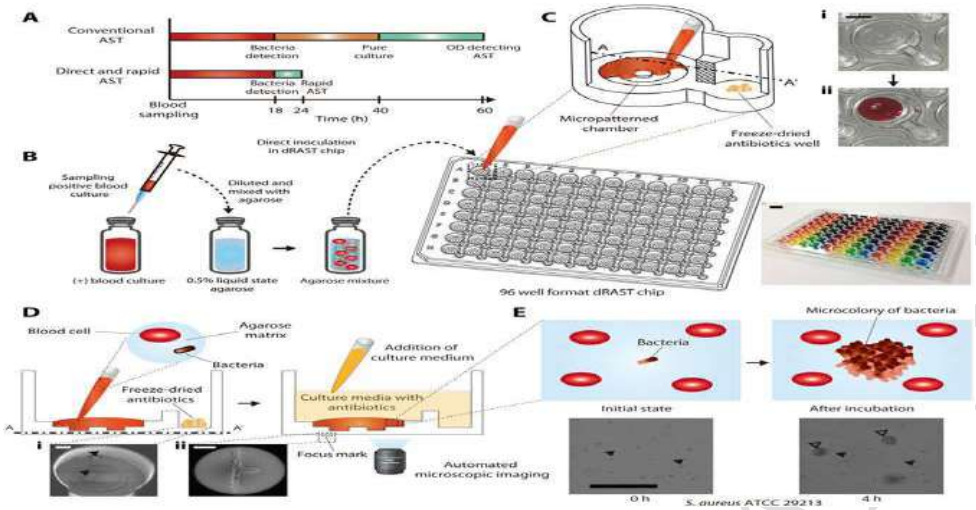
NOVEL TECHNOLOGIES IN ID

- Live bacteria are captured on microarrayed specific antibodies (spotted in triplicate onto the biochip surface) during the enrichment step. SPRi data are treated and plotted as variations of light reflectivity (ΔR (%)) over time for each region-of-interest (corresponding to antibody spots arrayed on the sensor).



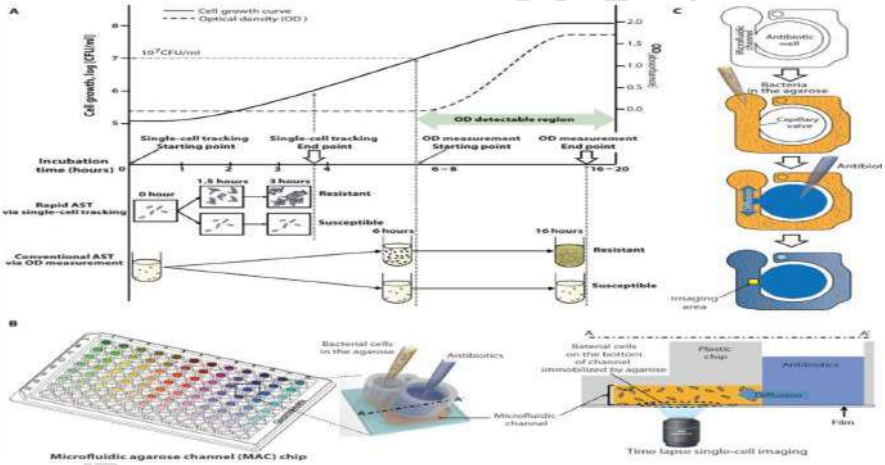
DIRECT RAPID ANTIMICROBIAL SUSCEPTIBILITY TESTING[DRAST]

- AST from positive blood culture bottles, it uses microscopic image analysis, used for Growth detection and time lapse for MIC calculation.



MAC CHIP

- Micro Fluidic Agarose Channel chip, Rapid AST by single cell tracking



❖ AUTOMATION IN BLOOD CULTURE

Bact/ALERT (biomerieux), BACTEC 9000 SYSTEM (BD), Versa TREK

PRINCIPLE:

- Culture bottles contain ^{14}C labelled substrates – organisms metabolise & produce $^{14}CO_2$
- CO_2 diffuses into gas permeable sensor matrix at the bottom of bottle to dissolve in H_2O, H+ ions produced reducing pH

BACTEC SYSTEMS:

- Fully automated with incubator, shaker and detector, Consists of Fluorescent sensors: reduced pH increases fluorescent output.
- BACTEC 9240(240 vials), BACTEC 9120 (120 vials), BACTEC-NR-860

BACT/ALERT MICROBIAL DETECTION SYSTEM

- Measures CO2 derived pH changes colorimetric sensor in the bottom of each bottle ,Blue → light green → yellow: as pH decreases,Bottle reflectance monitored & measured every 10 minutes

VERSA TREK SYSTEM (THERMO SCIENTIFIC)

- Monitors bacterial growth by detecting pressure changes in the headspace of the blood culture bottle secondary to gas consumption/production.

AUTOMATED SYSTEMS IN MYCOBACTERIOLOGY

BACTEC MGIT 960:

- Automated liquid in-vitro diagnostic instrument 960-tube capacity.4 ml enriched Middle brook 7H9 broth with 0.25% glycerol and added oleic acid, albumin, dextrose, and catalase (OADC) and PANTA antibiotics. Fluorescent indicator quenched by oxygen - embedded in silicone on the bottom of round-bottom tube. Initial concentration of dissolved oxygen quenches the emission from the compound, and little fluorescence detected. Actively growing and respiring microorganisms consume the oxygen, which permits the compound to fluoresce.
- BACTEC MGIT 960 SIRE susceptibility test: first line drugs Streptomycin, INH, Rifampicin, Ethambutol

AUTOMATION IN VIROLOGY:

PCR AND NUCLEOTIDE SEQUENCING :

It is achieved through the use of cycle sequencing reaction in the PCR reaction mixture. The resulting reaction products can be analyzed by polyacrylamide gel electrophoresis followed by automated laser scanning& sequence assembly.

MICROARRAY TECHNOLOGY:

GENE CHIPS : It consists of thousands of hybridization sites ,each of which provides information regarding the nucleotide composition of each amplicon

AUTOMATION IN MYCOLOGY

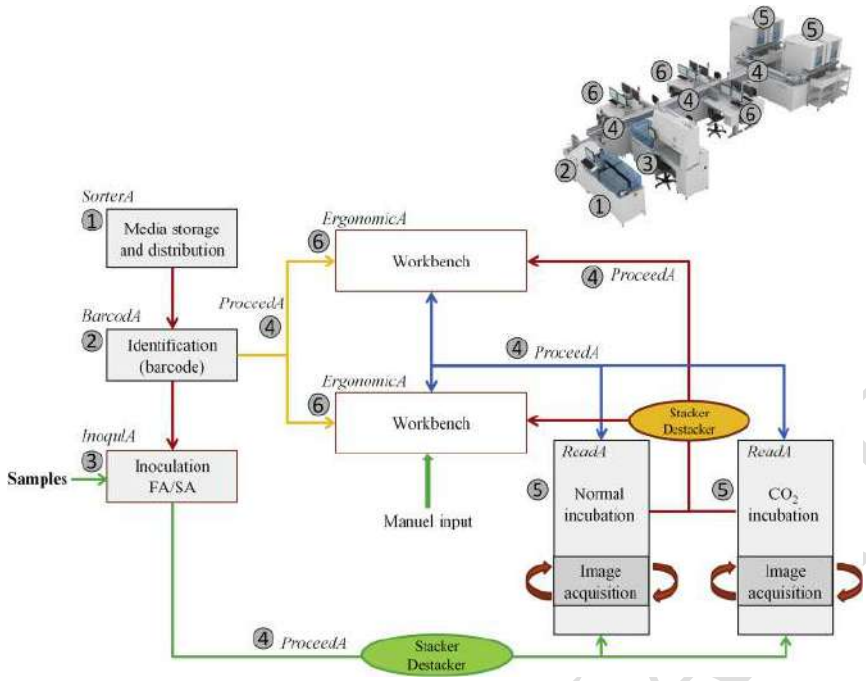
MICROSCAN YEAT IDENTIFICATION PANEL: 96 well, microtiter plate containing 27 dehydrated substrates, It uses chromogenic substrates to assess specific enzymatic activity, Results obtained within 4hours.

UNI-YEAST TEK SYSTEM: used to indicate carbohydrate utilization, nitrate utilization, urease production & morphology

API 20C AUX YEAST SYSTEM: Strip contains 20 micro-cupules: 19 contain dehydrated substrates for utilisation by yeasts & reactions compared with growth in first cupule lacking Carbohydrate.Most yeast identified in 48 hours; Cryptococcus & Trichosporon sps in 72 hours

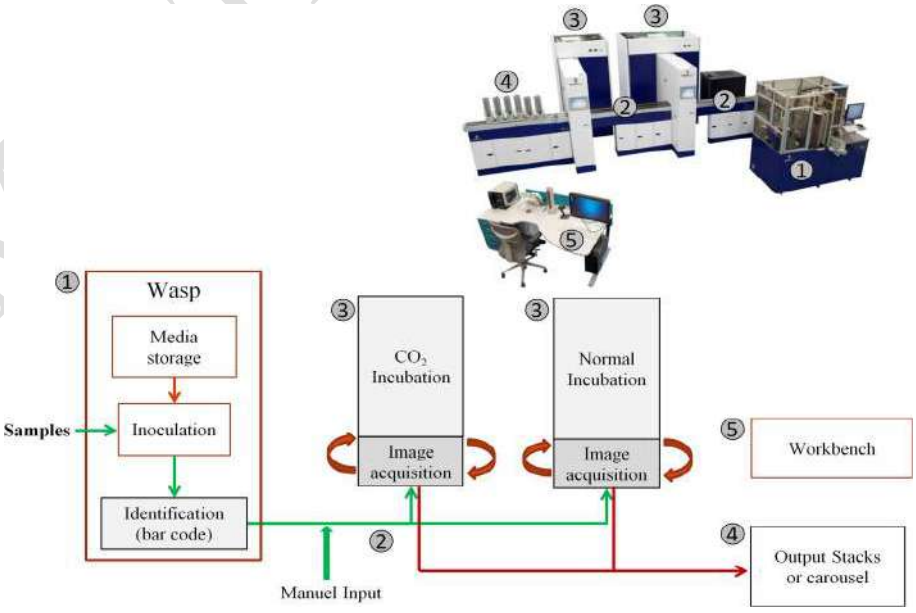
TOTAL LAB AUTOMATION:
1) Kiestra TLA
2) FMLA (Full Microbiology Laboratory Automation)
3) WASP lab
COMMON ELEMENTS: Conveyer /track system
Digital camera
Automated incubator
Digital reading station
Software
KIESTRA TLA: 5 modules-linked by conveyer

- Sorter A
- Barcod A
- Inoql A –processor
- Read A –incubator with digital imaging equipment
- ergonomics A –work benches



FMLA: Previ isola automated plate streaker,Smart incubator system. Linked via conveyer /track system

WASP LAB: CO2 & nonCO2 incubator .Linked by conveyer system.Image acquisition station-capture image, Plate with growth is reloaded on WASP lab for automated broth inoculation and Kirby bauer disk dispensing



NEW TREATMENT OPTIONS FOR INFECTIONS WITH CARBAPENAMASE RESITANCE BACTERIA

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Introduction

Carbapenem are β-lactam Antibiotics that act as cell wall synthesis inhibitors and inhibit the final transpeptidation step in peptidoglycan synthesis. Effective against infection caused by Gram negative bacteria like *Klebsiella pneumonia*, *Pseudomonas aeruginosa, Acinetobacter baumanii complex* and Gram positive bacteria like methicillin sensitive *Staphylococcus aureus.*

In the present decade Carbapenem resistant bacteria has become major problem in hospitals especially in Intensive Care units due to inadequate and inappropriate treatment options. Among them occurrence of Carbapenem resistant Enterobacteriaciae (CRE) is reported as a worldwide threat among the nations.

Defination of Carbapenem-Resistant Bacteria

US CDC defines CRE as enterobacteria non-susceptible to any carbapenem (Imipenem, Meropenem, Doripenem, Ertapenem) or found to produce a carbapenemases regardless of Carbapenem minimum inhibitory concentrations.

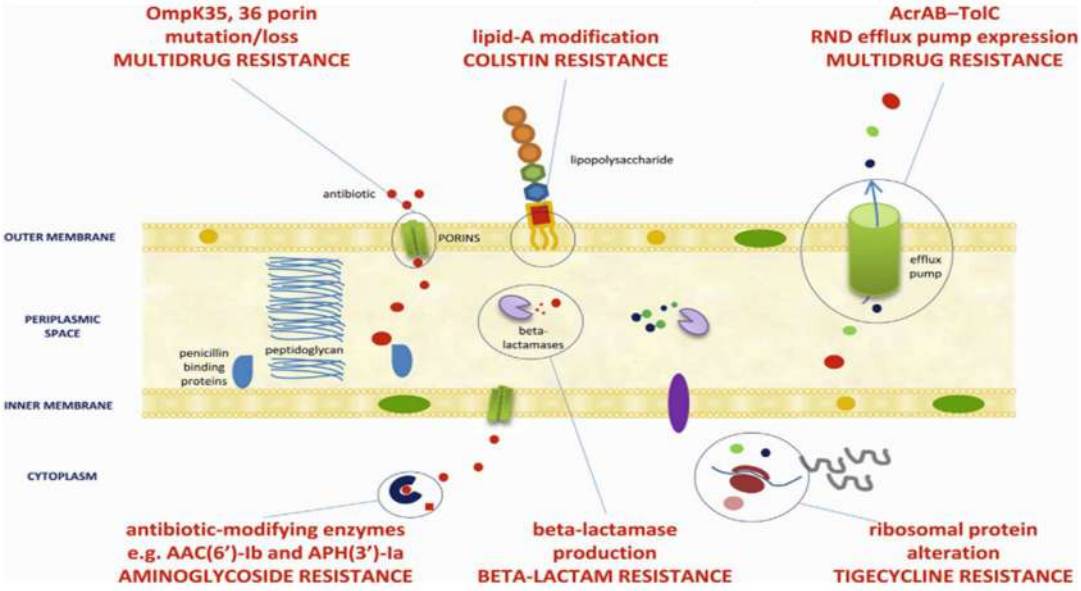


Figure 1 :- Major mechanisms of antimicrobial resistance, including carbapenem resistance, in Enterobacteriaceae[6]

For Gram-positives there are alternatives to Carbapenems (e.g. Glycopeptides, Daptomycin). Carbapenemase-producing Gram-negatives in particular are resistant to almost all β-lactams.

Classification of β-Lactamases with Carbapenemase Activity

β- lactamase Class	Gram-Negative Bacteria Where Enzyme Detected	Examples
A	*Klebsiella pneumonia*, Enterobacteriaceae	KPC, SME, NMC-A, IMI, PER, GES, SFO, SFC, IBC

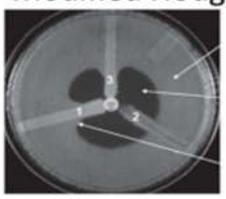
B	Enterobacteriaceae, *Pseudomonas aeruginosa, Acinetobacter species*	Metallo-β-lactamases (MBLs) inhibited by EDTA (IMP, VIM, NDM), SPM, SIM, GIM
D	*Enterobacteriaceae, Acinetobacter baumannii*	OXA-23, OXA-40,OXA-58, PSE

Methods of Detection of the Carbapenemases

Phenotypic tests

Test	**Principle**	**Detection of carbapenemases**	**Turn around Time**	**Interpretation of results**
Modified Hodge test (MHT)	Based on whether the growth of the indicator strain is enhanced at the junction of the inhibition zone growth line produced by the indicator strain and the test strain	KPC Carbapenemases	48hrs	Indentation of Zone diameter of Escherechia Coli toward Carbapenem disc along Streaked isolate.
CarbaNP Test	• Colorimetric microtube Assay • Based on in vitro hydrolysis of imipenem • Carbapenemases detected by changes in pH values using indicator phenol red.	KPC, NDM , VIM , IMP , SPM , SME type Carbapenemases Poor ability to detect oxa-48 type carbapenemases	2 hours	Color change from red to yellow or blue to yellow depending on the pH indicator used.
mCIM (modified Carbapenem Inactivation Method)	Inactivation/hydrolysis of meropenem by Carbapenemases after 24h of incubation .	KPC, NDM, VIM, IMP, SPM,SME as well as OXA type carbapenemases	18-24 hours	Positive→zone diam of 6–15 mm; indeterminate→ 16–18 mm; negative→19 mm; requires initial setup and then plating of disk onto lawn of E. coli following 4 hrs of incubation
eCIM (EDTA Carbapenem Inactivation Method)	➢ If the test isolate produces Metallo-β-lactamase, activity of Carbapenemase will be inhibited in presence of EDTA , such that meropenem disk will not be hydrolysed	Differentiates detection of MBLs i.e (NDM, VIM, IMP) from serine Carbapenemases (KPC, OXA, SMC)	18-24 hours	
Lateral flow immunoassays	➢ Antibody-based methods→ identify the presence of carbapenemase		15 minutes	Positive results based on the presence of visible lines specific for carbapenemase-type

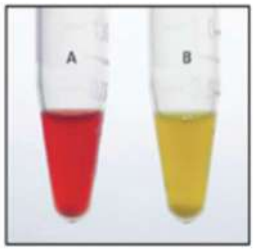
Modified Hodge Test

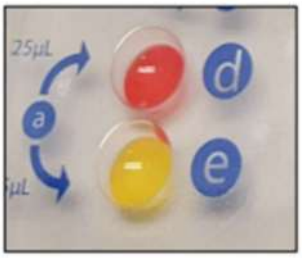


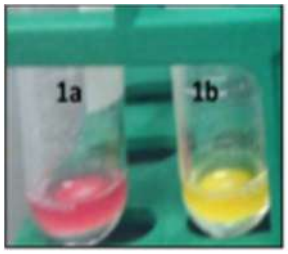
A lawn of carbapenem susceptible *E. coli* ATCC 25922

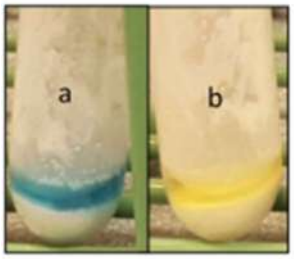
Zone of inhibition of *E. coli* ATCC 25922 by ertapenem

Indentation of *E. coli* ATCC 25922 growth (clover leaf appearance) around the streak line of the carbapenemase-producing *K. pneumoniae* ATCC BAA-1705.

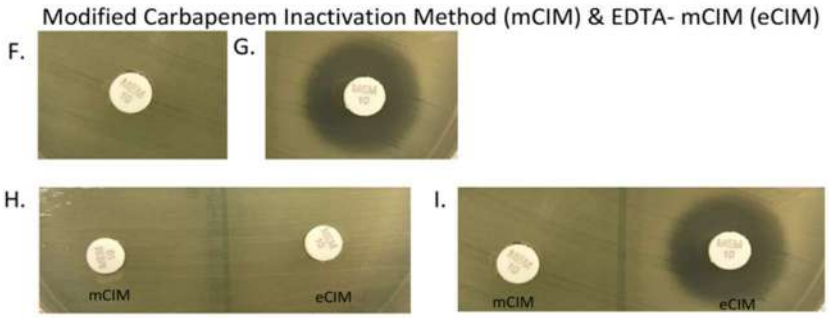








Carba NP test and Variants[7]



- MALDI–TOF-based method→ can be used for detecting KPC and NDM.

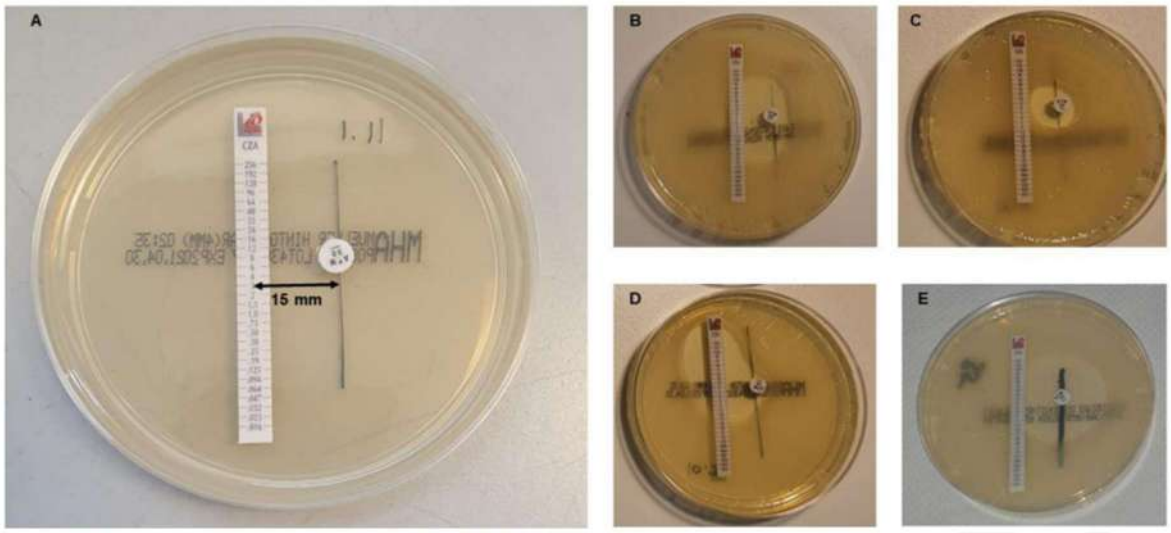
- **Molecular-based detection methods→**
  PCR- based methods-> Multiplex PCR; Real time PCR
  Microarrays
- ✓ Gold standards method
- ✓ Detects all carbapenemase genes

Benefits of combination therapy→

- ✓ Reduction of initial inappropriate Antimicrobial therapy
- ✓ Synergistic effect
- ✓ Suppression of emerging resistance

For example monobactam Aztreonam is resistant to hydrolysis by MBLs but susceptible to hydrolysis by serine class A, C and D ESBLs. In contrast, ceftazidime-avibactam, a third-generation cephalosporin combined with β-lactamase inhibitor, has broad activity against serine beta-lactamases but is hydrolysed by MBLs.

Screening for in-vitro synergy between CAZ/AVI and ATM for MBL-producing Enterobacterales can be done with Modified E-test/Disk diffusion method. This combination cane be used in NDM-producing Enterobacteriaciae.[8]



Novel antibiotic therapeutic for Carbapenem resistance bacteria

Drug	Class	Carbapenemases spectrum	Indications proposed
Ceftazidime avibactam	3rd generation Cephalosporin-β-lactamase inhibitor	• Activity against KPCs and OXA-48 is present • Not active against MBLs, *Acinetobacter baumanii, S. maltophila* • Activity→ NDM- *P.aeruginosa, Klebsiella pneumonia*	cUTI/AP, cIAI, HABP/VABP
Ceftaroline avibactam	Cephalosporin-β-lactamase inhibitor	• Activity against KPCs and OXA-48 is present • Not active against MBLs	cUTI
Plazomicin	Aminoglycoside	➢ Active against most KPCs ➢ Not active against many NDMs ➢ Activity → *P.aeruginosa (variable), Proteus mirabilis, Enterobacter spp, E.coli, Klebsiella pneumonia*	cUTI along with Acute Pyelonephritis cUTI/AP
Eravacycline	Tetracycline	Active against KPCs Activity → *Acinetobacter baumanii, S. maltophila*	cIAI cUTI
Aztreonam–avibactam	Monobactam-β-lactamase inhibitor	Inhibition of KPC, MBLs, ESBLs, OXA	
Meropenem–vaborbactam (Boronic acid derivative)	Carbapenem-β-lactamase inhibitor	Inhibition of KPC	cUTI/AP
Imipenem–relebactam	Carbapenem-β-lactamase inhibitor	Inhibition of KPC Activity→ *Klebsiella spp; E.coli, Enterobacter cloacae, Citreobacter freundii, P.aeruginosa*	cUTI/AP, cIAI, HABP/VABP

Cefiderocol	Novel parenteral siderophore Cephalosporin	Avoid resistance induced by porin channel mutations and efflux pumps overproduction Activity→ *Klebsiella spp; E.coli,Enterbacter spp, Proteus spp, Providencia spp*	cUTI/AP, HABP/VABP

Abbreviations: cIAI→complicated intra-abdominal infection; cUTI→ complicated urinary tract infection; HABP→hospital-acquired bacterial pneumonia; KPC→Klebsiella pneumoniae carbapenemase; NDM→New Delhi metallo-β-lactamase; NP→ nosocomial pneumonia; OXA→oxacillinase; P. aeruginosa→ Pseudomonas aeruginosa; S. maltophilia→Stenotrophomonas maltophilia; VABP→ ventilator-associated bacterial pneumonia.

Agent	**Adult dosage (with normal liver and kidney function)**	**Target organism**
Amikacin	Cystitis: 15 mg/kg/dose d IV once. All other infections: 20 mg/kg/dose d IV x 1 dose	CRE, DTR-Pseudomonas aeruginosa
Ampicillin-sulbactam	9 g IV q8h over 4 hours OR 27 g IV q24h as a continuous infusion	CRAB
Cefiderocol	2 g IV q8h, infused over 3 hours	CRE, DTR-P. aeruginosa, CRAB
Ceftazidime-avibactam	2.5 g IV q8h, infused over 3 hours	CRE, DTR-P. aeruginosa
Ceftazidime-avibactam and aztreonam	Ceftazidime-avibactam: 2.5 g IV q8h, infused over 3 hours + Aztreonam: 2 g IV q8h, infused over 3 hours, administered at the same time as ceftazidime-avibactam, if possible	Metallo-β-lactamase-producing CRE
Eravacycline	1 mg/kg/dose IV q12h	CRE, CRAB
Gentamicin	Cystitis: 5 mg/kg/dose d IV once All other infections: 7 mg/kg/dose d IV x 1 dose	CRE, DTR-P. aeruginosa
Imipenem-cilastatinrelebacta	1.25 g IV q6h, infused over 30 minutes	CRE, DTR-P. aeruginosa
Meropenem	2 g IV q8h, infused over 3 hours	CRE and CRAB
Meropenem-vaborbactam	4 g IV q8h, infused over 3 hours	CRE
Plazomicin	Cystitis: 15 mg/kg d IV x 1 dose All other infections: 15 mg/kg d IV x 1 dose	CRE, DTR-P. aeruginosa
Tigecycline	200 mg IV x 1 dose, then 100 mg IV q12h	CRE, CRAB

CRE: Carbapenem-resistant Enterobacterales; CRAB: Carbapenem-resistant *Acinetobacter baumannii*; DTR-P. aeruginosa: *Pseudomonas aeruginosa* with difficult-to-treat resistance; E. coli: Escherichia coli; IV: Intravenous; MIC: Minimum inhibitory concentration; PO: By mouth; q4h: Every 4 hours; q6h: Every 6 hours; q8h: Every 8 hours; q12h: Every 12 hours, q24h: Every 24 hours.

- β-lactam–sulfone+ β-lactamase inhibitor combinations → cefepime–tazobactam and cefepime–enmetazobactam; β-lactam–diazabicyclooctane+ β-lactamase inhibitor combinations including cefepime–zidebactam ; Sulbactam–durlobactam, meropenem–nacubactam and cefpodoxime proxetil- and β-lactam–boronate+ β-Lactamase inhibitor combinations → cefepime–taniborbactam are under clinical trials, which could be promising future treatment options against Carbapenemase-producing bacteria.[9]

- **Nanoparticles→**
  Bactericidal action of Graphene oxide/ Cu/Ag NPs against E. coli and K. pneumonia has recently been discovered. Mechanism of action of Nanoparticles is to kill bacteria, causes damage to membrane load cells as well as the produce free oxygen radicals.
- **Phage Therapy→**
  Use of phages as an alternative to antibiotics for treating multidrug resistant Shigella dysenteries isolated from wastewater has been studied.

Randomized clinical trials of CRE treatment

- ✓ Multinational AIDA trial →compared the effectiveness and safety of colistin alone with those of a colistin and high-dose meropenem combination against Carbapenem resistant Gram-negative bacilli.
- ✓ TANGO II open label RCT →assessed effect and safety of meropenem-vaborbactam with various CRE infections.

Antimicrobial resistance is dynamic.The best therapy should be delivered based on clinical reasoning, considering the severity of the patient illness, the isolate susceptibility, the site of infection, the available drugs and the optimal approach. Optimization of dosing of available agents and using combination therapy are the most appropriate treatment strategies that can be used at this time. A lot of efforts must be devoted to designing and conducting randomized clinical trials for the newer drugs for treating Carbapenem resistant bacteria in a better perspective.

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MICROBIOMES AND HUMAN HEALTH

By Dr Rajesh Kanna K
1st Year PG
AIIMS Bibinagar

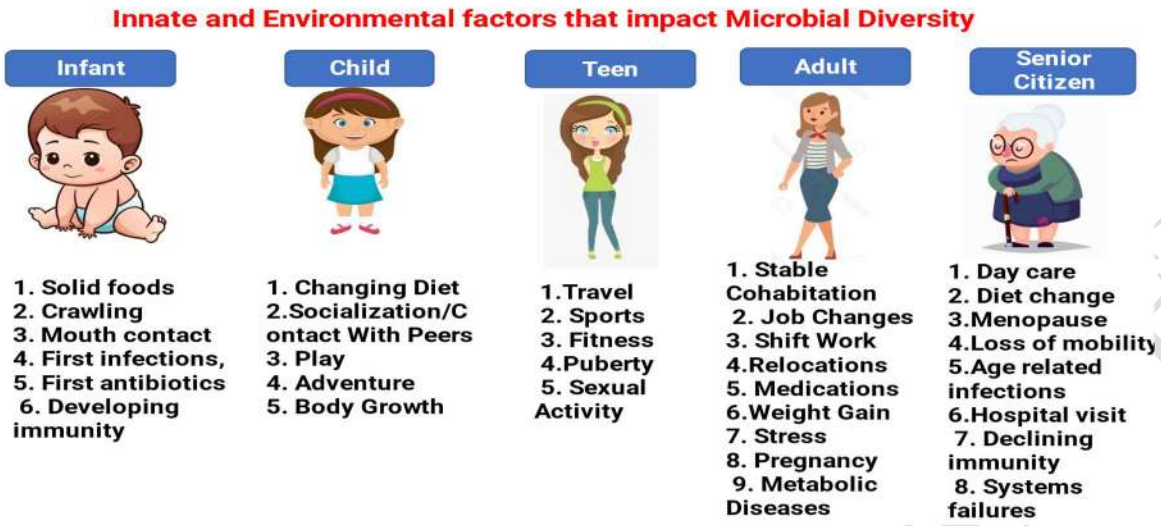
1.Introduction:

Microbiome is a term that describes the genome of all the microorganisms, symbiotic and pathogenic, living in and on all vertebrates. In 2001, renowned microbiologist and **Nobel laureate Joshua Lederberg** coined the term **Microbiome** to describe all the microbes "that literally share our body space." Humans cannot live a normal life without their microbial partners—that we are holobionts: hosts and microbes living together, and importantly, evolving together.

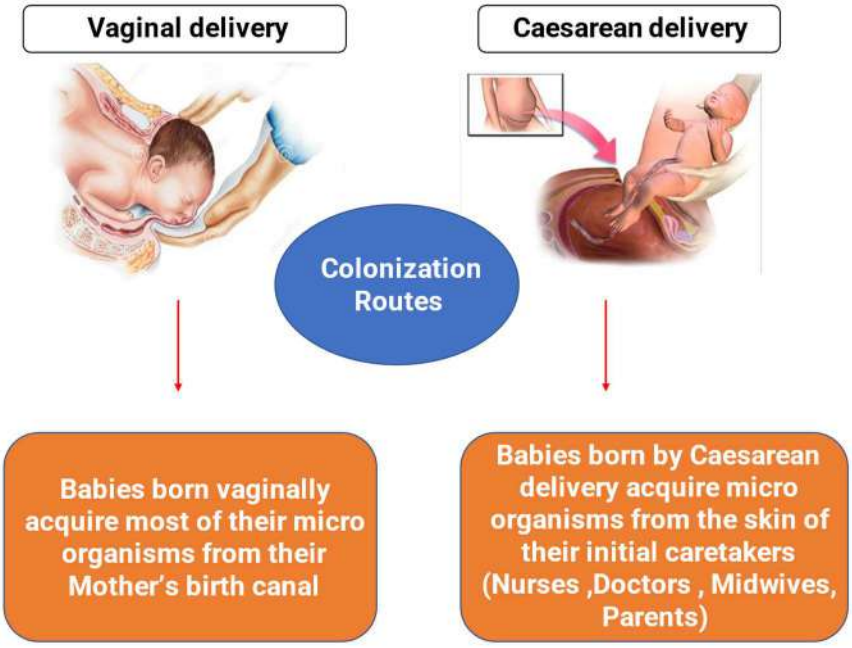
2.Human Normal Microbiota routinely cultured from various body sites:

Body systems	Body site	Micro organisms
Eye	Conjunctiva and outer ear	*Coagulase negative Staphylococcus, Hemophilus spp, Streptococcus spp, Staphylococcus aureus*
Skin	Non mucous membrane surfaces	*Coagulase negative Staphylococcus, Staphylococcus aureus, Streptococcus spp, Diphtheroid, Bacillus Spp, Malassezia furfur, Cutibacterium acnes*
Respiratory tract	Nose	*Coagulase negative Staphylococcus, Streptococcus spp, Neisseria spp, Hemophilus spp*
Gastro intestinal tract	Mouth and oropharynx	*Fusobacterium spp, Prevotella spp, Neisseria sp, Porphyromonas spp, Actinomyces spp, Eiknella corrodens, Veillonella*
	Stomach	*Staphylococcus spp, Streptococcus spp, Peptostreptococcus spp, Lactobacillus spp*
	Small intestine	*Lactobacillus spp, Clostridium spp, Bacteroides spp, Enterococcus spp*
	Large intestine	*Escherichia coli, Klebsiella spp, Proteus spp, Pseudomonas spp, Bacteroides spp, Fusobacterium spp*
Genito urinary tract	Distal urethra	*Bacteroides Spp, Fusobacterium spp, Peptostreptococcus spp, Streptococcus Spp, Diphtheroid*
	Vagina	*Lactobacillus Spp, Diphtheroid, Peptostreptococcus spp, Streptococcus Spp, Candida spp, Gardnerella vaginalis*

3.Factors that impact Microbial Diversity:

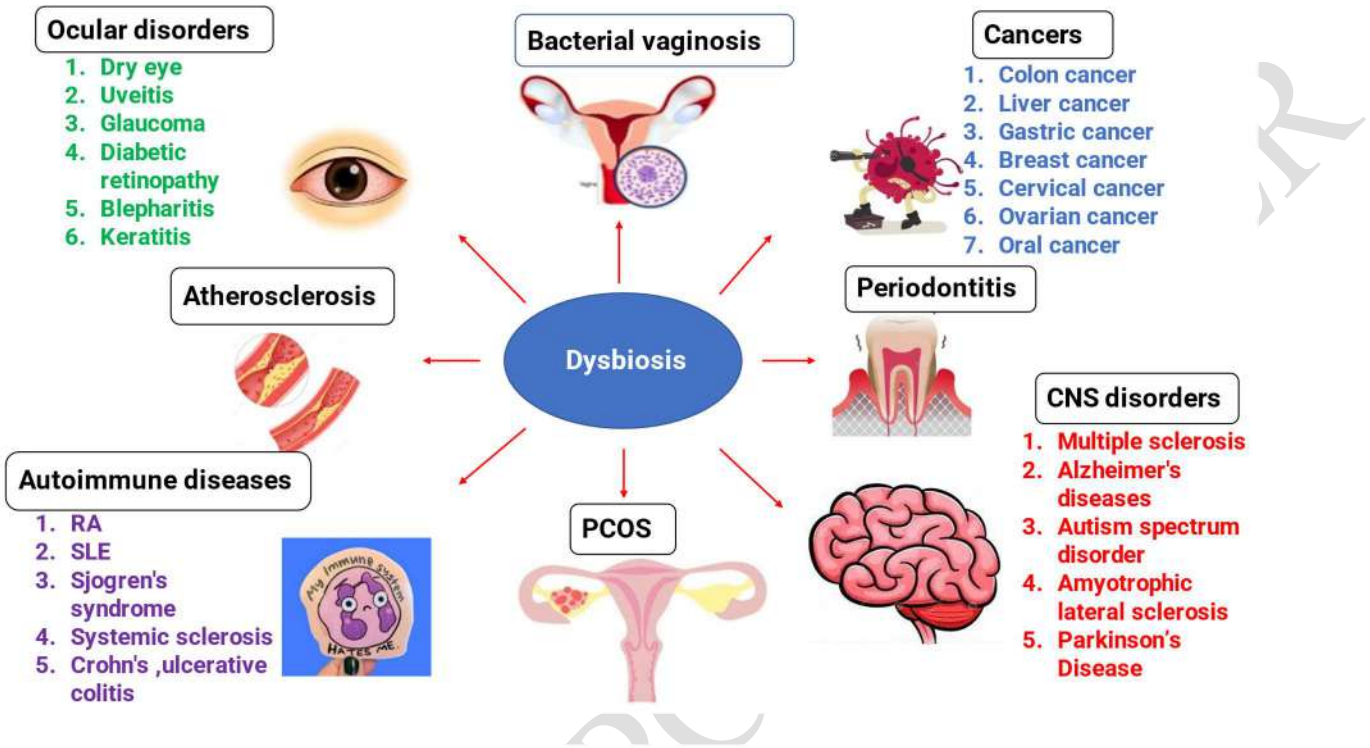


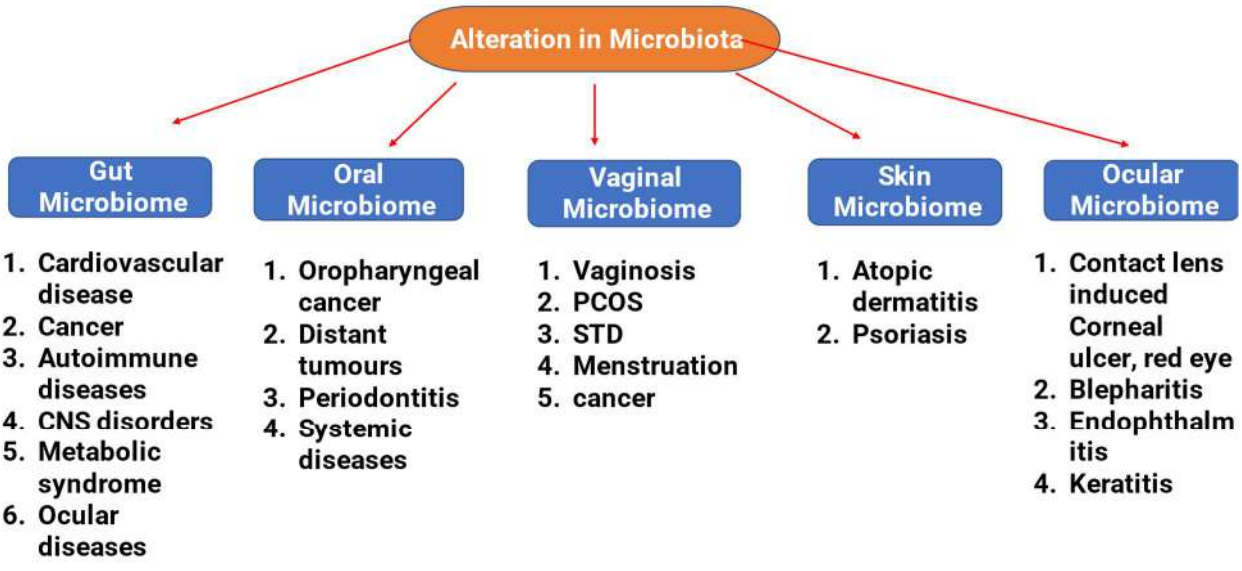
4.Colonization Routes of Microbiome to New-born:



5.Dysbiosis:

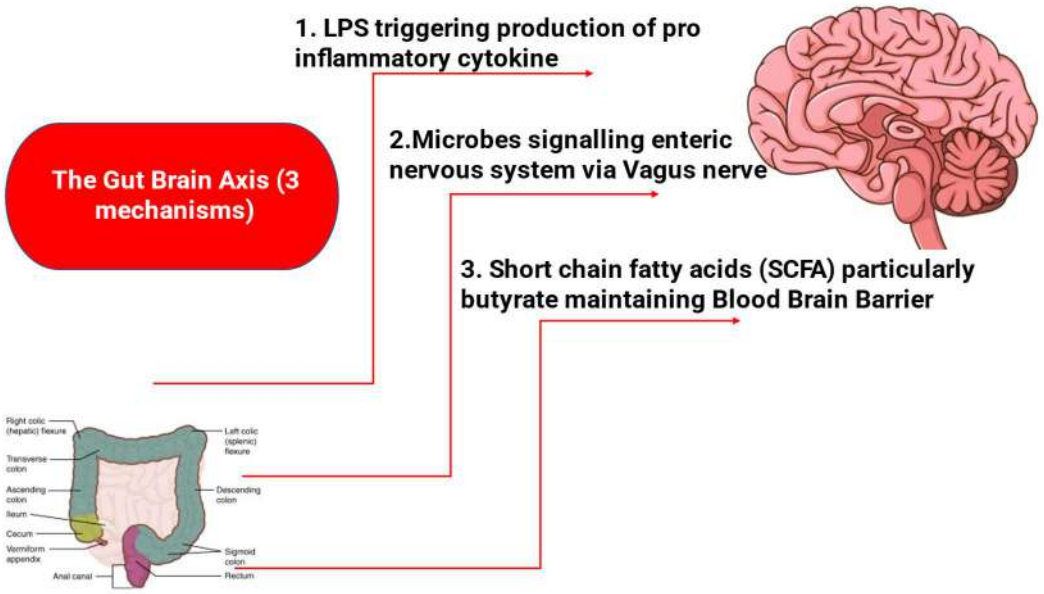
An unbalanced proportion of bacteria causing an imbalance in the microbial equilibrium is termed "dysbiosis". Disruption of the normal microflora (commonly referred to as dysbiosis) can lead to disease by the elimination of needed organisms or allowing the growth of inappropriate bacteria.





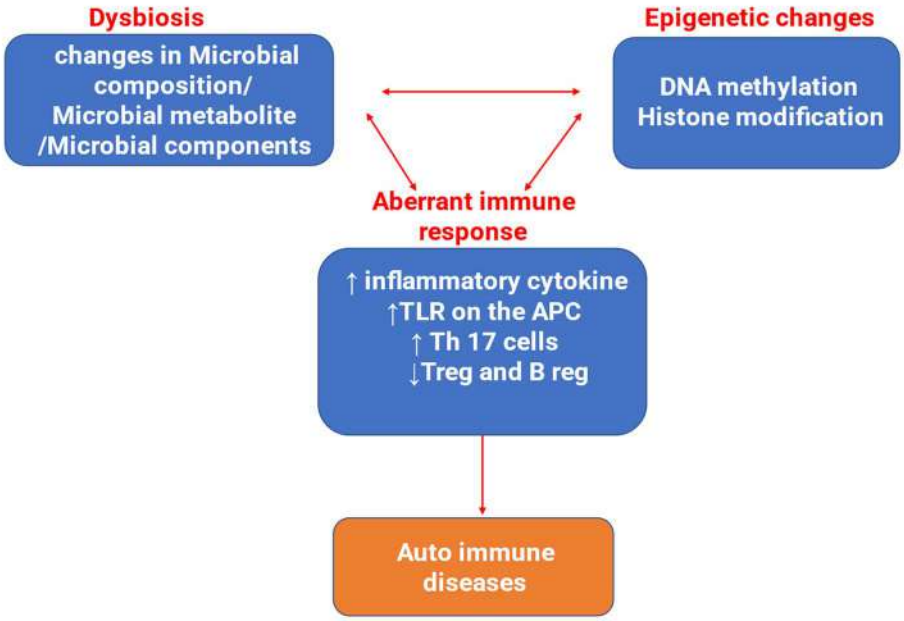
5.1 Gut Microbiome - Dysbiosis

5.1.1 CNS Disorders:



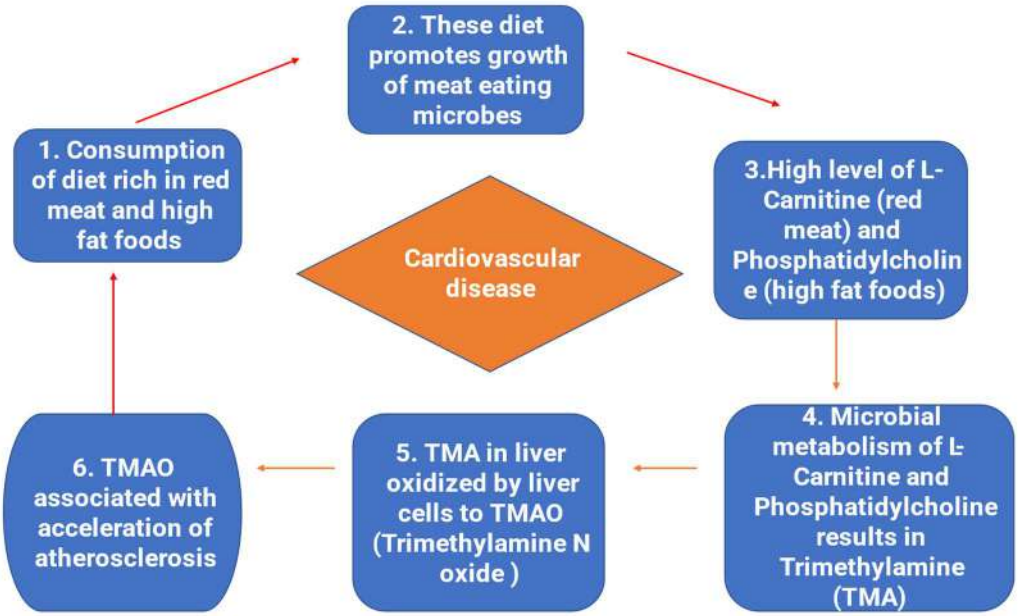
Neurological Disorders	Alteration in Microbiota Composition	
	Increase in	**Decrease in**
Multiple Sclerosis	*Pseudomonas, Mycoplana, Haemophilus, Blautia Methanobrevibacter, Enterobacteriaceae and Akkermansia*	*Bacteroides, Faecalibacterium, Clostridia clusters XIVa and IV*
Autism Spectrum Disorder (ASD)	4-ethylphenyl sulfate *(Bacteroides ovatus and Lactobacillus plantarum)*	-
Amyotrophic Lateral Sclerosis (ALS)	-	*Akkermansia muciniphila*
Parkinson's Disease	*Blautia, Ralstonia, Lactobacillus spp., Proteobacteria*	*Bacteroides, Prevotella, Faecalibacterium*
Alzheimer's Disease	*Blautia, Bacteroides*	*Firmicutes, Bifidobacterium, Dialister*
Stroke And Brain Injury	*Proteobacteria*	*Bacteroides, Prevotella, and faecalibacterium*

5.1.2 Autoimmune Diseases:

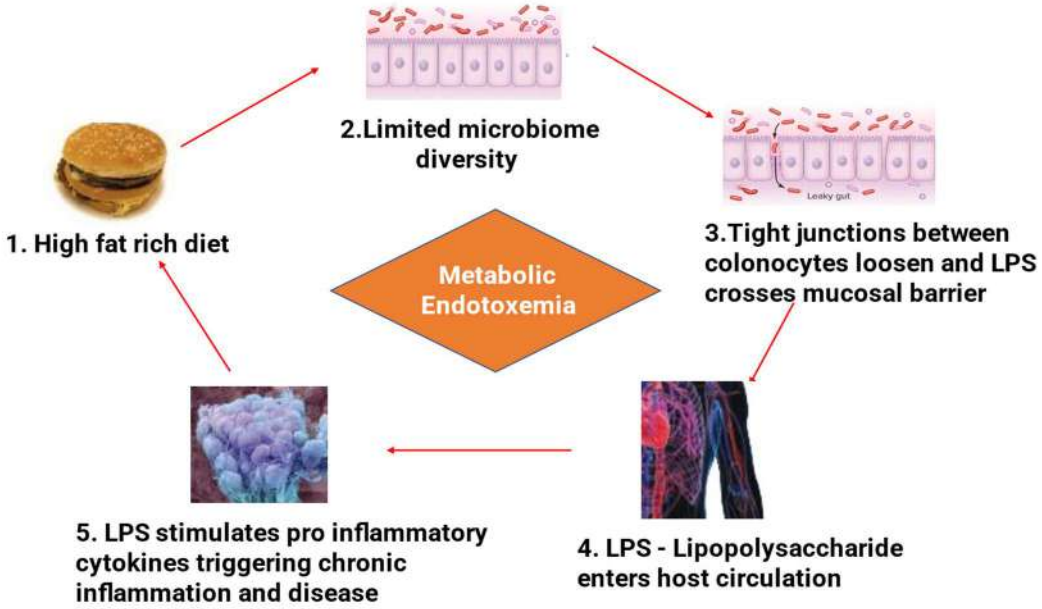


Autoimmune Diseases	Alteration in Microbiota Composition	
	Increase in	Decrease in
Crohn`s And Ulcerative Colitis	*Enterobacteriaceae family Fusobacteriaceae Adherent-invasive E. coli, Yersinia and Clostridium difficile*	-
Rheumatoid Arthritis	*Firmicutes Ruminococcaceae, Lachnospiraceae, Faecalibacterium prausnitzii and Desulfovibrinocaceae*	*Bacteroidaceae, Clostridium coccoides, Eubacterium rectal*
Systemic Lupus Erythematosus (SLE)	*Rhodococcus, Eggerthella, Klebsiella, Prevotella, Eubacterium, Lachnospiraceae and Flavonifractor*	*Dialister, Lactobacillaceae and Pseudobutyrivibrio*
Sjögren's Syndrome (SS)	*Firmicutes, Bacteroidetes,*	*Faecalibacterium prausnitzii, Alistipes finegoldii, Bifidobacterium, Escherichia, Shigella, Enterobacter, Leptotrichia, Fusobacterium*
Systemic Sclerosis (Ssc)	*Fusobacterium, Bifidobacterium, Lactobacillaceae*	*Faecalibacterium prausnitzii, Bacteroides fragilis*

5.1.3 Cardiovascular Diseases:



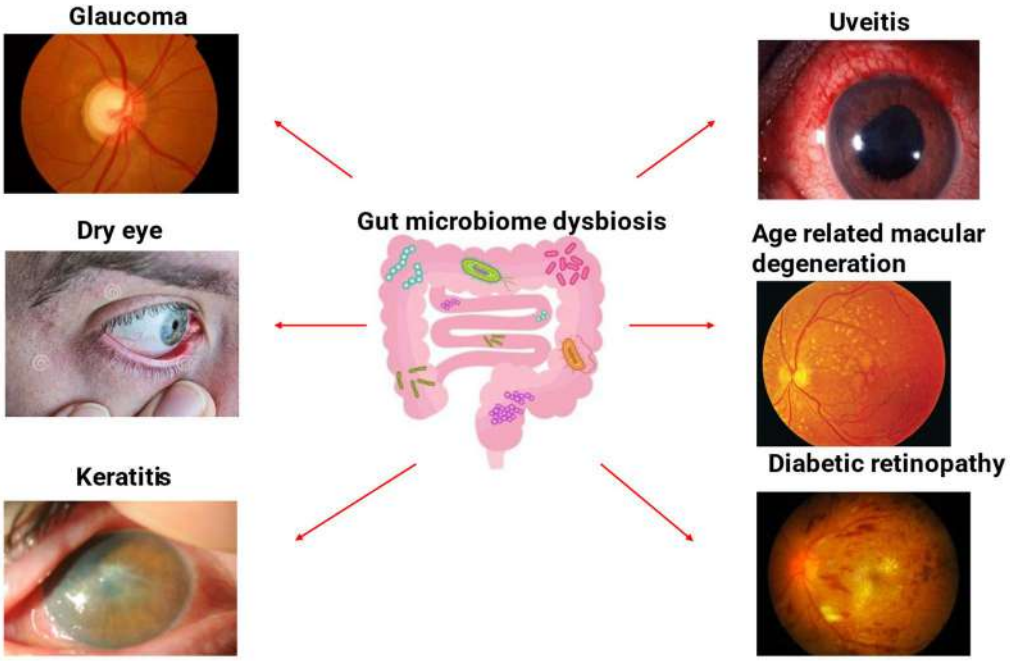
5.1.4 Metabolic Syndromes:



5.1.5 Cancers:

Cancer type	Intestinal microbes	Tumour promoter or suppressor	Mechanism
Colon Cancer	*Fusobacterium nucleatum*	Promoter	In order to advance a tumour, *Fusobacterium nucleatum* specifically attracts myeloid cells that infiltrate the tumour and multiplies the number of tumours.
Colon Cancer	*Escherichia coli*	Promoter	Senescent cells develop as a result of colibactin-producing E. coli, which improves tumour promotion via growth factor secretion.
Colon Cancer	*Bacteroides fragilis*	Promoter	In colonic epithelial cells, Bacteroides fragilis toxin (BFT) initiates a pro-carcinogenic, multi-step inflammatory cascade that calls for IL-17R, NF-B, and Stat3 activation.
Gastric Cancer	*Helicobacter pylori*	Promoter	H. pylori increase proliferation in a strain-specific manner in a novel gastroid system. In addition, *H. pylori* affects claudin-7 expression and localization in human epithelial cells and gastroids through the activation of snail and β-catenin.
Liver Cancer	SCFA-producing bacteria	Promoter	*Dietary soluble fibres are digested into SCFAs by gut bacteria, which encourages liver fibrosis, hepatic fibrosis, and cholestatic liver cancer.*
Breast Cancer	Gut microbiome	Promoter	*Commensal dysbiosis generated considerable myeloid infiltration into the mammary gland and breast tumour and promoted early inflammation within the mammary gland. It also increased fibrosis and collagen deposition both systemically and locally within the tumour microenvironment.*
Pancreatic Cancer	*Bifidobacterium pseudolongum*	Promoter	*The microenvironment of the Pancreatic ductal adeno carcinoma (PDAC) tumour was immunogenically reprogrammed, and this was linked to a unique gut microbiome. The macrophage M1 differentiation of Bifidobacterium pseudolongum was supported.*
Melanoma	*Bifidobacterium*	Suppressor	*Bifidobacterium had a favourable correlation with antitumor T cell responses within the tumour and enhanced dendritic cell production of genes related to antitumor immunity.*

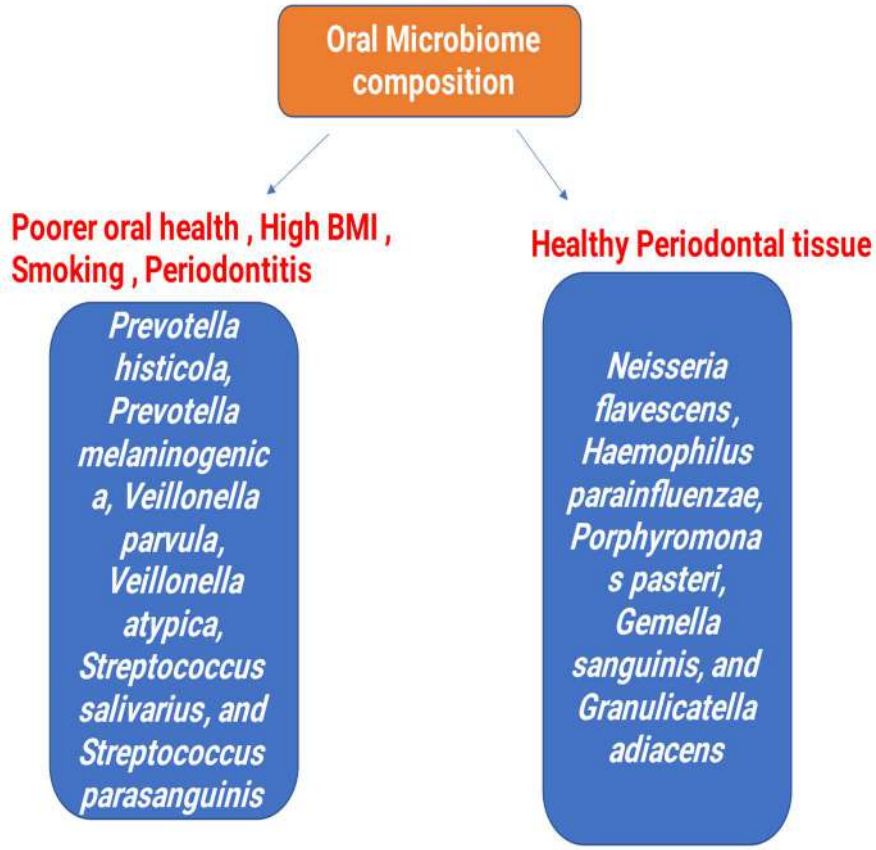
5.1.6 Ocular Diseases:



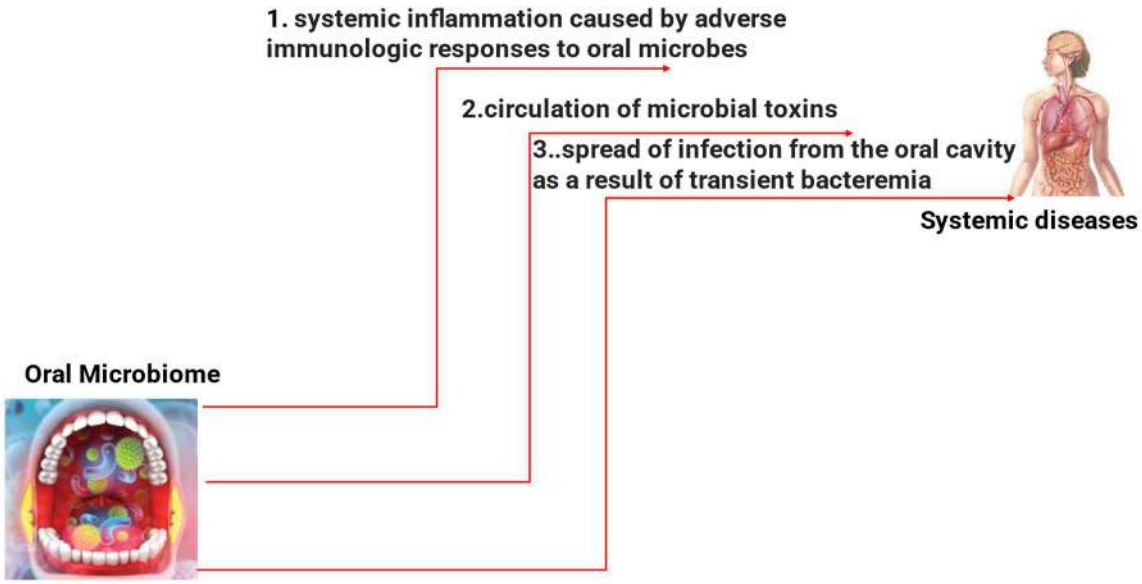
Ocular Diseases	Alteration in Microbial Composition	
	Increase in	Decrease in
Dry Eye	*Enterobacter, Escherichia/Shigella, and Pseudomonas (pathogens)*	*Clostridium (commensal)*
Uveitis	*Paraprevotella, Fusobacterium and Enterobacteriaceae (known gut pathogens)*	*Rikenellaceae*
Diabetic Retinopathy	*Prevotella copri, Lactobacillus*	*Firmicutes, Bacteroidetes*
Age-related Macular Degeneration	*Prevotella, Anaerotruncus, Oscillibacter, Ruminococcus torques and Eubacterium ventriosum*	-
Glaucoma	*Bacteroides and Prevotella*	-
Bacterial Keratitis	*Proteobacteria and Firmicutes*	-

5.2 Oral Microbiome -Dysbiosis

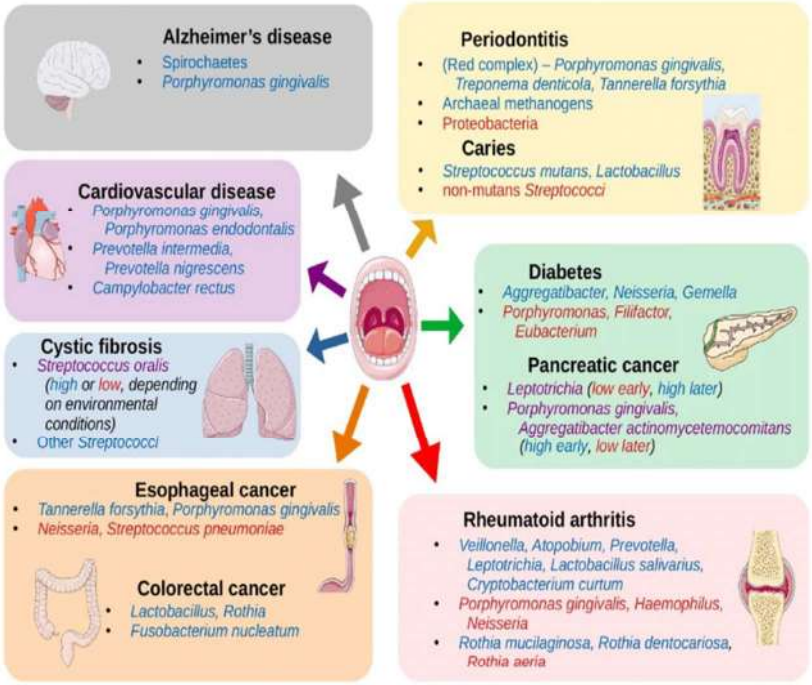
The oral cavity stands out among digestive organs because it contains teeth and other hard structures. Because of the various environmental factors present at each site, the oral microbiome composition at each site varies. Plaque on tooth surfaces has different bacterial populations in the supragingival and subgingival areas. The degree of the compositional difference is positively correlated with periodontal pocket depth and periodontitis development.



5.2.1 Oral Microbiome leading to Systemic Diseases:



Oral and Systemic Diseases associated with Oral Microbiome:



Blue - increased in abundance in the oral cavity in individuals presenting with the noted disease

Red -decreased.

Purple - either increased or decreased depending on the conditions or progression of the disease.

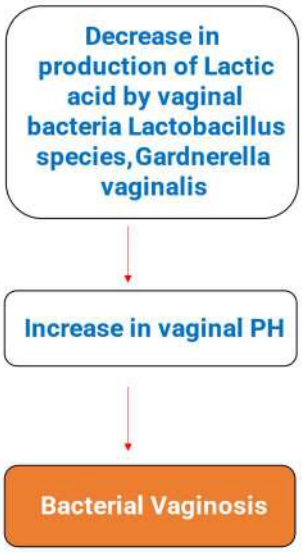
5.2.2 Oral Microbiome and associated Cancers:

Oral organisms in oropharyngeal cancer	
Cancer	**Organisms**
Head And Neck Squamous Cell Carcinoma (HNSCC)	Streptococcus sp. and Lactobacillus sp.
Oral Squamous Cell Carcinoma (OSCC)	Capnocytophaga gingivalis, Prevotella melaninogenica, Streptococcus mitis Bacillus, Enterococcus, Parvimonas, Peptostreptococcus, Slackia
Oral Organisms Associated with Distant Tumors.	
Cancer	**Organisms**
Esophageal Cancer	Streptococcus anginosus, S. mitis, Treponema denticol, T. forsythia and P. gingivalis
Pancreatic Cancer	Streptococcus mitis, Neisseria elongate, Leptotrichiato Porphyromonas
Lung Cancer	Capnocytophaga sp., Veillonella sp.
Colorectal Cancer	Lactobacillus and Rothia

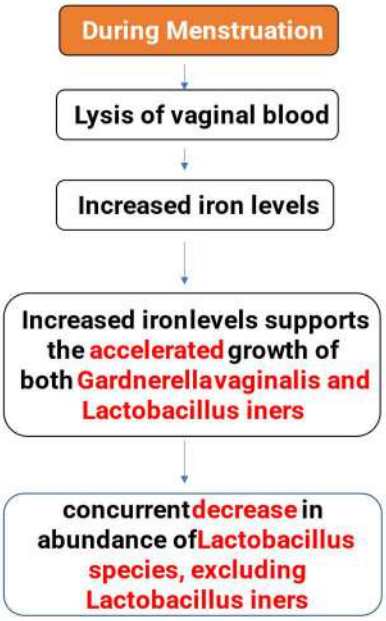
5.3 Vaginal Microbiome-Dysbiosis

A particular region of the human microbiome is the vaginal microbiome. It is distinguished by a limited number of microbial species, mostly lactobacilli, that can use glycogen, which is regulated by oestrogens. The production of lactic acid by vaginal epithelial cells, lactobacilli, and other fermentative bacteria is what makes the vaginal environment acidic.

5.3.1 Bacterial Vaginosis



5.3.2 Menstruation



5.3.3 Potential biomarker constituents of the Vaginal Microbiota for clinical features and Hormone levels of PCOS patients:

Clinical features in PCOS	Microbiota involved
Acanthosis nigricans	*Lactobacillus gasseri*
Intermenstrual bleeding	*Streptococcus* *Megasphaera*
Pregnancy history	*Streptococcus sp*
Testosterone	*Lactbacillus crispatus*
Anti-müllerian hormone	*Mycoplasma*

5.3.4 Vaginal Communities and Risk of Sexually Transmitted Diseases (STDs):

In a study done on asymptomatic North American women from four ethnic groups. The authors found that the vaginal communities in these women clustered into five core vaginal microbiomes, which they termed ***community-state types.***

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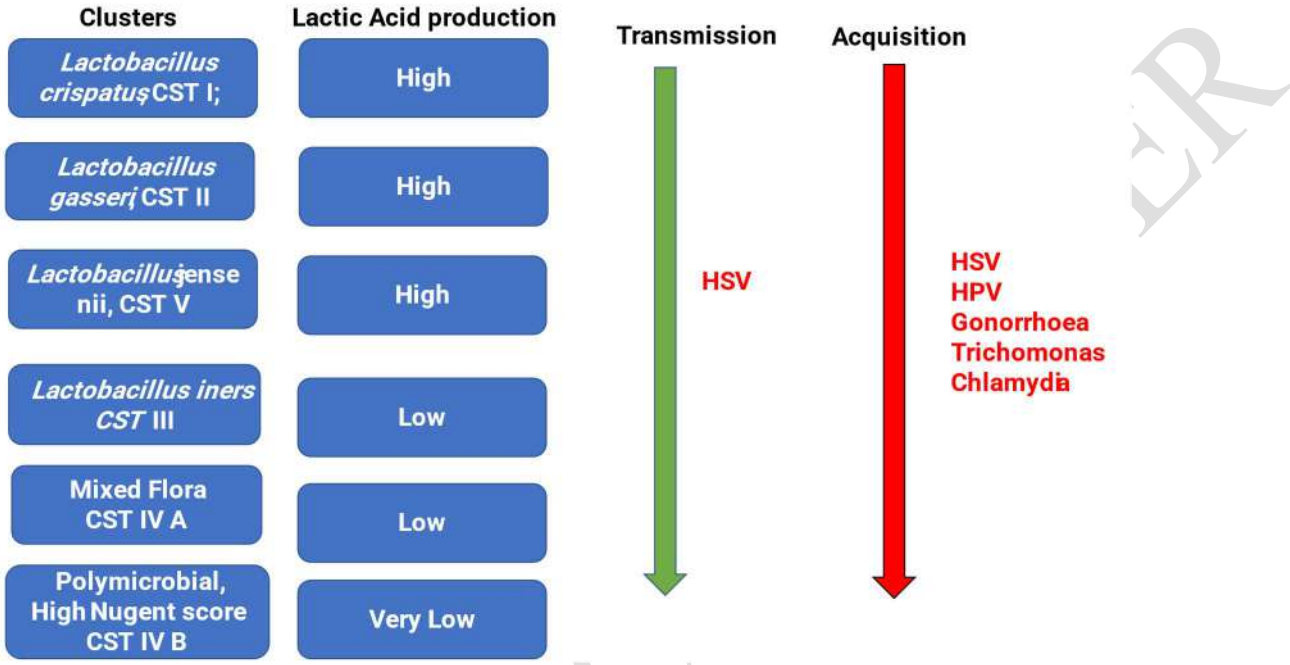
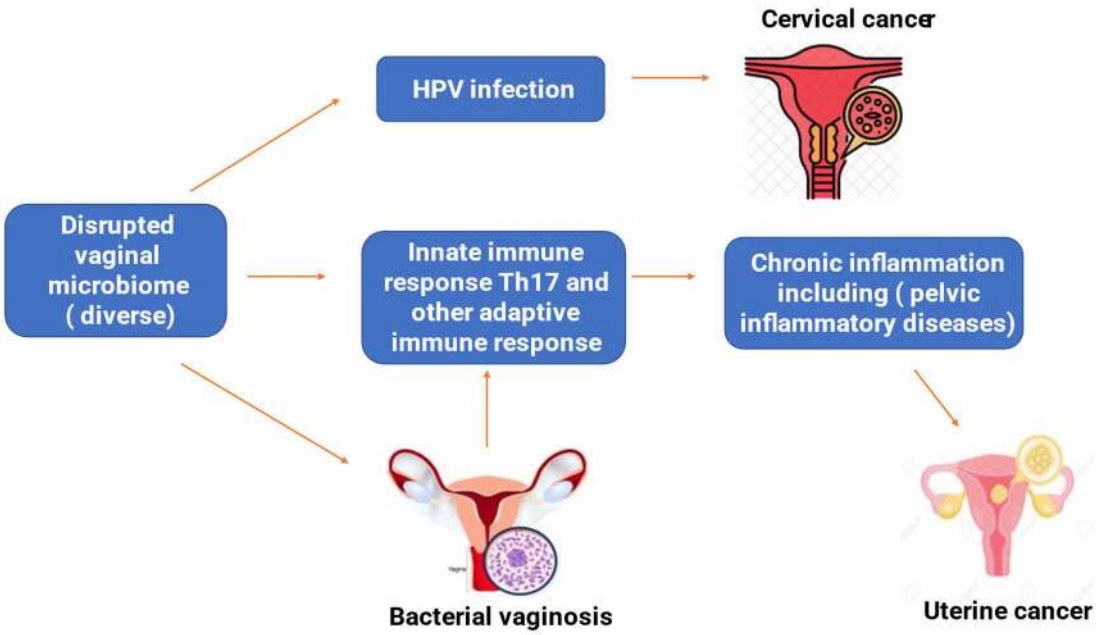


With greater vaginal flora diversity comes a higher risk of contracting and spreading STDs, while Lactobacillus crispatus-dominant communities have the lowest risk.

The generation of lactic acid is conserved across healthy vaginal populations, and higher amounts have been shown to be closely associated with vaginal health. The vaginal milieu may work differently for L-and D-lactic acid isomers, and the ratio between them may affect the immune system`s response and host gene expression.

The crucial discovery that 20–30% of women at any given time have a Lactobacillus–poor, varied microbiome has not been regarded as healthy

5.3.5 Vaginal Microbiome on Carcinogenesis:



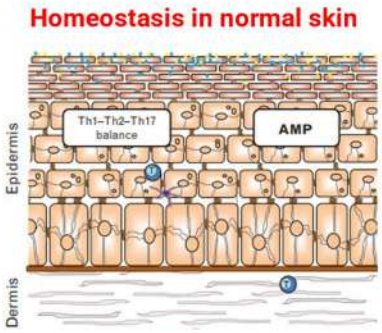
Vaginal Microbiome involved		
Association with HPV	**Association with HPV Clearance**	**Association with CIN**
1.Lactobacillus gasseri 2. Gardnerella vaginalis 3.Atopobium vaginae 4. Fusobacterium 5. Sneathia	1.Lactobacillus iners 2. Lactobacillus gasseri	1.Lactobacillus iners 2. Gardnerella vaginalis 3.Atopobium vaginae 4. Fusobacterium 5. Sneathia

5.4 Ocular Microbiome – Dysbiosis

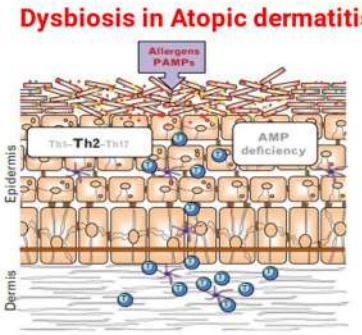
The commensal bacteria on the ocular surface probably contribute to both innate and adaptive immunity, with the tear film serving as an important physical barrier between the eye and its surroundings. Healthy corneal and conjunctival epithelial cells did not exhibit an inflammatory response to commensal bacteria like Staphylococcus epidermidis or Propionibacterium acnes in a cell culture model. When exposed to infections like Pseudomonas aeruginosa, on the other hand, epithelial cells secreted inflammatory cytokines (IL-6 and 8).

Ocular Diseases	Alteration in Microbial Composition	
	Increase in	Decrease in
Contact lens associated corneal ulcer	*Pseudomonas, Acinetobacter, and Methylobacterium*	
Contact lens induced red eye	*Haemophilus influenza*	
Blepharitis	*Staphylococcus, Streptophyta, Corynebacterium, and Enhydrobacter*	*Propionibacterium*
Dry eye	*Staphylococcus aureus, coagulase negative Staphylococcus, Corynebacterium, Propionibacterium, Rhodococcus and Klebsiella oxytoca*	
Keratitis	*Staphylococcus lentus, Pseudomonas aeruginosa and Staphylococcus epidermidis*	
Endophthalmitis	torque teno virus in all of the culture-negative samples	

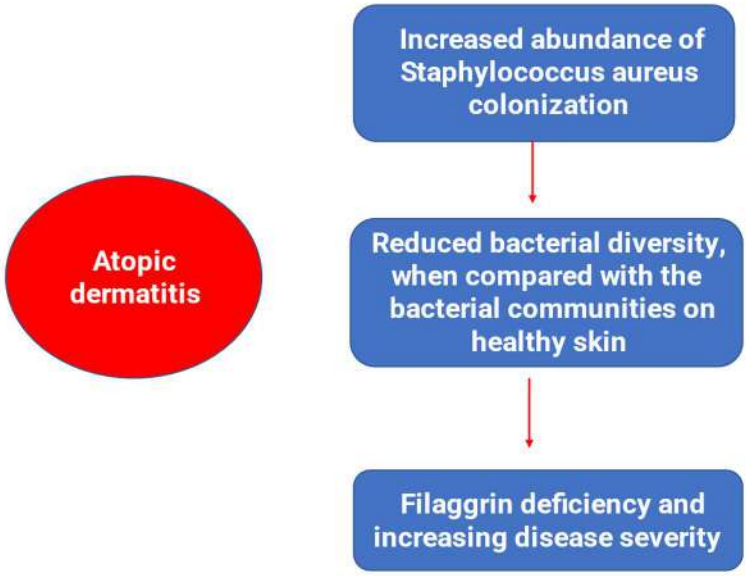
5.5 Skin Microbiome – Dysbiosis



1. Microbiome of healthy skin
2. Intact epithelial Barrier
3. Adequate production of antimicrobial peptides
4. Adequate response of innate and adaptive immune system (balanced Th1 Th2 Th17 response)

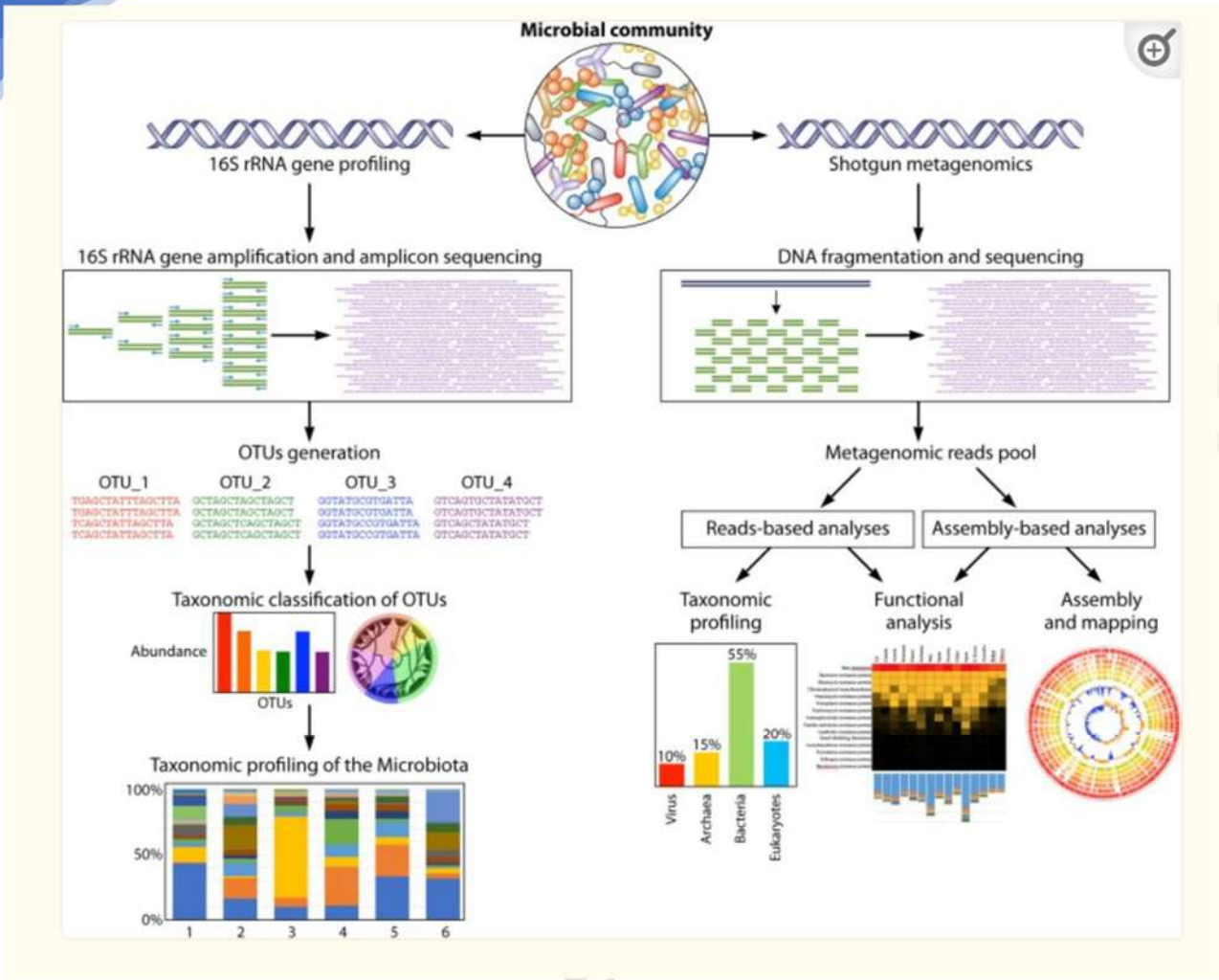


1. Colonization by S .aureus
2. Poor skin barrier
3. Inadequate production of antimicrobial peptides.
4. Penetration of allergens and microbial components.
5. Th2 cytokine response predominant leading to poor host defence system



6.Technical Approaches for Microbiota Determination:

The 16S rRNA gene-based microbial profile analysis have been used in several human gut microbiota research. Nine separate variable sections, designated V1 to V9, make up the 16S rRNA gene, and each one is surrounded by highly conserved DNA sequences that are good fits for PCR primer binding. Shotgun metagenomic sequencing is an alternative to 16S rRNA gene microbial profiling for cataloguing the human gut microbiota. With this method, gene-specific amplification is avoided, and any (fragmented) DNA taken from the examined environmental sample, including DNA from unclassified bacteria and viruses, may be sequenced. Compared to 16S rRNA gene-based microbial profiling, shotgun metagenomics much more information is available, including insights into the functional characteristics of the microbial community.



7.Microbiome as Therapeutics:

Diet:

In autoimmune uveitis mouse models, oral solutions of SCFAs containing sodium propionate, sodium butyrate, or sodium acetate enhanced the presence of Treg cells in the gut and decreased the migration of effector T cells between the intestine and the spleen, which decreased uveitis manifestations in the eye.

Humans on a Paleolithic diet showed improvements in multiple sclerosis-related functional, cognitive, and psychosocial characteristics.

Probiotics:

Synbiotics are foods or supplements that include both a prebiotic and a probiotic.

Prebiotics encourage the growth of helpful microorganisms, whereas probiotics deliver microbial components with established positive activities.

Probiotics are commonly gram-positive bacteria (e.g., *Bifidobacterium, Lactobacillus*) and yeasts (e.g., *Saccharomyces)*. Many of these microbes are found in ingestible capsules and as food supplements (e.g., yogurt, kefir). Probiotics have been used to treat *C. difficile*–associated diarrhoea and inflammatory bowel disease, to provide protection from Salmonella and Helicobacter pylori disease, as therapy for paediatric atopic dermatitis, autoimmune diseases, and even for reduction in dental caries.

Faecal Microbiota Transplantation (FMT):

FMT is a treatment where donor faeces are given to a recipient in order to alter the recipient's gut microbial makeup and to improve their health.

The most well-developed model of this therapy is the treatment of *C. difficile* colitis, for which one FMT has approximately 90% effectiveness in eradicating infection.

Increased microbiota diversity, which restores protective synergistic function to the gut, and recovery of bile acid metabolism, which raises levels of SCFAs like acetate, propionate, and butyrate, which are known to inhibit *C. difficile* growth, are two of the proposed mechanisms for this treatment.

Oral Microbiota Transplantation (OMT):

Human OMT has been hypothetically suggested by Floyd Dewhirst and Diane Hoffmann. It aims at transferring oral biofilms from a healthy donor to a patient with either caries or periodontitis.

Supragingival plaque is collected from a caries-free donor (perhaps a relative of the recipient patient), stored in saltwater, and then applied to the teeth of a caries-active patient using a nylon swab.

The donor should, in accordance with their suggested methodology, have a healthy oral microbiome that is devoid of cariogenic bacteria like Streptococcus mutans and has a small pH drop in response to a sugar challenge.

Chemotherapy and Immunotherapy:

The composition of the gut microbiota regulates the effectiveness of cancer treatment and immunotherapy, according to recent research in experimental animals and to some extent in patients, and addressing the microbiota may boost the success rate of immunotherapy.
The microbiome can be used as a prognostic biomarker, serving as a guide for choosing the best preventive and treatment measures for certain people.
It can be employed as a target and a treatment, as well as a primary, secondary, and adjuvant therapeutic measure.

8.Conclusion:

It is a known fact that conventional Microbiology focused on the detection of infectious agents. Recent advances in Holobionts have enabled us to understand the spectrum of Human Microbiome extending from normal physiological functions to infectious diseases and specifically non-communicable diseases. This data has shed some light on the avenues of Microbiology in Non-communicable diseases. It also underlines the fact that interpersonal variation, geographical variation, and ecological variation in microbiota may influence the disease pattern and treatment outcomes respectively. Hence, the association between microbiome and human health needs Personalised Microbiology in correlation with Personalised Medicine inevitably.

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MONKEYPOX AN EMERGING VIRAL INFECTION

Dr Grace Earnest (Final year Post Graduate)
Moderator: Dr G Jyothi Lakshmi
Professor, Osmania Medical College, Hyderabad

Introduction: Infectious diseases do not respect international borders. Especially in this era of globalization and rapid international travel, any infectious disease in one country can become a potential threat to the entire globe. The emerging diseases are threat to community as it increases morbidity and mortality and even increases bioterrorism potential. Global analyses of emerging infectious diseases (EIDs) suggest that around 60% of them are zoonotic and are rapidly increasing over time. A major portion of the global burden of endemic zoonosis is from India which is one of the several 'EID hotspots'. The population in these regions live in conditions of poor sanitation, inadequate nutrition, and lack of access to public health care systems despite many of these diseases being preventable and or treatable through specific low-cost interventions.

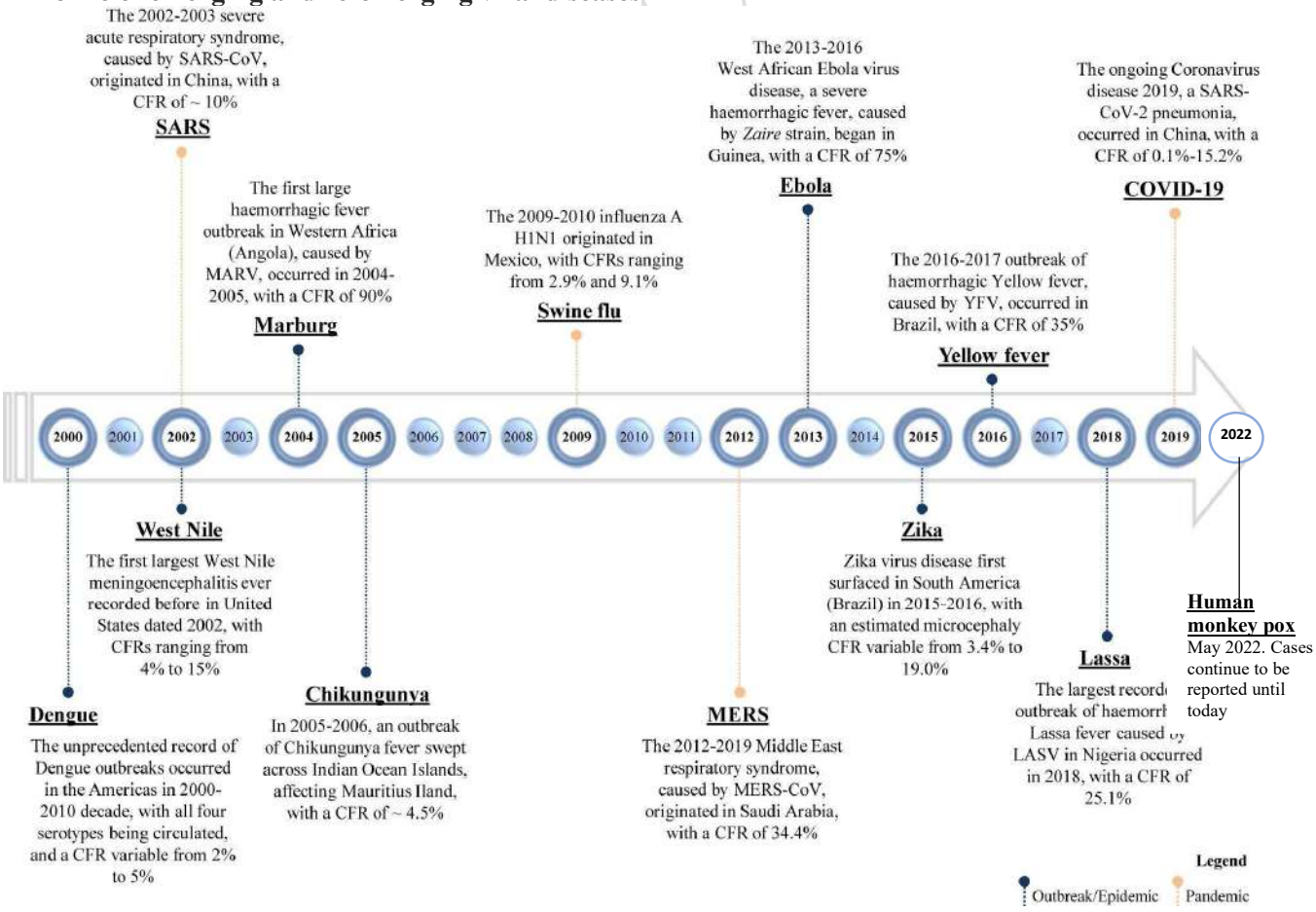
WHO has declared seven PHEIC (Public Health Emergency of International Concern) till date-Swine flu in 2009, Polio in 2014, Ebola and Zika in 2016, Kivu Ebola in 2019, the ongoing 2019-20 coronavirus pandemic and Monkeypox in 2022.

Monkeypox is a viral zoonosis with symptoms similar to those seen in the past in smallpox patients, although it is clinically less severe. With the eradication of smallpox in 1980 and subsequent cessation of smallpox vaccination, monkeypox has emerged as the most important Orthopoxvirus for public health.

What are emerging and reemerging infectious diseases?

According to the National Institute of Allergy and Infectious Diseases (NIAID), emerging infectious diseases are commonly defined as outbreaks of previously unknown diseases, Known diseases that are rapidly increasing in incidence or geographic range in the last 2 decades or persistence of infectious diseases that cannot be controlled. On the other hand, Reemerging diseases are diseases that reappear after they have been on a significant decline. Reemergence may happen because of a breakdown in public health measures for diseases that were once under control. They can also happen when new strains of known disease-causing organisms appear.

Timeline of emerging and re-emerging viral diseases



Why Do New Viruses Emerge?

In most cases, outbreaks have been known to occur in tropical regions in which there were no human inhabitants. An increase in contact with wild animals, due to the expansion of the human habitat, is believed to be the main cause for the emergence of new viruses. Climate changes such as global warming are another cause for the emergence of new viruses.

What are the emerging and re-emerging viruses?

Emerging viruses	Reemerging viruses
MERS-CoV	Human Monkey pox
Influenza : H10N8,H7N9,H5N1,H1N1,H3N2	Dengue virus
SARS	Chikungunya virus
Nipah virus	Measles
Hendra virus	Zika virus
Hanta virus	Marburg virus
Bourbon virus	Ebola virus
Enterovirus	Crimean-congo haemorrhagic fever (CCHF)
Akhmeta virus	Kyasanur forest disease (KFD)/ monkey fever
	Japanese encephalitis
	Chandipura virus
	Adenovirus 14
	Enterovirus 71
	Rift Valley fever virus
	West Nile virus
	Yellow fever

Influencing Factors

Viral factors
Environmental factors
Host factors
4. Disease control related Factors
5. Social health-related risk factors

1. Viral factors

Evolution of an emerging infection on an imbalance between infection and immunity

An emerging infection is usually caused by the naive immunity of human beings encountering a novel pathogen arising from microbial mutation, vector-borne, or/and zoonotic transmission.

Most of the common emerging infections are mediated by RNA viruses, which pose a higher rate of immune evasion such as

Genetic mutation
Sequence deletion
Recombination
Reassortment of RNA virus codes.

Emerging infections	Genetic changes	Vector-borne	Reservoir
Mutation			
1.Avian Flu	Mutation		Birds/Ducks
2.Swine Flu	Reassortment		Birds/Pigs
3.SARS- CoV-1	Deletion/recombinations		Civet cats/Bats
4.SARS-CoV-2	Mutation/recombinations		Pengolin/Bats
5.Enterovirus 71	Mutation		
Vector-borne			
1. West Nile virus		Mosquito	
2. Dengue fever		Mosquito	
3. Yellow fever		Mosquito	
4. Zika fever		Mosquito	
Zoonotic			
1. Ebola			Vertebrates
2. Lassa			Rodents
3. Hantavirus			Rodents
4. Monkeypox			Rodents, squirrels

2. Environmental Factors

Effect of changes of ecosystems: In addition to virus mutation, temperature and humidity are known to affect human-human transmission of emerging infections. Environmental factors include:

Seasonal Weather Influences Aerosol Transmission

Climate Changes and Global Transportation Enhance Vector-Borne Diseases

Urbanization and Environmental Changes Enhance Zoonotic Infections

3. Host Factors

Herd Immunity - Herd immunity is another key factor that determines the endemic or epidemic spread of an emerging infection.

Genetic Polymorphisms Associated With Infectivity and Immunopathology - Genetic polymorphisms of immunity genes and virus receptors also affect infectivity and fatality of an emerging infection.

Culture, Occupation, and Social Events - affect exposure and the spread of emerging infections

4. Disease control related Factors

High cost and potential environmental impact of vectorcontrol measures

Possible insecticide resistance

Lack of adequate public health and health system infrastructure

Lack of awareness and education

5. Social health-related risk factors

Poverty

Globalization

Rapid urbanization

Lack of adequate vector control

Poor hygiene and sanitation

Migration and mass movement of populations

Travel and tourism Loss of agriculture/livestock

Large social gatherings/events

IMMUNOPATHOGENESIS OF DIFFERENT EMERGING INFECTIONS:

An emerging infection can rapidly lead to a pandemic with high fatality rates. Each individual emerging infection has its unique pattern of infectivity related to virus-host interactions underlying ligation of pathogen-associated molecular pattern (PAMP) to pattern

recognition receptor (PRR) for the signalling of immune responses toward proper defence or morbidity. These are:

1) Deficient immunity with disseminated viremia
2) Pneumocytotropism with/without later hyperinflammation
3) Augmented immunopathology
4) Antibody-dependent enhancement of infection with altered immunit

Mechanisms of different emerging infections

Diseases	Immunity	Tissue response	
		Regional	Systemic
Mechanism 1: Defective immunity with systemic dissemination			
Ebola	B cell defect	Hemorrhage	Shock
Lassa	T cell defect	Hemorrhage	Shock
Enterovirus 71	T cell defect	Neurotropism	Brain-pulmonary Syndrome
WNV	B cell defect	Neurotropism	Encephalitis
Mechanism 2: Pneumocytotropism with/without hyperinflammation			
SARS-CoV-1	Proinflammation	Pneumocytotropism	ARDS
Swine flu	Immunosuppression	Pneumocytotropism	ARDS
SARS-CoV-2	Proinflammation	Pneumocytotropism	Hyperinflammation
Mechanism 3: Augmented immunopathology			
Hantavirus	Augmented inflammation	Renal/lung damage	Shock/ARDS
Avian Flu	Augmented inflammation	ARDS	Hemophagocytosis
Mechanism 4: Immune cross-enhancement of infection with altered immunity			
Dengue	Antibody-dependent	Hemorrhage	Shock
Ross River virus	Antibody-dependent	Rashes	Polyarthritis

Source: Immunopathogenesis of Different Emerging Viral Infections: Evasion, Fatal Mechanism, and Prevention by Betsy Yang and Kuender D. Yang

MONKEY-POX

Monkeypox was first discovered in 1958 in colonies of monkeys kept for research, hence the name 'monkeypox.' The first human case of monkeypox was reported from Democratic Republic of the Congo (DRC) in 1970. Monkeypox virus is an enveloped double-stranded DNA virus that belongs to the Orthopoxvirus genus of the Poxviridae family.

Current Scenario: According to Centers for Disease Control and Prevention, till 24.08.2022-
• Global Scenario: Over 44,500 confirmed Monkeypox cases worldwide. 12 confirmed deaths among which 5 are from areas not historically reported previously (emerging virus) and 7 in previously reported areas (re-emerging)
• In India: 9 confirmed Monkeypox

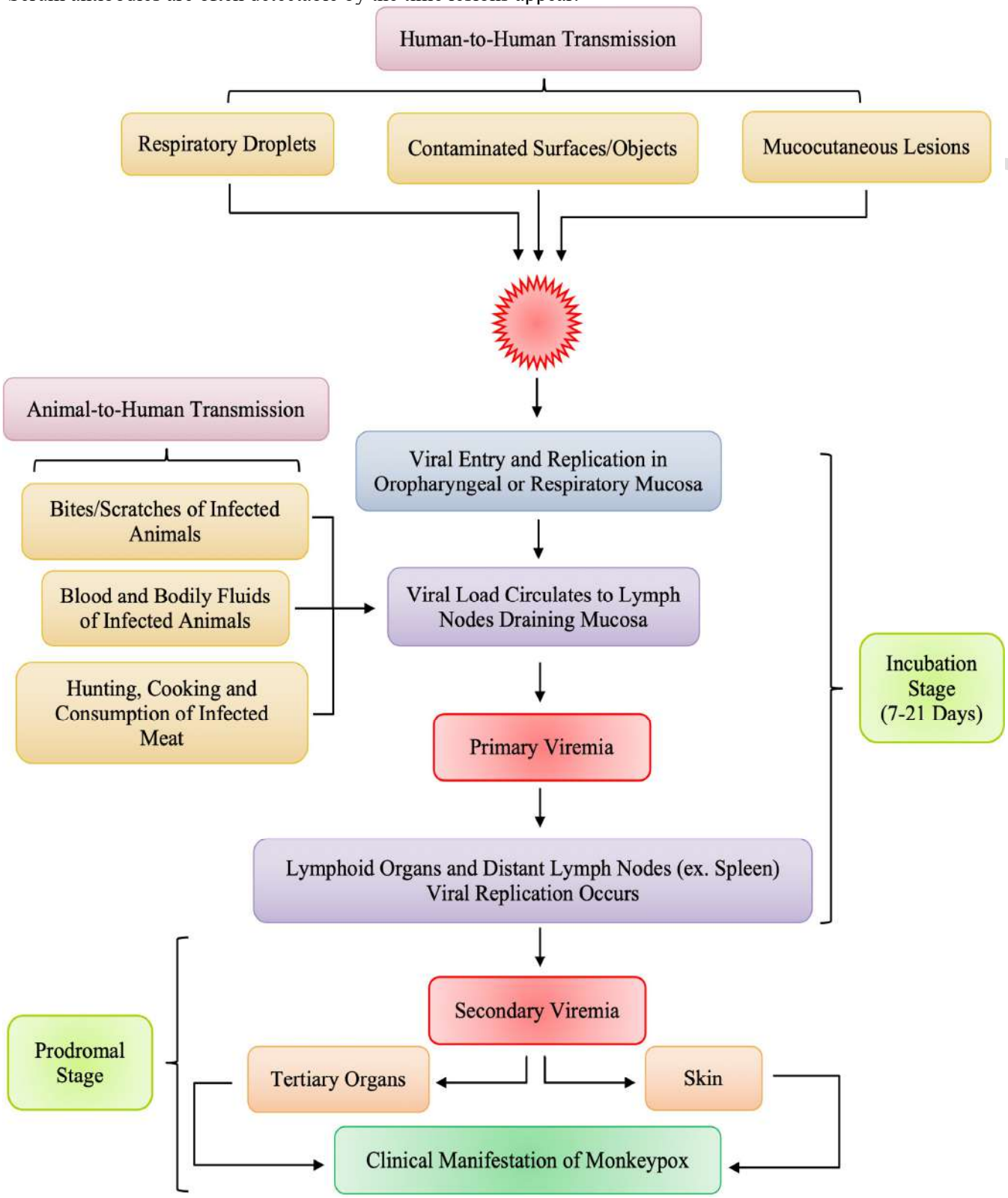
Transmission:

Animal-to-human (zoonotic) transmission can occur from direct contact with the blood, bodily fluids, or cutaneous or mucosal lesions of infected animals.
Human-to-human transmission can result from close contact with respiratory secretions, skin lesions of an infected person or recently contaminated objects.
Transmission via droplet respiratory particles usually requires prolonged face-to-face contact, which puts health workers and other close contacts of active cases at greater risk
Mother to fetus occurs via the placenta which can lead to congenital Monkeypox.
Eating inadequately cooked meat and other animal products of infected animals is a possible risk factor.

Pathogenesis

Reservoir – Squirrel, Rodents
The incubation period - 6 to 13 days but can range from 5 to 21 days
Route of entry - oropharynx, nasopharynx, or intradermal
Following viral entry from any route, virus replicates at the inoculation site then spreads to local lymph nodes.
Next, an initial viremia leads to viral spread and seeding of other organs.

Symptom onset correlates with a secondary viremia leading to 1 to 2 days of prodromal symptoms such as fever and lymphadenopathy before lesions appear.
Infected patients may be contagious at this time.
Lesions start in the oropharynx then appear on the skin.
Serum antibodies are often detectable by the time lesions appear.



Source : Monkeypox: A Comprehensive Review of Transmission, Pathogenesis, and Manifestation
Jasndeep Kaler, Azhar Hussain, Gina Flores, Shehreen Kheiri, Dara Desrosiers

Clinical Features:

Initial symptoms include fever, headache, myalgia, fatigue, and lymphadenopathy, a key differentiating feature of monkeypox from smallpox.

After 1 to 2 days, mucosal lesions develop in the mouth closely followed by skin lesions of the face and extremities (including palms and soles) and are centrifugally concentrated.

The rash may or may not spread to the rest of the body, and the total number of lesions may vary from a small amount to thousands.

Over the following 2 to 4 weeks, the lesions evolve in 1 to 2-day increments through macular, papular, vesicular pustular and crust phases.

The condition resolves around 3 to 4 weeks after symptom onset in most cases. Patients are no longer considered infectious after all crusts fall off.

Lab Diagnosis:

Collect lesion specimens for Monkeypox testing at Laboratory Response Network (LRN) laboratories located at public health department or authorised commercial laboratories

Specimen - Two swabs from each Skin lesion material from different locations is recommended.

Total of 4 swabs (4 web swabs or 4 dry swabs)

Using sterile, dry synthetic swabs with a plastic, wood, or thin aluminium shaft

Cotton tipped swabs not recommended

Swab the lesion vigorously, to ensure adequate viral DNA is collected.

Both dry swabs and swabs placed in viral transport media (VTM) can be use

End of each swab's applicator is broken off into a 1.5- or 2-mL sterile screw-capped tube.

Only one should be tested and the second should only be tested in case the first provides inconclusive results.

Types of specimens are accepted.

Dry swabs of lesion material

Swabs of lesion material in viral transport media (VTM)

Lesion crusts

Oropharyngeal swab.

EDTA blood (purple vacutainer) may support detection of virus ,but may not contain the high level of virus found in lesion samples, as any viremia occurs early in the course of infection, usually in the prodromal period, and before skin lesions become manifest.

Serum (Yellow vacutainer)

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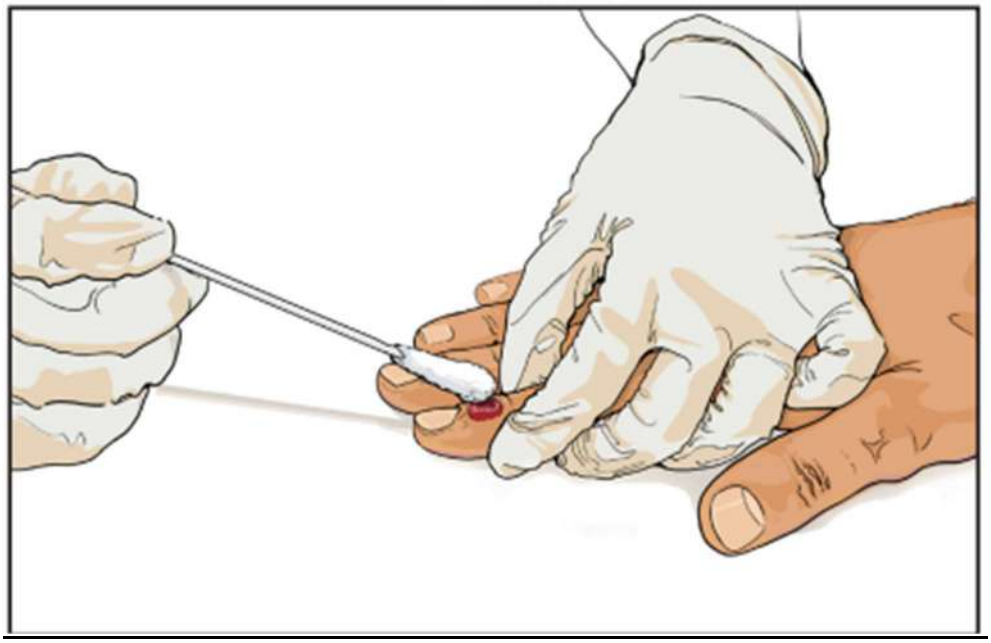


Storage and Transportation of specimen

Specimens can be shipped as Category A, UN2814 infectious substance, affecting humans.

Stored refrigerated (2-8°C) or frozen (-20°C or lower) within an hour of collection

If transport exceeds 7 days for the sample to be tested, specimens should be stored at -20 °C or lower.

Longer term specimen storage (>60 days from collection) is recommended at -70°C.
Refrigerated or frozen specimens should be sent to
Specimens received outside of acceptable temperature ranges are rejected

Testing methods

Electron microscopy.- to evaluate the sample for a potential poxvirus, but high technical skills and facility required, this method is not routinely used for the diagnosis of poxviruses.

Viral culture - not recommended as a routine diagnostic procedure and should only be performed in laboratories with appropriate experience and containment facilities.

Molecular method - Confirmation of MPXV infection is based on nucleic acid amplification testing (NAAT), using real-time or conventional polymerase chain reaction (PCR)

PREVENTION AND TREATMENT

Environmental :

Strategies to improve environmental sanitation and human hygiene are critical components of addressing several risk factors associated with transmission.

eliminating human contact with the vector

Better access to clean water, practicing hand washing, and hygienic disposal of waste and human excreta, reduces the chances of human-to-human transmission.

Infrastructure:

Encouraging and funding health care institutions, clinics, and community health facilities to engage in surveillance, prevention, and treatment programs

Vaccines:

1. JYNNEOS

Preferred vaccine to protect against virus
Is a two-dose vaccine.
It takes 14 days after getting the second dose for its immune protection to reach its maximum.

2. ACAM2000

Is a single-dose vaccine
It takes four weeks after vaccination for its immune protection to reach its maximum.
Has the potential for more side effects and adverse events than JYNNEOS.

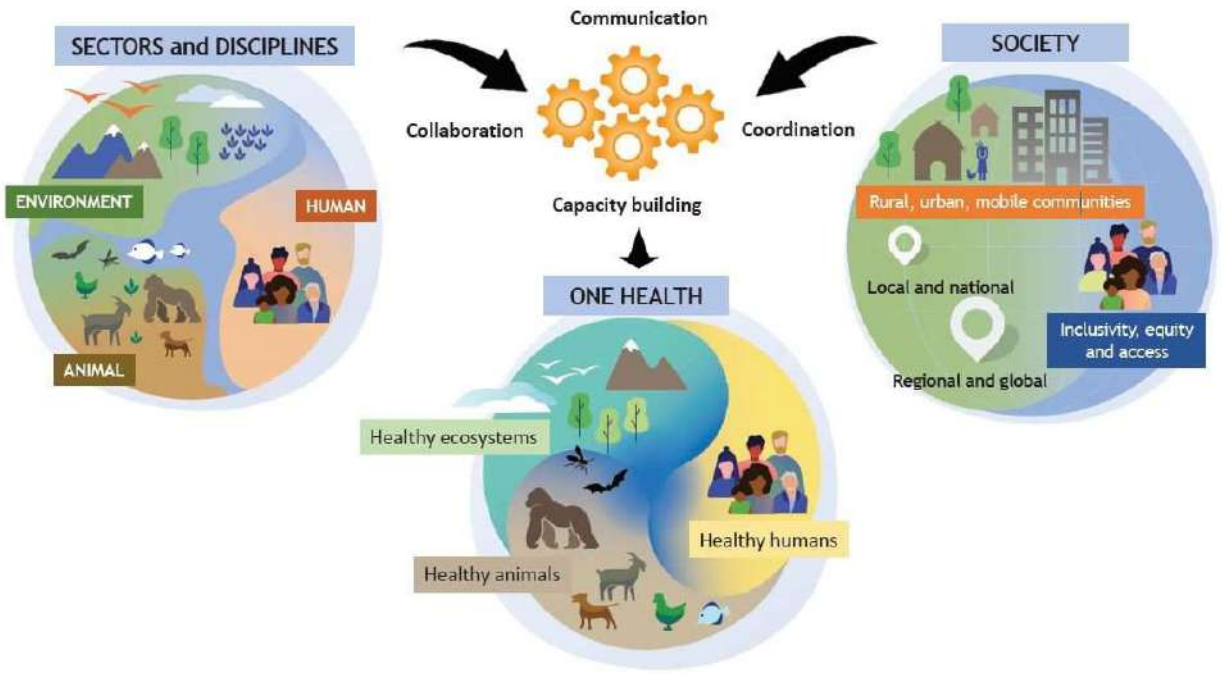
Methods to combat viruses:

One Health approach is an integrated, unifying approach that aims to sustainably balance and optimize the health of people, animals and ecosystems.

It recognizes that the health of humans, domestic and wild animals, plants, and the wider environment (including ecosystems) are closely linked and interdependent.

By linking humans, animals and the environment, One Health can help to address the full spectrum of disease control – from prevention to detection, preparedness, response and management – and contribute to global health security.

This concept of one health is for well being of human, animal and plants through a collaborative universal approach



Rapid Response Teams (RRTs)

In November 2004 **Integrated Disease Surveillance Project (IDSP)** with financial help from **World Bank** commenced disease surveillance system for epidemic prone diseases
To strengthen decentralized laboratory based Information Technology
To detect and respond to outbreaks in early phase with the help of Rapid Response Teams (RRTs).
It presents weekly update on outbreaks in the country

National Institute of Virology (NIV) Pune.
It is identified today as WHO Collaborating Center for arboviruses and haemorrhagic fever reference
The centre studies about viral diseases, investigates outbreaks and provides diagnosis for viral diseases and development of indigenous diagnostic tests.

Viral Research and Diagnostic Laboratory Network (VRDLN).
MOHFW, Government of India, in 2013 made a vision to establish and strengthen the network of laboratories across the country
100 laboratories in India with the objectives to create infrastructure and identify viruses, for capacity building, to develop diagnostics, trainings, and meetings of health officials and professionals and for research

Global Virus Network (GVN)
Essential and critical in the preparedness, defence and first research response to emerging, exiting and unidentified viruses that pose a clear and present threat to public health
No single institution in the world has expertise in all viral areas GVN

The most essential aspects of prevention of viral outbreaks lie in surveillance of agent, host and environment.
WHO since long has a mandate for promoting and supervising surveillance activities which has been emphasized further in recent times as evident from International Health Regulations (IHR) and pandemics of Public Health Emergency of International Concern (PHEIC).

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Monkeypox: A Comprehensive Review of Transmission, Pathogenesis, and Manifestation Jasndeep Kaler, Azhar Hussain, Gina Flores, Shehreen Kheiri, Dara Desrosiers

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RECENT APPROACHES ON VACCINE DEVELOPMENT IN VIEW OF EPIDEMICS

Dr Grace Olive, Microbiology postgraduate final year, Gandhi Medical College, Secunderbad.

INTRODUCTION - Traditional Vaccine technologies have been used for both bacterial and viral pathogens. Yet in many cases they have not been successful, causing persistence of infections and disease burden in the community. Evolving pathogens with high complexity, variability causing emerging infections have given the need to accelerate vaccine development. Novel technologies such as nucleic acid and viral vector vaccines offer the potential to revolutionize vaccine development as they are well-suited to address existing technology limitations.

VACCINE was first devised by Edward Jenner from Small pox of cow(*Variolavaccinae*). Louis Pasteur coined the term "VACCINE". Vaccines have given the world a whole new hope to combat pathogens and increase the life span of humans.

Historically, vaccines were deemed for children alone, but now a days adult vaccines are becoming increasingly necessary. Influenza vaccines available since 1940's is now being recommended for adults.Vaccines including hepatitis A, hepatitis B, pneumococcus, and meningococcus are recommended for sub-groups of the adult population.

EPIDEMIC- Unexpected rise in the number of cases with rapid spread of a particular disease in a defined area is called an epidemic.

The epidemics that have seen light in 2022 are New variants of Covid 19, Ebola, Monkey pox, Dengue, Malaria, Tomato fever (Hand Foot Mouth disease), Chikungunya, Avian Influenza, Seasonal Influenza, Zika virus, Yellow fever, MERSand Cholera

TYPES OF VACCINES-

- ✓ Inactivated vaccines
- ✓ Live-attenuated vaccines
- ✓ Messenger RNA (mRNA) vaccines
- ✓ Subunit, recombinant, polysaccharide, and conjugate vaccines
- ✓ Toxoid vaccines
- ✓ Viral vector vaccines
- ✓ Recombinant vector vaccines
- ✓ DNA vaccines

Recommended List of vaccines for Adolescents and Adults for year 2022

Recommended Vaccines	Catch up vaccines	Sub Groups
Tdap (2005)	MMR	Hepatitis A
HPV (2006 in females, 2009 in males)	Hepatitis B	Pneumococcus
Meningococcal conjugate (serogroups A, C, W, Y)	Varicella	
Influenza	Polio	
Meningococcal serogroup B (2014)	Zoster vaccine	
Covid 19 vaccine		

THE NEED FOR ACTION

There has been tremendous rise in industrialization, use of resources, urbanization, transportation and people trying to explore various places with tourism industry on the rise in every possible manner. There is exchange of culture, material and also carrying of pathogens and viruses from one place to the other.

The pandemic of Spanish flu 1919, the population of the world was around 1.8 billion comparing it to the present day of 7.9 billion during which the pandemic of Covid 19 was witnessed. The disease burden is high and so is the responsibility in giving a proper care and solution to combat such situations.We need to push the Vaccine development and research to newer heights to cater to the needs to the world.

EMERGING AND RE-EMERGING INFECTIOUS DISEASE

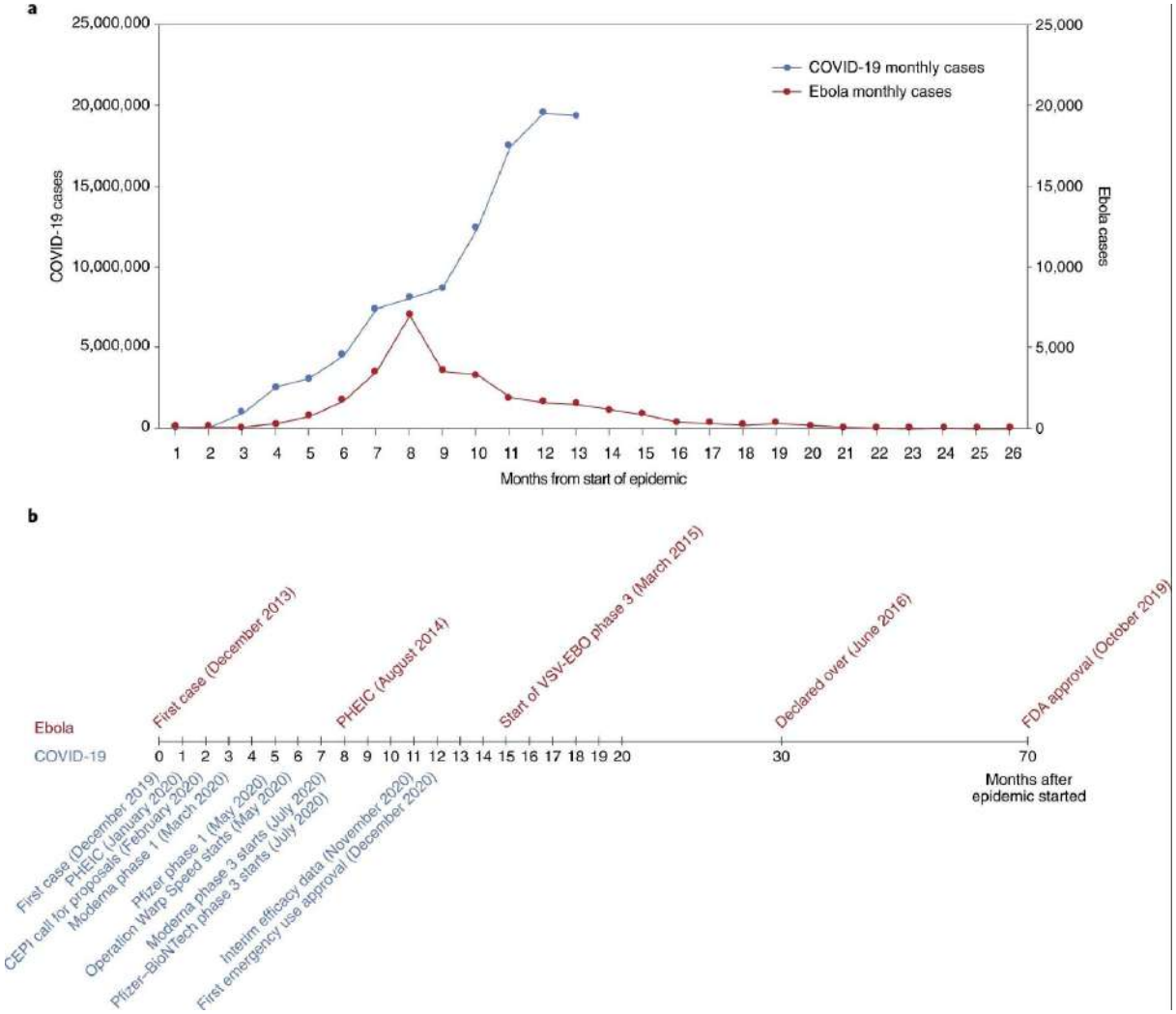
Year of Description	Name	Year of Description	Name
1918	Spanish Influenza	1996	Avian Flu
1931	Rift valley fever	1999	Nipah fever
1937	West Nile fever	2002	SARS
1967	Marburg Haemorrhagic Fever	2009	H1N1, H7N9 Swine flu
1969	Lassa Fever	2012	MERS
1969	Acute Haemorrhagic fever	2015	Zika
1981	HIV/ AIDS	2019- ongoing	COVID 19

Apart from many viral infections, dengue is an increasing global public health threat with four dengue types (DENV1-4). Two other major mosquito borne viral infections are yellow fever and dengue. A single dengue vaccine, Sanofi Pasteur's Dengvaxia based on the yellow fever 17D backbone, has been licensed in 20 countries, but uptake has been poor. Cholera, caused by pathogenic strains of *Vibrio cholerae*, is currently in its seventh global pandemic since 1817; notably, the seventh pandemic started in 1961. Global mortality due to cholera infection is very high probably due to delay in hydration.

MODEL FOR VACCINE DEVELOPMENT

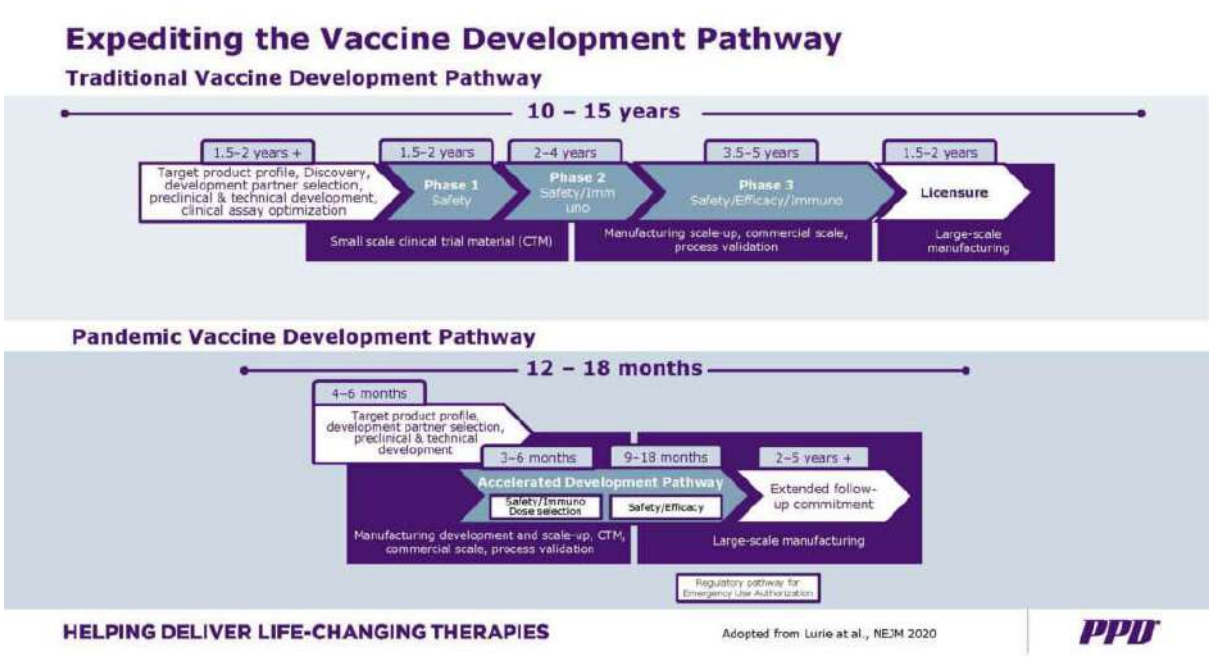
The understanding of emerging diseases has evolved over the past two decades more so after the pandemic of Covid 19. Initially, though SARS CoV was seen in 2002, the need for vaccine development for it soon ended, as the epidemic stopped and wet markets were closed, everything seemed to be under control. And the need for Vaccine was not given importance.

Following of traditional methods for research and development takes a 5-10-year timeline which might no longer be ideal in view of Emerging and Re-emerging diseases. An example comparing the Ebola outbreak and Covid 19 pandemic and their vaccine development is given below, which clearly shows the pace in with which Covid 19 vaccine was developed compared to Ebola.



Perceiving these shortcomings in vaccine development during public health emergencies, there arose the Coalition for Epidemic Preparedness Innovations (CEPI), a non -profit organization dedicated to timely vaccine development.

CEPI initially focused on diseases from a list of WHO priority pathogens for EIDs—Middle East respiratory syndrome (MERS), Lassa fever, Nipah, Rift Valley fever (RVF) and chikungunya. CEPI has been able to fund several vaccine development efforts, among them product development by Moderna, Inovio, Oxford–AstraZeneca and Novavax are prime. Providing upfront funding helped these groups to advance vaccine candidates to clinical trials and develop scaled manufacturing processes in parallel.



VACCINE PLATFORMS

Vaccines are the cornerstone of the management of infectious disease outbreak and can defuse pandemic and epidemic risk. The faster a vaccine is deployed, the faster an outbreak can be controlled. The standard vaccine development cycle is not suited to the needs of explosive pandemics.The new vaccine technologies provide a platform for reducing the cycle and help in developing vaccines faster and effectively.

While several DNA vaccines are licensed for veterinary applications, and DNA vaccines have shown safety and immunogenicity in human clinical trials, no DNA vaccine has reached licensure for use in humans. Recombinant proteins vary greatly in design for the same pathogen (for example, subunit, virus-like particles) and are often formulated with adjuvants but have longer development times. Virus-like particle-based vaccines used for hepatitis B and human papillomavirus are safe, highly immunogenic, efficacious and easy to manufacture in large quantity. The technology is also easily transferable.

Two COVID-19 vaccines were developed using mRNA technology (Pfizer–BioNTech and Moderna), both showing safety and high efficacy. While COVID-19 mRNA vaccines are a useful proof of concept, gathering lessons from their large-scale deployment and effectiveness studies still requires more work and time.

The table below gives a list of most important technical vaccine platforms for emerging and re- emerging epidemics.

Vaccine platform	**Other specifications**	**Developed for**	**Under development OR stopped for**
Live attenuated		Influenza; yellow fever; poliomyelitis	COVID- 19, RVF(for human and veterinary use), Lassa fever, Chikungunya
Whole inactivated	With or without adjuvant	Influenza; poliomyelitis; COVID-19	SARS, Zika, RVF (veterinary use), chikungunya
DNA	Electroporation; adjuvant		SARS, MERS, zika, COVID 19
mRNA		COVID-19	Lassa fever, Disease X
Recombinant vectors Non replicating	**Recombinant vectors Non replicating**		
Ad5			COVID 19
ChAd3			Ebola
ChAdOx1		COVID-19	RVF, MERS,Lassa fever, Chikungunya
Ad26		Ebola; COVID-19	
Live attenuated			
MVA		Ebola	MERS
VSV		Ebola	COVID 19, Lassa fever, Nipah
Measles			MERS,Lassa fever, Nipah, COVID 19, Chikungunya
Protein based			
Virus-like particle	With adjuvant	COVID-19	COVID 19
Monomer; dimer; trimer	With adjuvant		COVID 19, RVF, Nipah
Molecular clamp	With adjuvant		Influenza, MERS, COVID 19

VACCINES BASED ON RECOMBINANT VECTOR PLATFORMS

These vaccines are subdivided into nonreplicating vectors (for example, adenovirus 5 (Ad5), Ad26, chimpanzee adenovirus-derived ChAdOx, highly attenuated vectors like modified vaccinia Ankara (MVA)) and live attenuated vectors such as the measles-based vector or the vesicular stomatitis virus (VSV) vector. Either each vector is designed with specific inserts for the pathogen targeted, or the same vector can be designed with different inserts for the same disease.

The development of the Merck Ebola vaccine is an example. ERVEBO is a live attenuated, recombinant VSV-based, chimeric-vector vaccine, where the VSV envelope G protein was deleted and replaced by the envelope glycoprotein of *Zaire ebolavirus*. ERVEBO is safe and highly efficacious, now approved by theUS FDA and the EMA, and WHO prequalified, making VSV an attractive 'platform' for COVID-19.

T-CELL RECEPTOR VACCINES

TCR peptide vaccination refers to the parenteral administration of small proteins derived from amino acid sequences of T cell receptors. They modulate cytokine production and improve cell mediated immunity.

Examples are: Atopic Dermatitis, Rheumatoid arthritis, Multiple Sclerosis.

VIRUS LIKE PARTICLE VACCINES

Virus like particles (VLP's) refers to particles that self-assemble as a result of the expression of proteins encoding capsids, cores or envelopes of viruses or even preparation of monolayered particles derived from multi-layered viruses. Examples are:

- ✓ Human papilloma virus vaccine -Gardasil,
- ✓ Hepatitis B virus (HBV) vaccine,
- ✓ HEV239

HETEROLOGOUS PRIME BOOST VACCINE(HPB)

HPB immunization is the administration of two different vectors or delivery systems expressing the same or overlapping antigenic inserts. This vaccine approach has been considered for HIV and Ebola vaccines.It is being investigated for COVID-19 vaccines with the Oxford–AstraZeneca AZD1222 and Gamaleya Sputnik V COVID-19 vaccines or with the Pfizer–BioNTech. Other HPB combinations might be considered involving mRNA, DNA, viral vector-based and protein-based vaccines. HPB offers potential benefit to improve immune system and avoid multidose reactogenicity. HPB possibilities deserves further consideration by manufacturers, funders and regulators supported by clinical trial studies and assessment of implementation challenges.

Licensed HPB vaccines which are approved by the U.S FDA are

- ✓ 9-valent HPV vaccine (Gardasil 9, 9vHPV),
- ✓ quadrivalent HPV vaccine (Gardasil, 4vHPV), and
- ✓ bivalent HPV vaccine (Cervarix, 2vHPV).
- ✓

EDIBLE VACCINES

Edible vaccines are subunit vaccines where the selected genes are introduced into the plants and the transgenic plant is then induced to manufacture the encoded protein. Disadvantage is that dose cannot be managed and cooking might alter vaccine properties, whereas Advantages are

- ✓ Easy to administer
- ✓ Easy to store
- ✓ Readily acceptable delivery systems for different age groups
- ✓ Cost effective

VACCINE	VECTOR USED	DISEASE/ CONDITION
Hepatitis B virus	Tobacco, Potato , Lettuce	Hepatitis B
Norwalk virus	Tobacco, Potato	Diarrhoea, Nausea, Stomach cramps
Rabies virus	Tobacco	Rabies
Transmissible GE, Corona Virus	Tobacco, Maize	Gastroenteritis
HIV virus	Tomato	AIDS
Vibrio cholera	Potato	Cholera

PHARMACOVIGILENCE FOR COVID 19

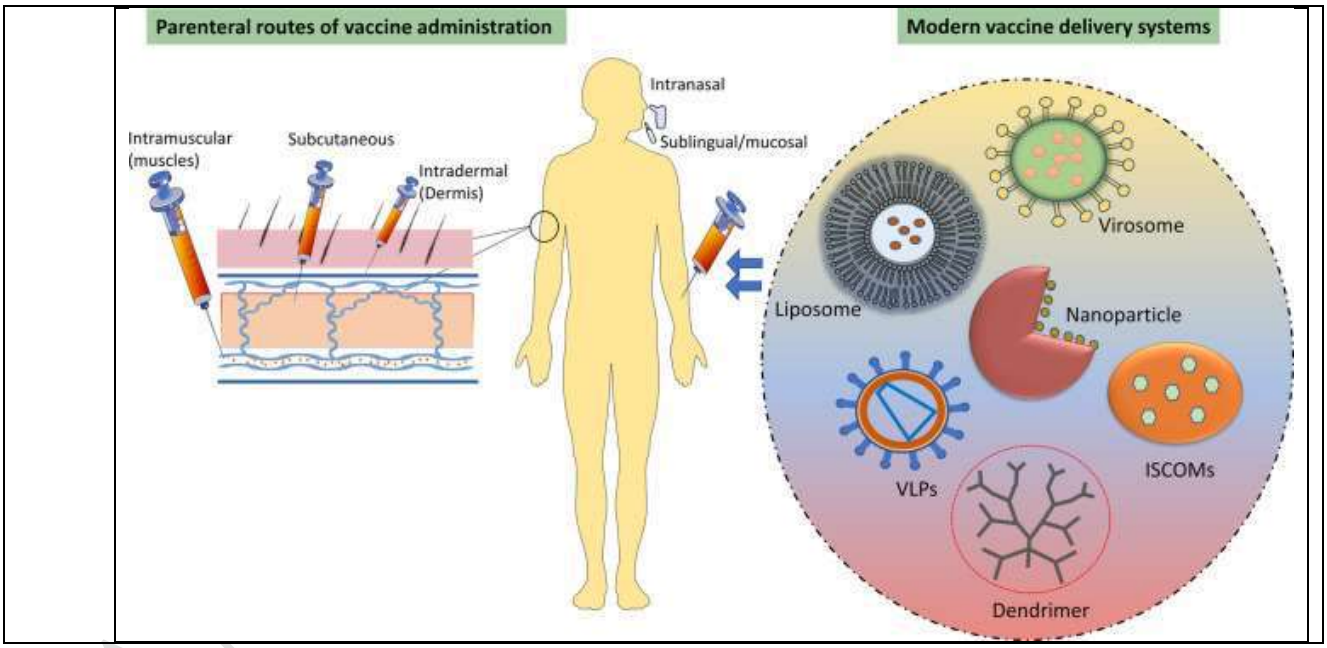
In May 2020, the 42nd Global Advisory Committee on Vaccine Safety addressed pharmacovigilance preparedness for the launch of the future COVID-19 vaccines. One of their recommendations was that infrastructure and capacity for

surveillance of the safety of COVID-19 vaccines should be in place in all countries and engaged before a vaccine is introduced. The WHO's COVID-19 vaccine safety surveillance manual develops the monitoring and reporting of adverse events following immunization and adverse events of special interest, data management systems and safety communication, and the need for post authorization safety surveillance studies. One critical element of this surveillance is the duration of the observation period.In view of the public health urgency and the extensive vaccination campaigns foreseen worldwide, the EMA and the national competent authorities in EU member states have prepared themselves for the expected high data volume by putting pharmacovigilance plans specific for COVID-19 vaccines in place. Good pharmacovigilance practices include detailed requirements and guidance on the principles of a risk management plan (RMP) and requirements for vaccines. In addition, core RMP requirements for COVID-19 vaccines have been developed to facilitate and harmonize the preparation of RMPs by companies and their evaluation by assessors.

OTHER CONSIDERATIONS FOR VACCINE DEVELOPMENT

- ✓ Speed of development
- ✓ Ease of Manufacturing
- ✓ Scale -up
- ✓ Ease of logistics (administration, storage, presentation)
- ✓ Technology transfer- to other countries
- ✓ Worldwide supply
- ✓ Cost of goods and manufacturing

NOVEL VACCINE DELIVERY SYSTEMS



LIST OF VACCINES APPROVED RECENTLY

GROUP	VACCINES
Bacterial	Pneumococcal Mycobacterium tuberculosis Mycobacterium leprae Vibrio cholerae
Viral	Dengue virus Influenza virus HIV Ebola virus Chikungunya virus Monkey pox Corona virus
Parasitic	Malaria

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