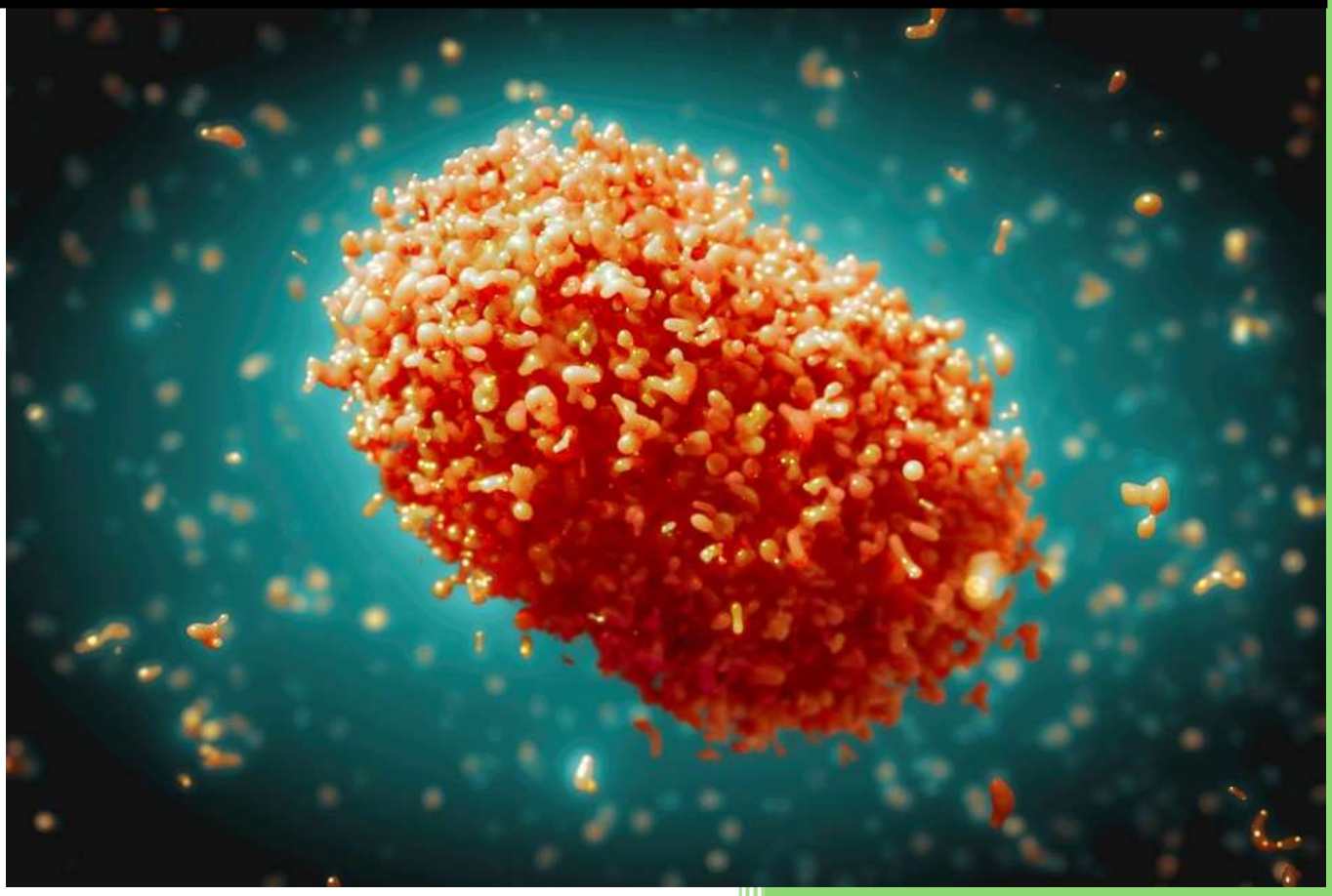
e-bulletin 2024

INDIAN ASSOCIATION OF MEDICAL MICROBIOLOGISTS – TELANGANA & ANDHRA PRADESH CHAPTER (IAMM-TAPC CHAPTER)



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September 2024; Issue No.: 1.0

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September 2024 Issue No. 1.0

FROM THE PRESIDENT IAMM TAPC CHAPTER

Dated:18.8.2024

Dr. Ranganathan Iyer MD FRCPath DNB DPB MAMS,
Consultant Clinical Microbiology, Infections & Infection Control,
President, IAMM TAPC Chapter

Dear Friends and Colleagues,

It gives me great pleasure writing to you as a president of the IAMM TAPC, welcoming one and all in the Twin states of Telangana and Andhra Pradesh to the IAMM TAPC State Chapter Conference at the Siddhartha Medical College and Hospital Vijayawada to be held on the 14th and 15th September 2024, preceded by Four Pre-Conference workshops of great interest on the 13th September 2024. It has been a fruitful and an eventful year studded with workshops and Guest lectures commencing last August in 2023. A number of topics and areas as yet unexplored have been covered over the year with faculty presentations both from within the state and outside of the state at a national level.

The year saw the emergence of Mpox as an health care emergency in many countries of the world. India under the guidance of the ICMR and the Ministry of Health and Family welfare has been in a state of preparedness to grapple with suspected cases, the diagnosis of the same and the way forward towards preventing the spread of this virus amongst the population in the country. AMR containment has gathered strength in many states of India. Diagnosis and management of individual patients along with the institution of appropriate infection prevention and control measures has been a priority in many states of India, with Kerala taking the lead in the country.

Our Twin states saw many viral infections with Influenza, Respiratory Syncytial virus and Mycoplasma infections afflicting children and adults alike. Myraid clinical presentations and unusual sequalae, possibly related to the COVID-19 pandemic have been noted in these infections, particularly in children. We do need a robust diagnostic and clinical management service in all Government and other hospitals to be able effectively tackle this problem in the population. The recent flooding in many areas of the twin states poses another challenge with many water-borne and food borne infections such as Salmonellosis and Shigellosis raising their head as potential outbreaks.

It is envisaged that the conference of the IAMM TAPC at the Siddhartha Medical College in the form of presentations, posters, talks and debates would open new vistas for us to acquire a new insight into these issues and get us prepared to tackle the same.

All the best to one and all in the IAMM TAPC. Hope to meet all of you at Vijayawada for the IAMM TAPC CHAPTER Conference

Dr. Ranganathan N Iyer
President IAMM TAPC

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SECRETARY'S MESSAGE

Dr Mohammed Khaleel
SECRETARY, IAMM TAPC State Association
Professor & HOD, Microbiology, MEU Co ordinator
Mahavir Institute of Medical Sciences, Vikarabad, Telangana State.

Respected Senior Faculty, Esteemed colleagues and Dear Postgraduates of the Association.

I as a Secretary had the privilege of working alongside with my Executive team witness the incredible progress. My experience includes significant contributions by getting our Association Registered and procure Pan card officially. Me along with Dr A. Kishore have made it possible to Transfer of Bank account to SBI, Bhoiguda Branch and making all transaction transparent through the Online banking system. Gradual increase in Membership strengthening the Association with creation of official Google group mail and social media (What's app) has been created to have the update on academic and share one's experience (research, awards, innovative work etc) and reach out to all the members through this platform. Heartful gratitude to BD Company for collaboration in conduct of monthly webinar for Post graduates and all life members with eminent speakers (A Big thanks) to share their experiences on most important topics (need of the hour). Their experiences have equipped us all with a deep understanding of the challenges and opportunities we face in routine. Another milestone was to create and design the Realtime official website to be available for effective dissemination of information, updates to all members by Dr Vijendra.

Thanks to the sincere efforts of previous Association bearers who have been mentors, guiding us for any queries.

My sincere thanks to the Senior faculty members President Dr Nagamani madam, Dr Jyothi Padmaja madam, Dr Ranganathan Iyer Sir ,Organising Committee of previous Conferences and EC Member : Jt Secretary- Dr Vijendra K ,Treasurer- Dr Kishore A Reddy, Members- Dr Sudha Madhuri madam ,Dr Lakshmi Jyothi madam, Dr Shabnum madam, Dr Y Uma Rani madam for their valuable contribution in conduct of PG Paedogugue, PG Write up, News bulletin, Website update and for initiating approval for various welcome change for benefit of the Association.

I believe that the journey has been quite challenging, but I was committed, accomplish my promise to fulfil as shared in manifesto being elected as Secretary. This has been done with IMMENSE SUPPORT, TRUST AND COOPERATION FROM ALL OF YOU!

I am passionate about the future of our Association making an impact at National level. As your Secretary, I humbly thank you ALL for your untiring support to let us move together & ahead for progress.

I look forward the opportunity to see you all at 27th IAMM TAPC State Annual Conference 13th – 15th September 2024, Siddhartha Medical College, Vijayawada for a very memorable experience.

LONG LIVE IAMM TAPC ASSOCIATION!

XXVI IAMM-TAPC ANNUAL STATE CONFERENCE 2023

Pre-Conference Workshops


XXVI I.A.M.M. TAPC State Chapter Conference
CSSD Workshop
" Good Practices in Sterilization Processes "


WELCOME

XXVI IAMM TAPC Chapter Annual State Conference Inauguration

Souvenir Launch by IAMM National President - 2023

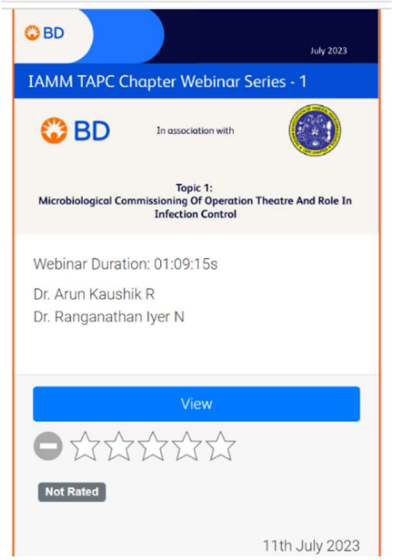
e-bulletin 2023 Release

Executive Team 2023 - 2024

IAMM TAPC Chapter WEBINAR SERIES - I IN ASSOCIATION WITH BD

(July 2023 to July 2024)

Topic – I



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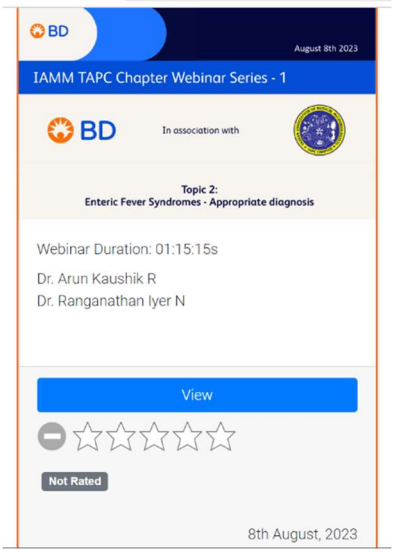

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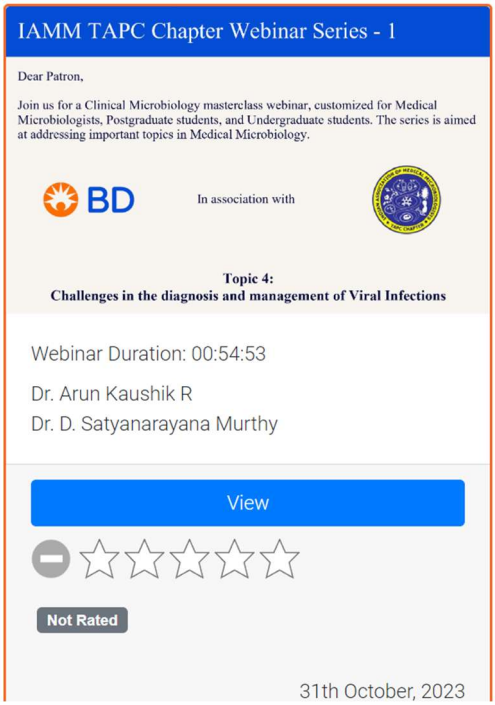

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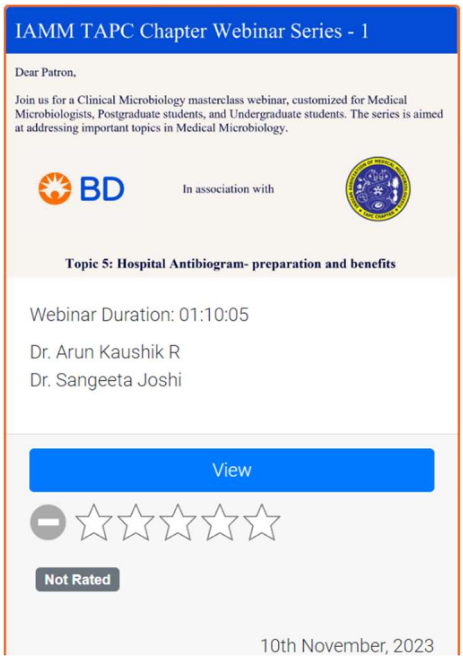

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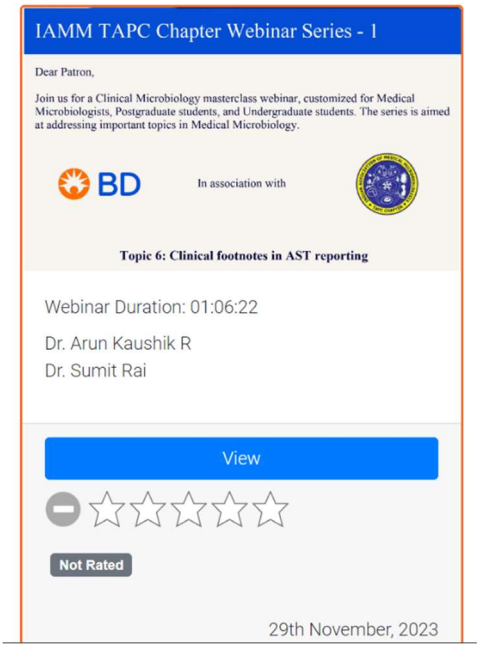

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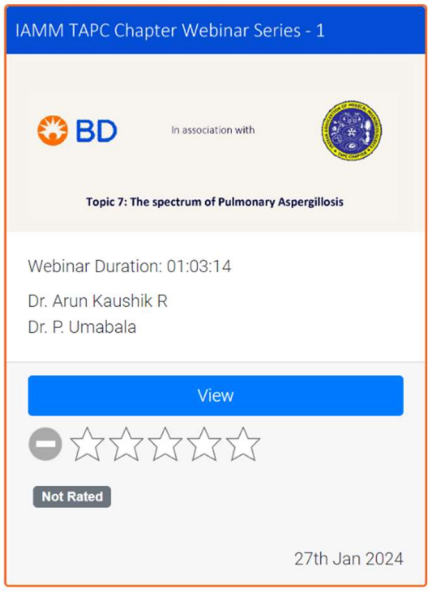

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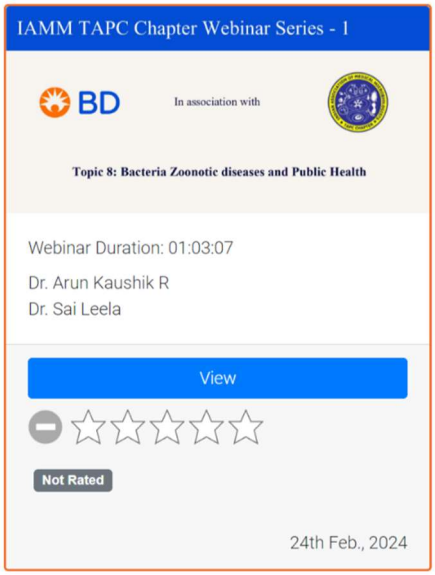

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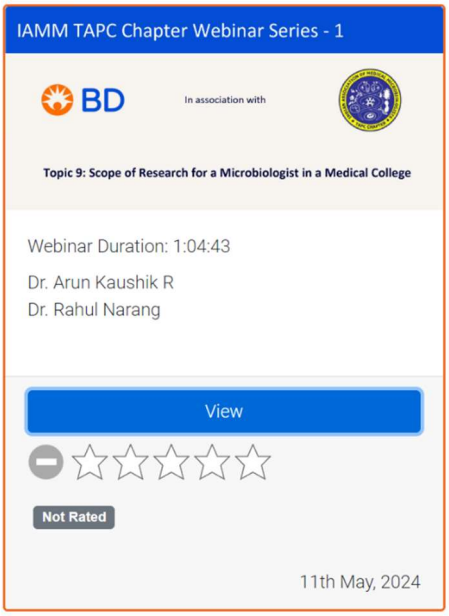

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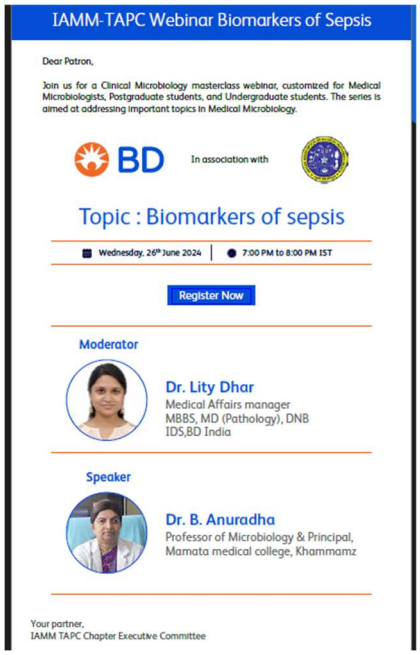


Topic – 11

"స్వాగతం సుస్వాగతం"

కూర్పు: డా. మజ్జి భారతి
(Prof& HOD, GMC,Rjy)

సిద్ధార్థ వైద్య కళాశాలలో నిర్వహించే
వత్సరాని కొకసారి వచ్చే వేడుక
IAMM-TAPC, 27వ వార్షిక సదస్సుకు స్వాగతం
మైక్రోబయాలజిస్టులందరికీ ఇదే మా ఆహ్వానం

రోగకారక క్రిములపై అలుపెరుగని పోరాటం
అదే సూక్ష్మజీవ శాస్త్రజ్ఞుల ప్రధాన లక్ష్యం
దానికై ఉద్దేశించబడిన ప్రి-కాన్ఫరెన్స్ విభాగం
ఉత్కంఠభరితంగా సాగే క్విజ్ కార్యక్రమం
ప్రముఖ మైక్రోబయాలజిస్టుల ఉపన్యాసాలు
నేర్చుకోవాలనే ఉత్సుకతను రేకెత్తిస్తే

యువ శాస్త్రజ్ఞులు, అధ్యాపకులు
పోటాపోటీగా సమర్పించే పరిశోధనా పత్రాలు,
టిబి వ్యాధికారక క్రిమి నిర్ధారణా పరీక్షలు,
క్యాన్సర్ చికిత్సలో రోగనిరోధక వ్యవస్థ పాత్ర,
లేబరేటరీ పరీక్షల్లో పాటించాల్సిన విలువలు,
ప్రస్తుత పరిస్థితుల్లో సూక్ష్మజీవశాస్త్ర ప్రాముఖ్యత,
వీటిపై వాడివేడిగా జరిగే చర్చాగోష్ఠులు, మేధను మథిస్తే

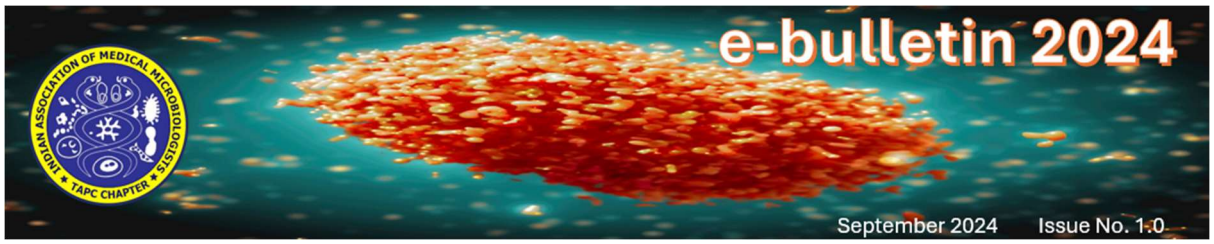
ఈ శాస్త్ర, సాంకేతికత అధ్యయనాల విందుతోపాటు...

ఇంద్రకీలాద్రిపై కొలువు దీరిన కనకదుర్గమ్మ
మంగళగిరిలో వెలసిన పానకాల నరసింహుడు
గుహలో వెలసిన గుణదల మేరీమాత
గలగలా పరవళ్ళు త్రొక్కుతూ కృష్ణమ్మ
సమున్నతమైన డా. బి.ఆర్. అంబేద్కర్ విగ్రహం
ఆధ్యాత్మిక, దర్శనీయ స్థలాలు కాగా

లక్షల ఎకరాలకు సాగునీటినందించే ప్రకాశం బ్యారేజ్
కమ్మరాజుల నిర్మిత శత్రుదుర్భేద్యమైన కొండపల్లి కోట
నదీ ద్వీపాల్లో ఒకటైన భవాని ఐలాండ్
ఉండవల్లి పురాతన గుహాలయాలు
కొండపల్లి కొయ్యబొమ్మలు, మంగళగిరి చేనేత వస్త్రాలు
చారిత్రక, భౌగోళిక ప్రసిద్ధాలు కాగా

వీటి సందర్శన కలిగించు మనసుకెంతో పసందు

ఈ అనుభూతులతో మీ హృది నిండాలని
శాస్త్ర, సాంకేతిక విషయాలు మీ మేధకు పదును పెట్టాలని
ఆకాంక్షిస్తూ మీ అందరికీ యిదే మా ఆహ్వానం
సూక్ష్మజీవ శాస్త్ర విభాగం, SMC, విజయవాడ
***IAMM-TAPC- Organising Committee**



UNVEILING THE POWER OF ARTIFICIAL INTELLIGENCE IN CLINICAL AND DIAGNOSTIC MICROBIOLOGY

Dr. MOHAMMED KHALEEL,
Professor & Head, Dept. of Microbiology, Mahaveer Institute of Medical Sicences, Vikarabad, Telanagana.

Artificial intelligence (AI) is a field, which combines computer science and robust datasets, to enable problem solving in Field of Microbiology. Artificial Intelligence (AI) reshapes medical diagnosis, enhancing accuracy and efficiency significantly. It accelerates processes, offering precise insights crucial for effective patient care. AI-driven technologies, like machine learning and deep learning, empower medical professionals profoundly. The amalgamation of AI and healthcare leads to quicker, more accurate diagnoses. It revolutionizes medical practices, transforming patient outcomes and healthcare efficiency. Big implications in Areas of Hospital Infection control policies and alarm of MDR Bugs in Hospital/Community, Antibiotic Stewardship, outbreaks control measures

AI is revolutionizing the field of medical microbiology, enabling faster and more accurate pathogen identification, antibiotic resistance prediction, and personalized treatment recommendations. This introduction explores the remarkable potential to transform the needs of Microbiology speciality which is NOW multi-disciplinary and multifaceted

- **Challenges in Medical Microbiology**

1. *Complex Pathogen Identification*

Accurately identifying the causative pathogen from a complex sample can be time-consuming and prone to error, especially for less common microorganisms.

Manual process involves man power with many interventions and diverse media and matrices

2. *Antibiotic Resistance Patterns*

Emerging antibiotic resistance poses a growing threat, requiring rapid detection to guide appropriate treatment.

3. ***Personalized Treatment Approaches***

Developing personalized treatment plans based on a patient's unique microbial profile and risk factors remains a significant challenge.

- **Benefits of Automation and AI:**

*Improved benefits and capacity***:** 43% reduction in SAMPLE/ PLATE handling time

***Reductions in human error*:** 52% reduction in touches per specimen

*Improved standardization and quality***:** Strict diagnostic algorithm adherence with machine assisted interpretation, reducing TAT (turnaround time) with superior organism's recovery/isolation including fastidious one.

End to end traceability from sample to report

- **Applications of AI in Pathogen Identification**

Automated Microscopy

AI-powered image analysis can rapidly and accurately identify pathogens from microscopic samples, significantly reducing manual effort and improving diagnostic speed.

Genomic Analysis

AI algorithms can interpret complex genomic data to detect the presence and characteristics of microorganisms, aiding in precise identification and strain typing.

Multimodal Integration

AI can integrate diverse data sources, such as clinical symptoms, laboratory results, and epidemiological information, to provide a comprehensive diagnostic solution.

- **AI-powered Antibiotic Resistance Prediction**

Genomic Profiling

AI models analyze microbial genomic data to identify genetic markers associated with antibiotic resistance.

Personalized Guidance

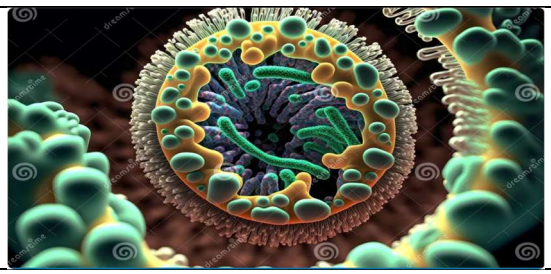
AI-driven decision support systems provide tailored recommendations for appropriate antibiotic selection and dosage, optimizing patient outcomes.

Phenotypic Correlation

AI algorithms correlate genomic profiles with observed antibiotic susceptibility patterns to improve resistance prediction accuracy.

According to the Centers for Disease Control and Prevention, The deaths and higher costs could be deflected by new robotic technology for hospitals, like “Little Joe”, that can sweep unoccupied rooms after use and disinfect them to pristine and safe levels. the disinfection robots eliminate Clostridium difficile (C. diff) in less than 4 minutes and MRSA in less than 2 minutes. “The robots are used for further disinfection in the operating suites and patient rooms including isolation, burn and transplant,”	

Automated Microscopy and Image Analysis

	
Robotic Microscopy	**Advanced Image Analysis**
AI-enabled robotic microscopes automate the imaging process, reducing manual labor and ensuring consistent, high-quality sample analysis.	AI algorithms can rapidly and accurately detect, classify, and quantify microbial structures in microscopic images, aiding in diagnosis and research.

- **Improving Diagnostic Accuracy and Speed**

Faster Turnaround
AI-powered diagnostic tools can significantly reduce the time required for pathogen identification and antibiotic resistance detection, enabling quicker treatment initiation MALDI -TOF
Enhanced Accuracy
AI algorithms consistently outperform traditional methods in accurately detecting and classifying microbial pathogens, improving diagnostic reliability.BIO ARRAY
Cost-Effectiveness
By automating labor-intensive tasks and reducing the need for repeat testing, AI-based diagnostics can lead to substantial cost savings for healthcare systems. CBNAAT/MGIT

- **Ethical Considerations in AI-based Medical Microbiology**

Privacy and Data Security	Ensuring the confidentiality and appropriate use of patient data in AI-powered systems is crucial to maintain public trust.
Algorithmic Bias	AI models must be carefully designed and validated to mitigate the risk of biases that could lead to disparities in diagnosis and treatment.
Transparency and Explainability	Healthcare providers and patients should have a clear understanding of how AI-based recommendations are generated to facilitate informed decision-making

- **Limitations:**

Machine assisted interpretive algorithms are only as good as the data that trains them- garbage in = garbage out

Current machine assisted interpretive algorithms require human intervention (Eg: No auto release functionality)

There are no existing guidelines or best practice recommendations for validating automated systems or machine assisted interpretive algorithms as not feasible for all labs and still has to be customised

Future Trends and Opportunities for Microbiology

- Large language models:

Replace /supplement administrative work in laboratory (Eg: SOP writing, verification protocols, workflow decision- making support, etc)

- Microscopy Imaging

Different stain for Bacteria, fungi, virus and stool parasitic machine assisted interpretation

- Analytics:

Real time result monitoring for continuous quality assurance

Pattern recognition and association studies for large datasets (Eg: Microbiome analytics, Microbe disease state associations etc)

The future promises remarkable advancements of AI's role in medical diagnosis, continue to evolve, becoming more adept at recognizing patterns and anomalies, expect to integrate seamlessly with existing Microbiological practices, aiding professionals extensively.

Reference:

Canadian Journal of Medical laboratory sciences: The future is Here: Artificial Intelligence in Medical Laboratory, Summer 2022, Vol 84, No 2

PG WRITE UPS

Topics:

1. Travel Medicine & Infectious Diseases
2. Role of Multiplex PCR in Syndromic Diagnostic Testing
3. Role of Diagnostic Stewardship in combating AMR
4. Emerging Resistance of Parasitic agents
5. Diagnostic Challenges due to Emerging Antifungal Resistance

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TRAVEL MEDICINE & INFECTIOUS DISEASES

Dr. Priyam Krishnatreya, 2nd year PG,
GUIDE: Dr. N Padmapriya, Prof & HOD
Department of Microbiology,
GMC, Ongole

"While travelling, the world opens up, but so does the risk of infectious diseases-reminding us, illness knows no borders"

INTRODUCTION: Every break that life has to offer people eagerly looks forward for an opportunity to travel which includes crossing borders, entering realms of other states, countries & continents. Along with the fun and adventure of the experience comes the risks of developing diseases Endemic to the respective country. It can be also true for global health workers, businessmen, missionaries & military personnel, etc who has to travel frequently.

Travel medicine is a multidisciplinary field that integrates epidemiology, clinical medicine, public health, tropical medicine, and preventive medicine to address the unique health risks of traveling across diverse regions and cultures.

In todays globalized world, it is of utmost importance to spread awareness about risks associated with infectious diseases and for protecting travellers from undesirable consequences.

Prevalence of infectious diseases associated with travel in various continents

CAUSATIVE PATHOGENS & PREVALENCE: The risks of contracting an infectious diseases largely depends on the destination, duration of stay, activities undertaken while travelling and immunization status. There are many microbial organisms that can lead to infections in a traveller.

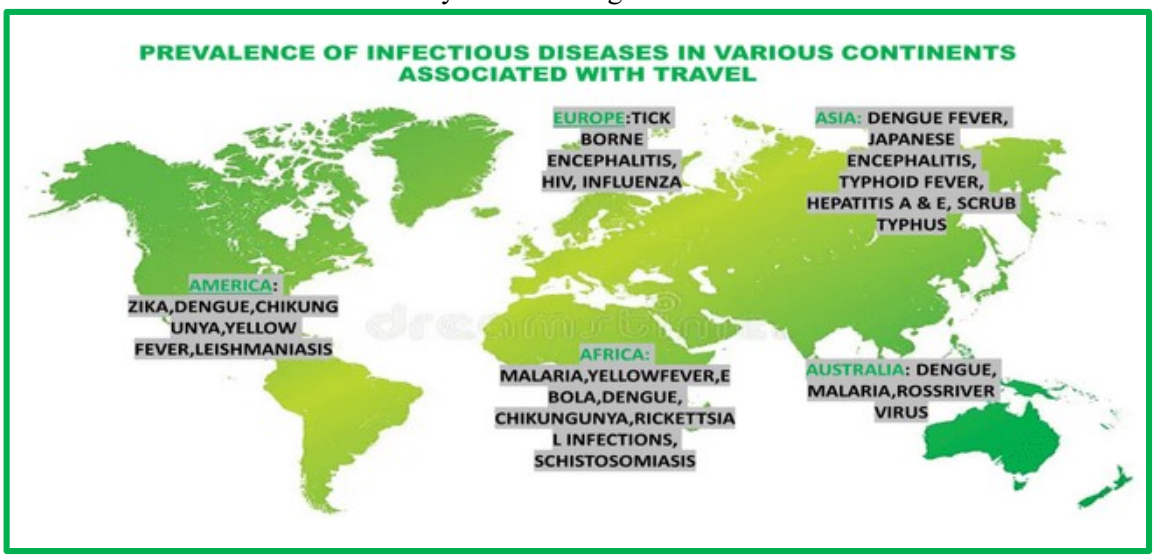


Table summarizes the common pathogens associated with infectious diseases

TYPE OF PATHOGEN	NAME OF PATHOGEN	DISEASE
BACTERIA	1. Salmonella(non-typhoidal) 2. Escherichia coli (ETEC) 3. Shigella 4. Rickettsia spp. 5. Mycobacterium tuberculosis	1. Salmonellosis 2. Traveller's diarrhoea 3. Shigellosis 4. Rickettsial infections 5. Tuberculosis
VIRUS	1. Dengue virus 2. Corona virus 3. Hepatitis A virus 4. Hepatitis E virus 5. Zika virus 6. Influenza virus 7. Flavivirus 8. Norovirus 9. Monkey pox virus 10. Human Immunodeficiency virus	1. Dengue fever 2. SARS-Cov 1,2 3. Hepatitis A 4. Hepatitis E 5. Zika fever 6. Influenza 7. Yellow fever 8. Viral gastroenteritis 9. Monkey pox 10. HIV-AIDS
PARASITES	1. Plasmodium species 2. Entamoeba histolytica 3. Trypanosoma cruzi 4. Trypanosoma brucei 5. Leishmania spp 6. Schistosoma spp 7. Strongyloides stercolis 8. Taenia solium 9. Echinococcus species 10. Giardia lamblia	1. Malaria 2. Amoebic dysentery 3. American trypanosomiasis 4. African trypanosomiasis 5. Leishmaniasis 6. Schistosomiasis 7. Strongyloidiasis 8. Cysticercosis 9. Echinococcosis 10. Giardiasis
FUNGI	1. Histoplasma capsulatum 2. Dermatophytes 3. Blastomyces dermatitidis 4. Coccidioides immitis 5. Talaromyces marneffii	1. Histoplasmosis 2. Dermatophytosis 3. Blastomycosis 4. Coccidiomycosis 5. Talaromycosis

FACTORS ASSOCIATED WITH INFECTION TRANSMISSION WHILE TRAVELING

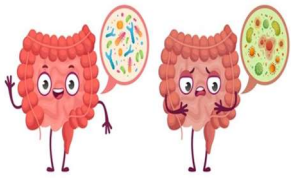
MODE OF TRANSMISSION:

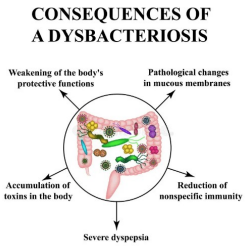
Contaminated food and water can spread bacterial infections like Salmonella, viral infections such as Norovirus, and parasitic infections like Giardia.
Improper food handling, inadequate storage, and undercooked meals contribute to contamination.
Water distribution systems can harbour biofilms, contaminating drinking water and causing infection.

CROWDING, CONTACT & OUTBREAK:

While traveling via airplanes & trains, people come in close contact with each other.
Due to close proximity and shared air people might develop infections transmitted through air droplets & aerosols.

Travellers unknowingly carry pathogens across borders that might lead to outbreak.
For example: Respiratory pathogens such as Influenza virus & SARS-CoV-2





BITES AND FOMITES:

Many infections associated with travel are vector borne. They are transmitted by bite of insect carrying the infection and cause serious illness. For example: Malaria, Dengue, Yellow fever.

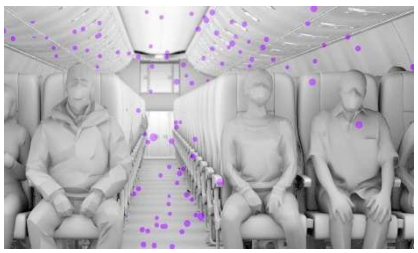
Lack of proper sanitary precautions and unhygienic practices might lead to transmission of pathogens. For example: Norovirus, Staphylococcus aureus

MICROBIOME DISRUPTION:

People traveling to a new place isn't accustomed to the food habits of that area.

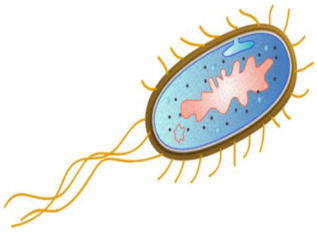
Gut microbiome might undergo dysbiosis due to the diet & water

Increase susceptibility to gastrointestinal infection



VIRULENCE FACTORS:

Pathogens possesses various virulence factors that helps in invasion and survival of infection inside the host. For example: *E. coli* strains causing traveller's diarrhoea produce enterotoxins that disrupt intestinal function.



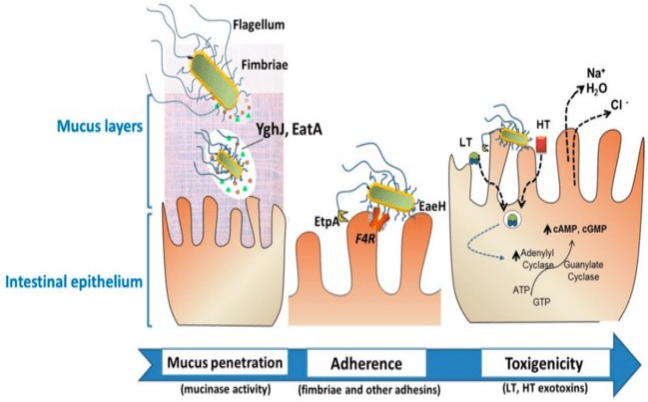
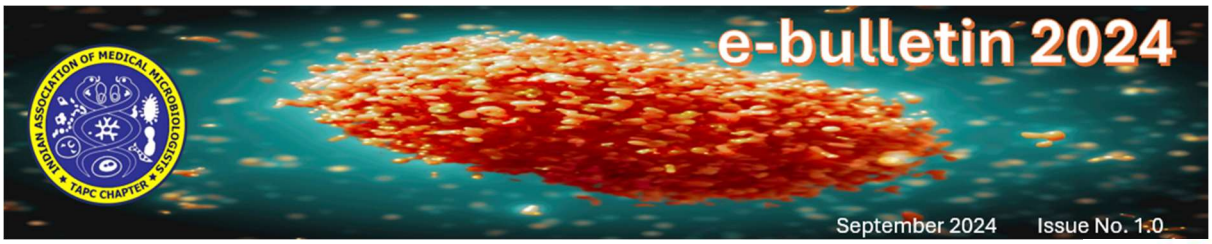
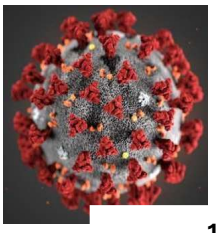
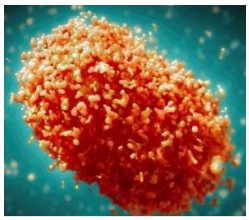
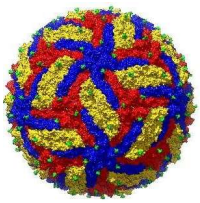


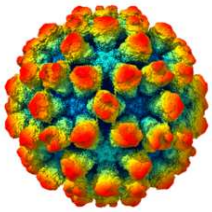
Fig-2.0: Pathway of Enterotoxin production by Enterotoxigenic E. COLI

VIRAL INFECTIOUS DISEASES ASSOCIATED WITH TRAVELLING

DISEASE	CAUSATIVE ORGANISM	INCUBATION PERIOD	MODE OF TRANSMISSION	VIRULENCE FACTORS	LAB DIAGNOSIS
COVID 19	SARS-CoV-2	2-14 days (average 5-6 days)	Respiratory droplets -coughing, sneezing -close contact	Spike Protein: Binds to ACE2 receptor High Replication Rate Immune Evasion: downregulating the expression of MHC Furin Cleavage Site Inhibition of Apoptosis	-NUCLEIC ACID AMPLIFICATION TESTING: Real-time RT-PCR TRUENAT CB-NAAT -ANTIGEN DETECTION: Detects nucleocapsid protein antigen Rapid chromatographic immunoassay ANTIBODY DETECTION: Sero surveillance & survey of high-risk groups
ZIKA VIRUS/ ZIKA FEVER	ZIKA VIRUS	3-14 days	-Mosquito bites (Aedes) -sexual transmission -vertical transmission	NS1 protein: Aids in immune evasion -marker of active infection NS2A, NS3, NS5 Envelope protein Neurotropism	RT-PCR ELISA: IgM & IgG detection PLAQUE REDUCTION NEUTRALIZATION TEST neutralizing ability of patient sera against Zika virus VIRAL CULTURE: inoculation of cell lines Vero or C6/36 cells
EBOLA VIRUS	EBOLA VIRUS	2-21 days (average 8-10 days)	Direct contact with infected bodily fluids, contaminated surfaces	Glycoprotein(GP): Viral entry Viral Protein 24 (VP24): Blocks interferon signalling Viral Protein 35 (VP35) aids viral replication Replication Strategy Immune Evasion	RT-PCR ANTIGEN DETECTION TESTS: (ELISA) or lateral flow immunoassays IgM AND IgG ANTIBODY DETECTION: ELISA VIRAL CULTURE: inoculation in Vero cell line
MONKEYPOX	Monkey pox virus	5-21 days (average 7-14 days	-contact with infected animal -contact with infected person	Envelope Protein Viral Protein C & E: Immune Evasion Viral Protein 24: budding of new viral particles	ELECTRON MICROSCOPY RT-PCR VIRAL CULTURE: NOT RECOMMENDED
NORWALK VIRUS	Norovirus	24-48 hours	Faecal-oral route, contaminated food/water, person-to-person	VP1 (Major Capsid Protein) VP2 (Minor Capsid Protein) NS6 (Non-structural Protein) RdRp (RNA-dependent RNA Polymerase	RT-PCR ENZYME IMMUNOASSAYS: *RIDA®QUICK Norovirus* test. Detect Norovirus antigens RT-LAMP

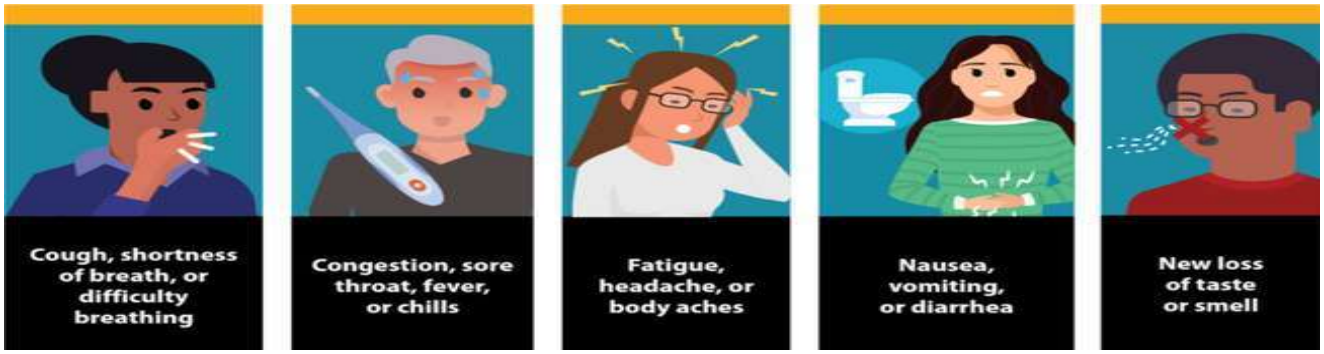


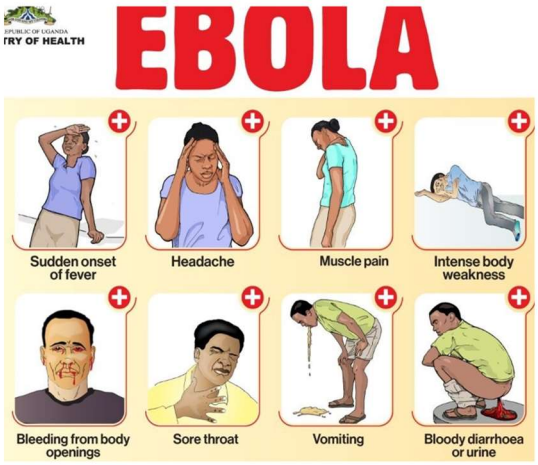




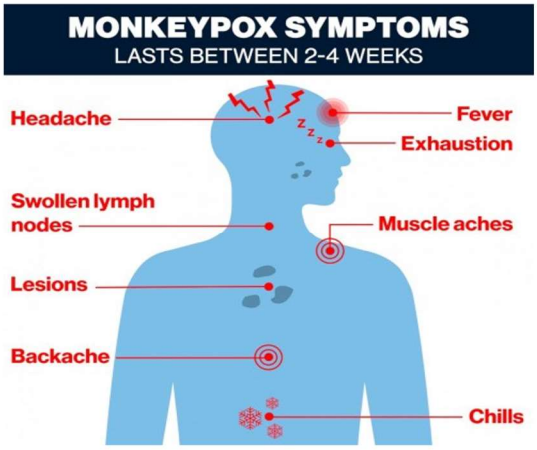


1.CORONAVIRUS 2. MONKEYPOX VIRUS 3. ZIKA VIRUS 4. EBOLA VIRUS 5. NOROVIRUS

COVID 19 SIGNS AND SYMPTOMS





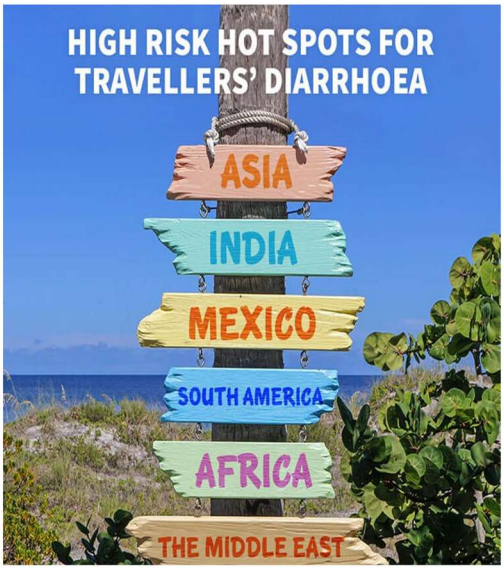


BACTERIAL INFECTIOUS DISEASES ASSOCIATED WITH TRAVELLING

DISEASE	CAUSATIVE ORGANISM	INCUBATION PERIOD	MODE OF TRANSMISSION	VIRULENCE FACTORS	LAB DIAGNOSIS
TRAVELLERS DIARRHOEA	Enterotoxigenic E. coli	1-3 days	Contaminated food/water	**Heat-labile toxin (LT) Heat-stable toxin (ST) Fimbriae (adhesins)**	Stool culture, detection of enterotoxins (e.g., ELISA), PCR
SALMONELLOSIS (GASTROENTERITIS	Salmonella (NON TYPHOIDAL)	6-72 hours	Contaminated food (poultry, eggs, dairy)	**Invasion plasmid antigens (Ipa) Type III secretion system**	Stool culture: XLD-produces black colonies serotyping, PCR
SHIGELLOSIS	Shigella	1-3 days	Feco-oral route (contaminated food/water	**Shiga toxin Invasion plasmid antigens Type III secretion system**	Stool culture: XLD or Salmonella-Shigella agar PCR Serotyping
CAMPYLOBACTERIOSIS	Campylobacter jejuni	2-5 days	Contaminated food (poultry, unpasteurized milk)	**Adhesins, enterotoxins Cytolethal distending toxin**	Stool culture: Campylobacter selective agar RT-PCR Antigen detection

"As of March 26, 2024, an Escherichia coli outbreak occurred in New Jersey and Colorado, USA, linked to the consumption of raw cheddar cheese among tourists"

- Single dose therapy:
  - Single dose of ciprofloxacin 500 mg or norfloxacin 400 mg
  - Azithromycin 500 mg or 1000 mg stat*
- 3-day treatment:
  - Ciprofloxacin 500 mg or norfloxacin 400 mg 12 hourly x 6 tabs
  - Azithromycin 500 mg daily x 3 tablets



"As of July 28, 2023, the UK Health Service Agency identified a Salmonella Enteritidis infection in travellers returning from Turkey, particularly from the Antalya region, with symptoms including watery diarrhoea and weakness"

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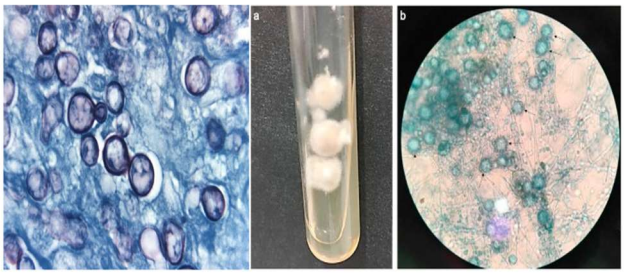
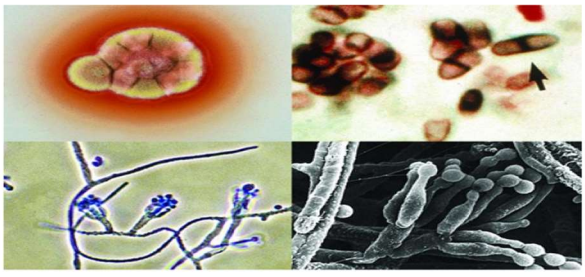
FUNGAL INFECTIOUS DISEASES ASSOCIATED WITH TRAVELLING

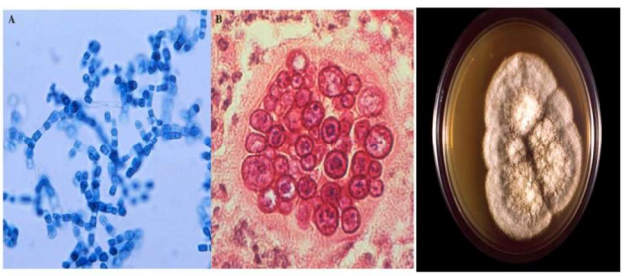
DISEASE	CAUSATIVE ORGANISM	INCUBATION PERIOD	MODE OF TRANSMISSION	VIRULENCE FACTORS	LAB DIAGNOSIS
HISTOPLASMOSIS	*Histoplasma capsulatum*	3-17 days	Inhalation of spores from contaminated soil	Dimorphic fungus Intracellular survival Ability to form a capsule in vivo	Sputum culture **Serology**: Urinary antigen test **Culture at 37°C:** Sabouraud dextrose agar, blood agar PCR
BLASTOMYCOSIS	*Blastomyces dermatitidis*	30-45 days	Inhalation of spores from soil/water	Dimorphic fungus Thick-walled yeast Proteolytic enzymes	Sputum culture Biopsy **Serology**: Antigen detection MEDIA: Sabouraud dextrose agar, blood agar Culture at 37°C, special stains (e.g., GMS)
TALAROMYCOSIS	*Talaromyces marneffii*	1-3 weeks	Inhalation of spores (endemic in Southeast Asia)	Dimorphic fungus Ability to survive inside macrophages	Sputum culture Biopsy Serology: Antigen detection PCR
COCCIDIOID-OMYCOSIS	*Coccidioides immitis*	1-3 weeks	Inhalation of arthroconidia from soil	Spherule formation Evade immune responses Endotoxin-like activity	Sputum culture Serology (IgM/IgG) PCR



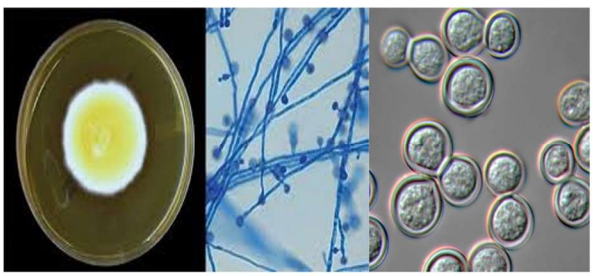
HISTOPLASMA CAPSULATUM



TALAROMYCES MARNEFFEI



COCCIDIODES IMMITIS



BLASTOMYCES DERMATITIDIS

PARASITIC INFECTIOUS DISEASES ASSOCIATED WITH TRAVELLING

DISEASE	CAUSATIVE ORGANISM	IP	MODE OF TRANSMISSION	VIRULENCE FACTORS	LAB DIAGNOSIS
CRYPTOSPORIDIOSIS	Cryptosporidium parvum	2-10 days	Faecal-oral route (contaminated water)	**Oocyst formation**: environmental resistance **Adhesion to intestinal epithelial cells**: facilitates colonization	**Stool examination**: Acid-fast staining techniques **Modified Ziehl-Neelsen stain**: for oocyst detection Immunofluorescence assays PCR
MALARIA	*.Plasmodium falciparum*	7-30 days	Anopheles mosquito bites	**PfEMP1** **RAP1** **Merozoite Surface Proteins (MSPs)** **Asexual Stage Antigens (GPI) Anchors**	Blood smear (thick and thin) for microscopy Rapid diagnostic tests (RDTs) detecting specific antigens PCR for confirmation.
LEISHMANIASIS	Leishmania spp. (e.g., Leishmania donovani, Leishmania major, Leishmania braziliensis)	2 weeks to several month	**Sandfly bites** (phlebotomine sandflies)	**Lipophosphoglycan (LPG)** **Proteinases** **Heat shock proteins** **Surface glycoproteins (e.g., gp63)** **Inhibition of macrophage activation** **Antigenic variation**	**Microscopic examination**- stained smears of lesions or blood **Culture:** on NNN media **Serological tests**: **rK39 antigen test** RT-PCR BIOPSY
TAENIASIS/ CYSTICERCOSIS	Taenia solium	8 to 14 weeks	**Ingestion of undercooked or raw pork** containing cysticerci (larval stage)	**SCOLEX** suction cups and hooks for attachment **Proglottids** reproductive segments for high fecundity **Antigenic variation** to evade immune response **Cysticerci** larval stage can survive in host tissues	**Stool examination:** for eggs or proglottids **Serological tests**: Antibody detection **Imaging studies:** CT or MRI for cysticercosis **Endoscopy**
GIARDIASIS	Giardia lamblia	1 to 3 weeks	**Faecal-oral route** (ingestion of cysts from contaminated water, food	**Adhesive disc**: attachment to the intestinal wall **Cyst formation**: protection in the environment **Antigenic variation**: evade host immune response	**Stool examination** (microscopic identification of cysts or trophozoites) **Serological tests** **RT-PCR** **Duodenal aspirate**

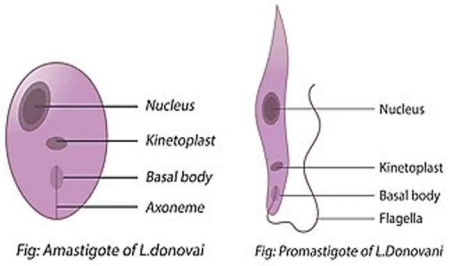
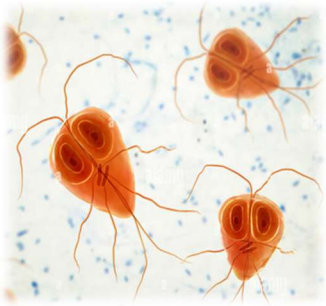
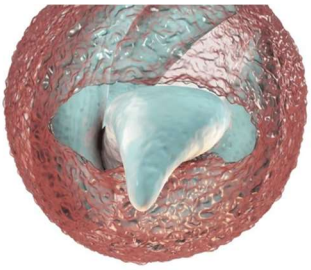


Fig: Amastigote of L.donovai

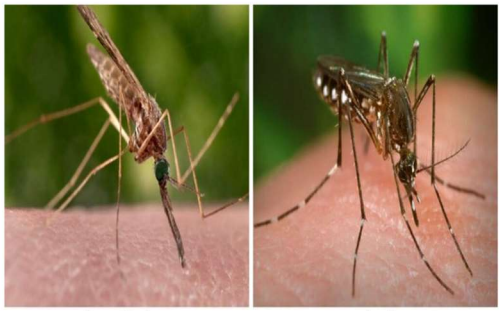
Fig: Promastigote of L.Donovani

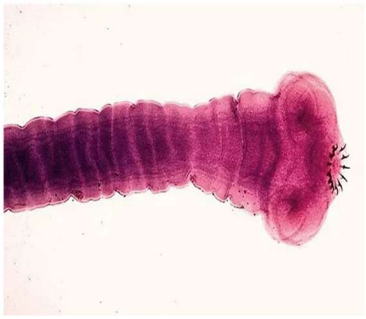


GIARDIA LAMBLIA



CRYPTOSPORIDIUM PARVUM





PREVENTION AND MANAGEMENT OF TRAVEL-RELATED INFECTIOUS DISEASES

VACCINATION: **Vaccination certificate**: Many countries require vaccination certificates for entry, particularly for diseases like yellow fever or COVID-19, making them essential for international travel compliance.

INFECTIOUS DISEASE	VACCINE	RECOMMENDED FOR TRAVELERS
HEPATITIS A	Hepatitis A vaccine (e.g., Havrix, Vaqta)	-at least 2 weeks before travel - 2 doses provide long-term protection.
HEPATITIS B	Hepatitis B vaccine (e.g., Engerix-B, Recombivax HB)	-Recommended for travellers with probable --- -sexual contact or exposure to blood 3 doses required
YELLOW FEVER	Yellow fever vaccine (e.g., YF-Vax, Stamaril)	-Some countries require proof of vaccination for entry -provides lifelong immunity after one dose.
DENGUE	Dengue vaccine (e.g., Dengvaxia)	-Recommended for individuals aged 9-45 -With previous history -3 doses needed
INFLUENZA	Influenza vaccine (e.g., flu shot)	-receive the vaccine at least two weeks before travel -allow time for immunity to develop
TYPHOID	Inactivated Typhoid Vaccine (e.g., Typhim Vi) Live Attenuated Typhoid Vaccine (e.g., Typhoral)	Inactivated Vaccine: 1 dose, given at least 2 weeks before travel. - Live Attenuated Vaccine: 4 doses, taken every other day (Day 1, 3, 5, and 7) at least 1 week before travel.

- **CHEMOPROPHYLAXIS**:

DISEASE	CHEMOPROPHYLAXIS	DOSAGE
TYPHOID FEVER	CIPROFLOXACIN	500 mg twice daily for 7-14 days
TRAVELLERS DIARRHOEA	CIPROFLOXACIN	500 mg twice daily for 3 days
LEPTOSPIROSIS	DOXYCYCLINE	200 mg weekly before travel to high-risk area
MALARIA	ATOVAQUONE-PROGUANIL	1 tablet daily, 1-2 days before, continue for 7 days after travel
LEISHMANIASIS	PENTAMIDINE	2-4 mg/kg IM or IV every 2-4 weeks during exposure risk.

HYGIENE AND SAFE PRACTICES:

- Travelers should drink bottled or treated water
- Avoid raw/undercooked foods
- Practice hand hygiene
- Use mosquito nets and insect repellents in areas with vector-borne diseases.

PRE-TRAVEL CONSULTATIONS:

- Consultations assess the traveller's health, itinerary, and destination-specific risks.
- Issuing travel advisories for regions with prevalent infections is essential.

POST TRAVEL SURVEILLANCE:

- Quarantine Protocols: Enforce mandatory quarantine for travellers from regions with outbreaks (e.g., Ebola, COVID-19) to prevent local transmission.
- Symptom Monitoring: Implement symptom checks (e.g., fever, cough) for a defined post-travel period (e.g., 14 days) to identify and isolate suspected cases.
- COVID-19 Testing: Enhance COVID-19 surveillance through pre-departure and post-arrival testing to quickly identify cases and prevent community spread.
- Contact Tracing: Establish contact tracing for travellers testing positive for infectious diseases to notify and manage potential exposures

ROLE OF MICROBIOLOGIST IN TRAVEL MEDICINE:

- Provide Health Advisories: Provide tailored consultations based on destination, activities, and health conditions.
- Detection: Identify pathogens in returning travellers using rapid diagnostic tests for early detection.
- Surveillance of Infectious diseases: Monitor pathogen distribution and transmission patterns
- Disease Prevention: Contribute to vaccine development for infectious diseases prevalent in travel destinations.
- Guidance on Empirical treatment: Recommend treatment before definitive test results to optimize patient care and reduce disease spread

ANTIMICROBIAL RESISTANCE

- Increased Exposure Risk: Travelers may encounter multi-drug resistant organisms (MDROs) in healthcare settings
- Inappropriate Antibiotic Use: Self-medication and unsupervised antibiotic use during travel can promote AMR by fostering the emergence of resistant strains.
- Cross-Border Pathogen Dissemination: Travel facilitates the global spread of resistant microorganisms
- Surveillance Gaps: Insufficient monitoring of AMR trends in travel-related infections hampers understanding and response.

ANTIMICROBIAL STEWARDSHIP:

- Prescription of antibiotics should be made only when necessary.
- Selection of correct drug & appropriate dosage helps in attaining therapeutic efficacy

CONCLUSION:

In a growing world with merged boundaries, education and awareness of travel-related illnesses are essential. Integrating artificial intelligence for disease prediction, along with rapid pathogen identification through new sequencing methods and point-of-care testing, enhances outbreak tracking. A comprehensive strategy of awareness, protective measures, and post-travel surveillance is vital to reducing cross-border infectious disease transmission. The future of travel medicine relies on advancements in microbiology and technology to improve public health.

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IMAGE LINKS: (SOURCE: GOOGLE)

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ROLE OF MULTIPLEX PCR IN SYNDROMIC DIAGNOSTIC TESTING

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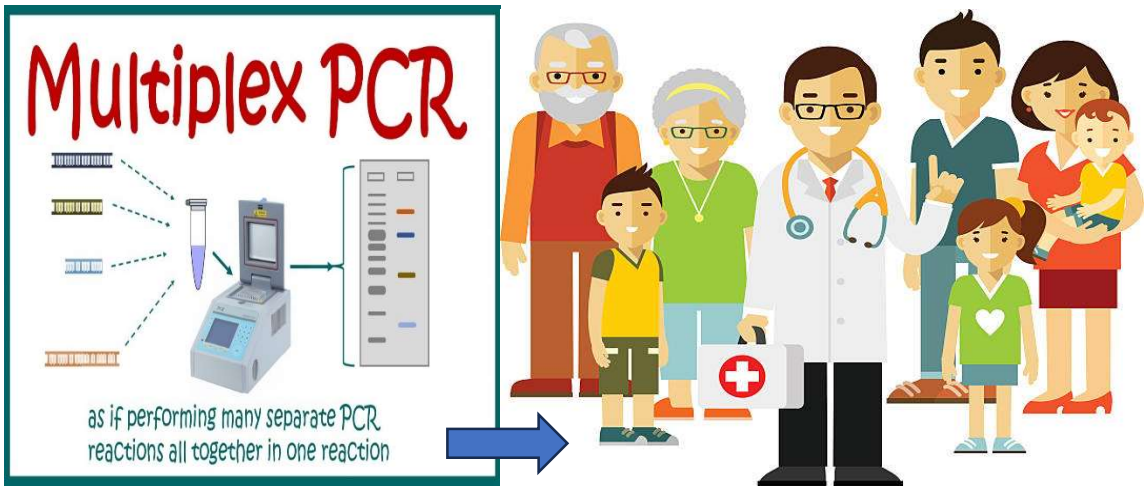
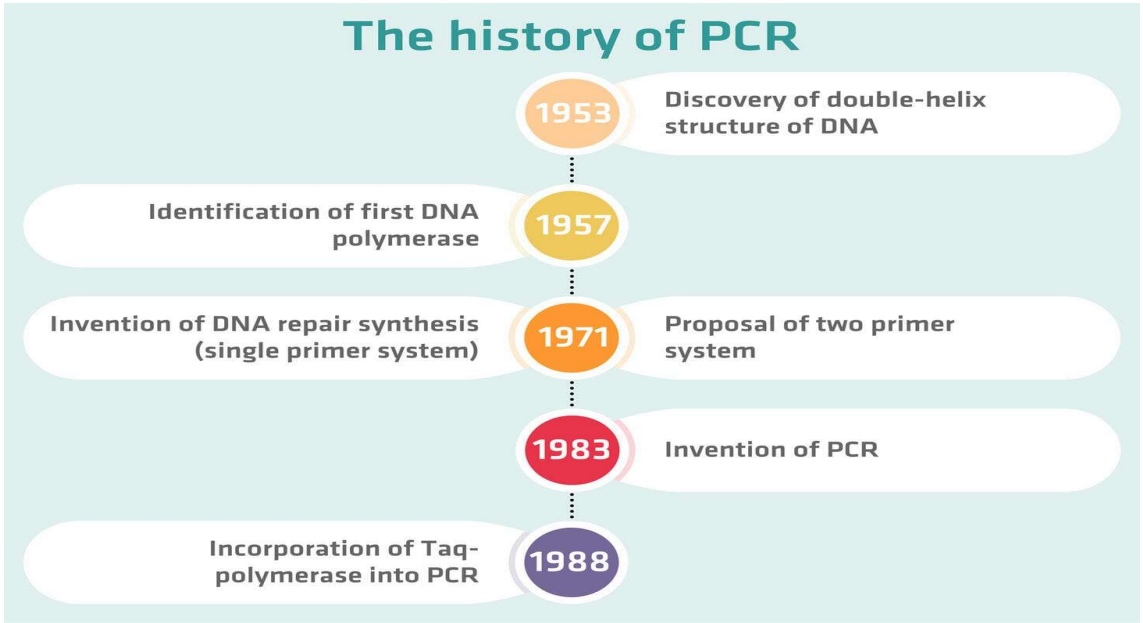
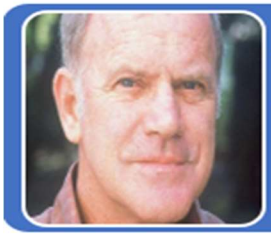
Mentor: Dr. K. Parameswari. HOD and Professor.
Dept. of Microbiology,
Guntur Medical College-Guntur



HISTORY



The history of PCR starts with the discovery of DNA by James Watson and Francis Crick.
The following are some of the scientists who contributed in invention and development of PCR



1983: Kary Mullis a Research Scientist at a California Biotech company

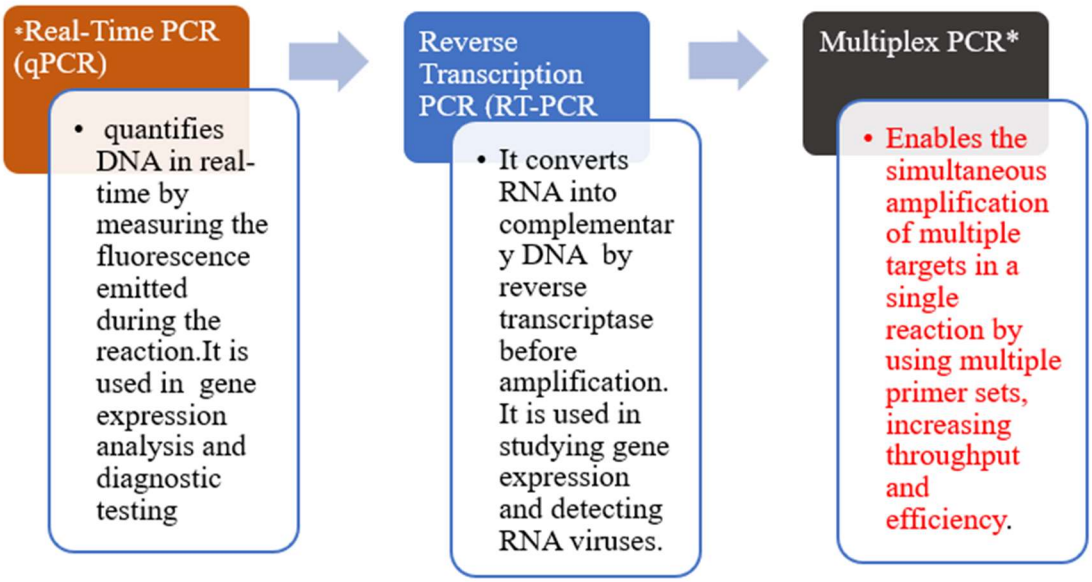
- invents the basic PCR technique. The method allows for the amplification of a specific DNA sequence using a pair of primers.
- Recieved NOBLE prize in chemistry in the year 1993

1986:Ronald R. D. Lee

- The concept of multiplex PCR begins
- using multiple primer pairs in a single reaction to amplify different DNA targets simultaneously

POLYMERASE CHAIN REACTION(PCR): It is a laboratory technique used to amplify specific DNA sequences, creating millions of particular DNA segments. This process involves:
1. Denaturation
2. Annealing
3. Extension

PCR has undergone numerous advancements to enhance its efficiency, accuracy, and applicability developments includes



Multiplex Polymerase Chain Reaction: A Cornerstone in Modern Molecular Biology
Multiplex PCR (Polymerase Chain Reaction) is an advanced variation of the traditional PCR. It allows simultaneous amplification of multiple DNA targets in a single reaction.

Principles of Multiplex PCR
Multiplex PCR operates on the same fundamental principles as standard PCR but involves the simultaneous use of multiple sets of primers, each specific to different target sequences within the DNA sample.

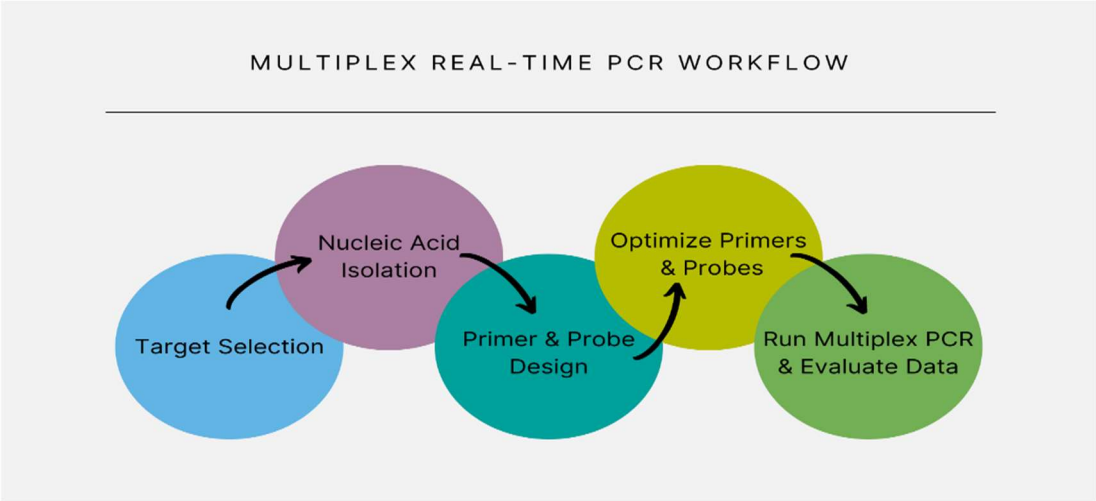
The core components of multiplex PCR include:

- Template DNA: The DNA sample containing the target sequences to be amplified.
- Multiple Primers: Pairs of primers designed to specifically bind to the different target regions of the DNA. The design of these primers is crucial to avoid non-specific binding and primer-dimer formation.
- DNA Polymerase: An enzyme, such as Taq polymerase, that synthesizes new DNA strands by extending from the primers.
- Nucleotides (dNTPs): The building blocks used by the DNA polymerase to synthesize new DNA strands.
- Buffer Solution: Ensures the optimal conditions for the PCR reaction, including the right pH and ionic strength.

The multiplex PCR process follows these steps:

1. Denaturation: Heating the reaction mixture to around 94-98°C to separate the double-stranded DNA into single strands.
2. Annealing: Cooling the mixture to 50-65°C to allow primers to bind (anneal) to their specific complementary sequences on the single-stranded DNA.
3. Extension: Raising the temperature to approximately 72°C, enabling the DNA polymerase to extend the primers and synthesize new DNA strands.

These steps are repeated for 25-40 cycles to exponentially amplify the target DNA sequences.



Development and Optimization

Multiplex PCR has undergone significant advancements to enhance its reliability and efficiency. Key aspects of development and optimization include:

- Primer Design: Designing primers that work well together in the same reaction is crucial. Primers must have similar melting temperatures (Tm) to ensure uniform annealing and minimize the formation of primer-dimers and non-specific products.

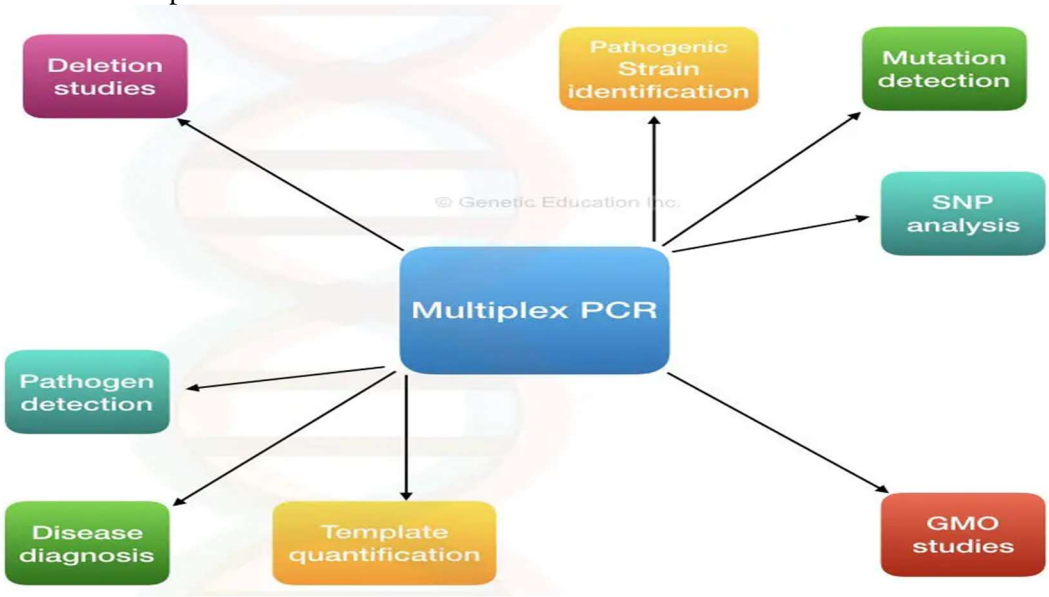
- Optimization of Reaction Conditions: Adjusting the concentrations of primers, dNTPs, magnesium ions, and the DNA polymerase to balance the amplification of multiple targets and avoid competition among primers.

- Thermal Cycling Conditions: Fine-tuning the thermal cycling parameters (temperatures and durations of each step) to accommodate the requirements of all primer sets involved.

Types of Multiplex PCR:

- Single template PCR reaction
- Multiple template PCR reaction

Applications of Multiplex PCR



The ability to amplify multiple targets simultaneously has made multiplex PCR invaluable in various fields:

- Clinical Diagnostics
- Multiplex PCR is extensively used in diagnosing infectious diseases by detecting multiple pathogens in a single test. For example:
- Respiratory Infections: Panels can simultaneously test for viruses and bacteria responsible for respiratory symptoms, such as influenza, RSV, and SARS-CoV-2.
- Sexually Transmitted Infections (STIs): Simultaneous detection of pathogens like Chlamydia, Gonorrhea, and Trichomoniasis.
- Gastrointestinal Infections: Panels can identify various bacteria, viruses, and parasites causing gastrointestinal symptoms.
- Genetic Research:
- In genetic research, multiplex PCR facilitates the analysis of multiple genetic markers, mutations, or polymorphisms in a single reaction. This is particularly useful for:
- Genotyping
- Gene Expression Studies
- Forensic Science

- Multiplex PCR is a cornerstone in forensic DNA analysis which is crucial for:
- Criminal Investigation.
- Paternity Testing.
- Environmental and Agricultural Science
- Multiplex PCR is used in environmental and agricultural studies to detect and quantify multiple microbial species in environmental samples. Applications include:
- Microbial Ecology.
- GMO Detection.

ROLE/Advantages of Multiplex PCR over RT-PCR in Syndromic Diagnostic Testing

Syndromic diagnostic testing aims to identify the pathogens responsible for a specific set of clinical symptoms by detecting multiple potential causes at once.

Both Multiplex PCR (Polymerase Chain Reaction) and RT-PCR (Reverse Transcription PCR) are valuable tools

However, multiplex PCR offers distinct advantages over RT-PCR, particularly in terms of efficiency, cost, and comprehensive pathogen detection.

The key advantages of multiplex PCR over RT-PCR in syndromic diagnostic testing:

1. Simultaneous Detection of Multiple Pathogens

Multiplex PCR can amplify and detect multiple DNA targets from different pathogens in a single reaction. Which is beneficial in syndromic testing, where symptoms can be caused by various pathogens.

Allows for the simultaneous detection of viruses, bacteria, and fungi in one test, providing a more comprehensive diagnostic tool compared to RT-PCR, which typically targets fewer pathogens per reaction.

2. Efficiency and Cost-Effectiveness

Single Reaction Setup: Multiplex PCR reduces the need for multiple separate reactions, saving time and resources. This streamlining is crucial in clinical settings where rapid diagnosis is needed.
Lower Costs: Fewer reagents and consumables are required compared to running multiple RT-PCR tests, making multiplex PCR more cost-effective for large-scale testing.

3. Speed of Diagnosis

Rapid Turnaround: Multiplex PCR can provide results more quickly by testing for multiple pathogens simultaneously. This rapid identification can lead to faster clinical decision-making and treatment initiation.
Reduced Labor: Less hands-on time is required to prepare and run a single multiplex PCR reaction compared to multiple RT-PCR tests.

4. Reduced Sample Volume Requirements

Multiplex PCR can analyse multiple targets from a single sample, which is particularly advantageous when sample volume is limited or difficult to obtain (e.g., CSF, paediatric samples).
Minimized Invasiveness: Reducing the number of samples thereby minimizes patient discomfort and the invasiveness of the diagnostic process.

5.High Sensitivity and Specificity

Precision: Multiplex PCR offers high sensitivity and specificity, ensuring accurate detection of low-abundance pathogens.

Reduced Cross-Reactivity: minimize the risk of cross-reactivity and false positives, which can be more challenging to manage in RT-PCR assays targeting multiple pathogens.

Multiplex PCR offers significant advantages over RT-PCR in syndromic diagnostic testing due to its ability to simultaneously detect multiple pathogens, increased efficiency, cost-effectiveness, rapid turnaround time, accurate and timely diagnosis of complex syndromes, improving patient outcomes, aiding in effective disease management.

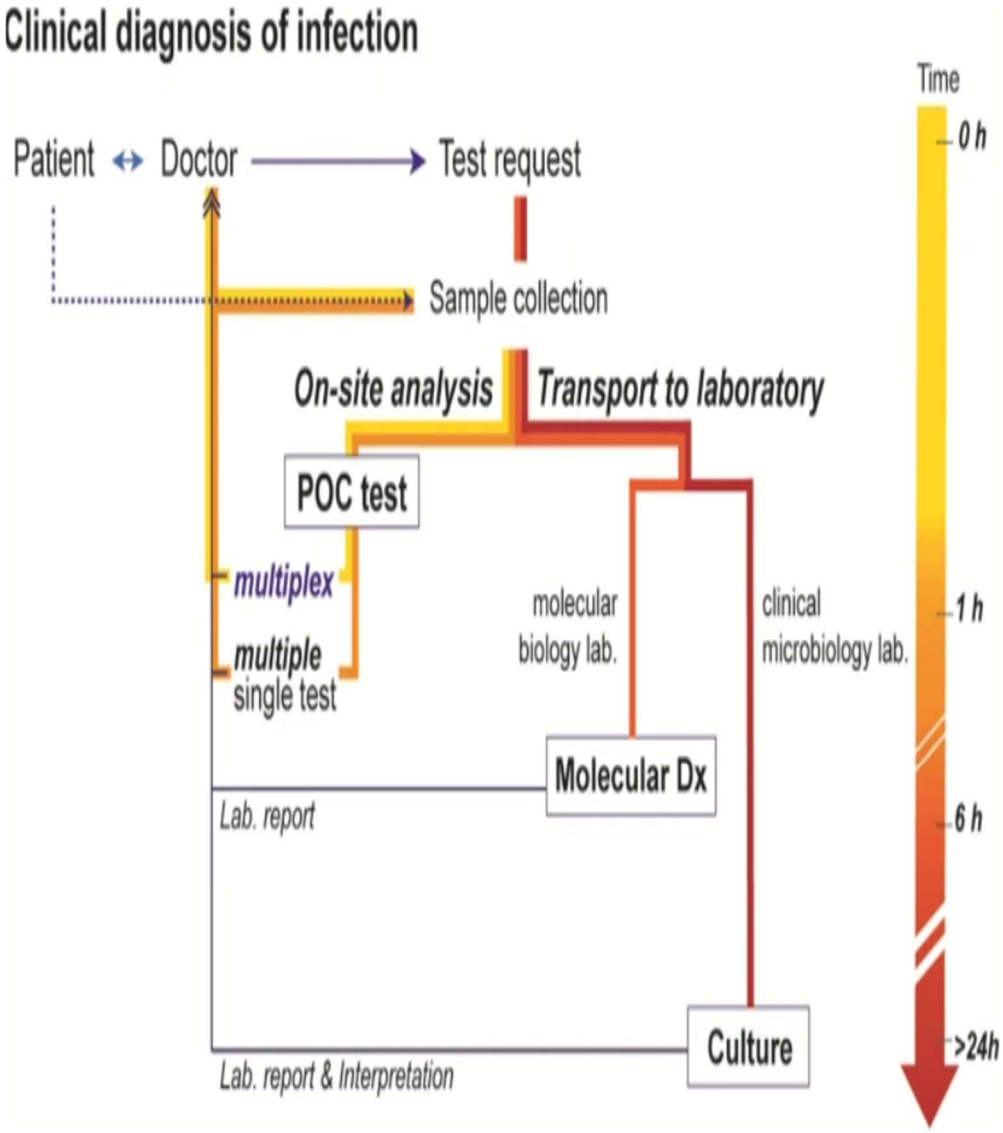
SYNDROMIC DIAGNOSTIC TESTING

One of the advanced technologies in microbiology is multiplex molecular assays i.e. Syndromic diagnostic tests is a novel approach to rapid diagnosis of common infectious diseases.

Syndromic panels are GAME CHANGERS in diagnosis of infectious diseases by

- Improving Patient outcomes through Reducing time to diagnose and clinical decision-making
- Optimize laboratory laboratory workflow
- Enhancing antimicrobial stewardship

Multiplex Molecular Point-of-Care Test for Syndromic Infectious Diseases



Clinical diagnosis of infection according to a diagnostic platform. The implementation of a multiplex point-of-care (POC) test reduces diagnostic cycles for faster and better treatment decisions

ROLE OF MULTIPLEX PCR IN SYNDROMIC DIAGNOSTIC TESTING

Blood Stream Infections

BioFire FilmArray role

1.Median time to optimal therapy shortened from 14.68 h to 4.65 h

2.Antibiotics adjusted in greater than 30% of patients.

3.Median time to pathogen identification: 1.58 h (96.2% of pathogens identified)

Verigene BC-GN role

1.Median time to pathogen identification reduced to 11 hours

2.ICU LOS significantly shorter

3.30-day mortality significantly shortern

4.Estimated net cost saving for each patient in ICU decreases

Respiratory Tract Infections

Role of multiplex pcr in respiratory tract infections Panels can simultaneously test for viruses and bacteria responsible for respiratory symptoms, such as influenza, RSV, and SARS-CoV-2

BioFire FilmArray

1.Influenza result time decreases from 7 hrs to 1.5 hr

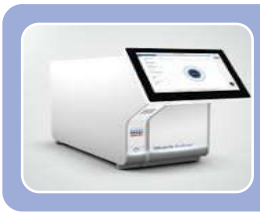
2.Non-influenza viruses diagnosis discharged home: 21% versus 5%

3.Average time to test result: 383 min versus 1119

Respiratory viral PCR test

1.47% of stewardship interventions accepted

2.Time to de-escalation of antibiotics were slightly reduced between pre- and post-multiplex PCR:



QIAstat-Dx Respiratory SARS-CoV-2 Panel

1.Median time to result of POC test: 1.7 h versus 21.3 h for control

2.Time to arrival in definitive clinical area: 8 h versus 28.8 h

Infectious Diarrhoea

Biofire FilmArray Gastrointestinal panel (GIP)

- *clostridioides difficile* testing results were excluded
- Stool positivity rate detection increased 5 times
- Multiplex estimated to decrease cost of care of the patient
- Percent positivity increased from 4.1% to 29.2%.
- Patients assessed via multiplex PCR were less likely to undergo endoscopy
- Patients assessed via multiplex PCR were less likely to be prescribed antibiotics.

Central Nervous System

The FilmArray Meningitis/Encephalitis (ME)

- ME panel was able to detect 141 of the most common pathogens associated with meningitis versus 104 pathogens with using traditional methods Negative predictive value was >99%
- ME panel identified 101 organisms.
- Breakdown of cases identified: 55 viral, 38 bacterial, 7 fungal and 1 polymicrobial
- ME detected more cases that are difficult to diagnose by conventional methods
- ME panel identified 56 pathogens
- 96.3% sensitivity
- 96.58 specificity

MULTIPLEX PCR'S VERSATILITY AND COMPREHENSIVE APPROACH POSITION IT AS A CORNERSTONE TECHNOLOGY IN MODERN SYNDROMIC DIAGNOSTICS.

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ROLE OF DIAGNOSTIC STEWARDSHIP IN COMBATING AMR

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Guide: N.Padmapriya, HOD & Professor,
Department of Microbiology,
GMC, Ongole

"Drug resistance follows the drug like a faithful shadow."

Paul Erhlich in 1908

Role of Diagnostic Stewardship in Combating AMR

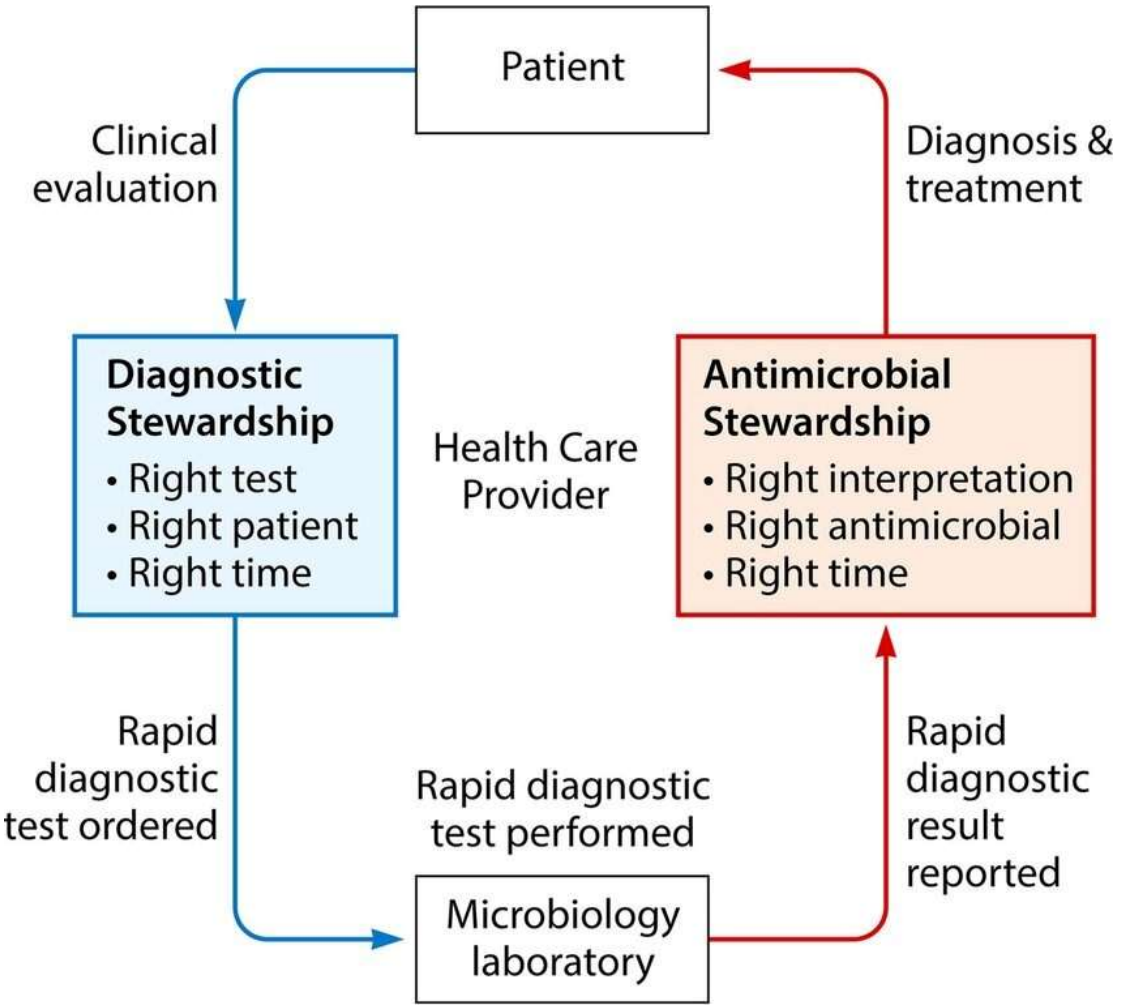
Introduction:

Antimicrobial stewardship (AMS) programs have been developed worldwide to help curtail the pandemic of antimicrobial resistance (AMR). The goal of these programs is to ensure that patients receive timely and appropriate therapy, while reducing overuse of unnecessary drugs, costs, and medication-related adverse events.

Diagnostic stewardship (DS) promotes the appropriate use of the right diagnostic tools for every patient, to limit overuse and guide timely patient management. This strategy also enables early discontinuation of antimicrobial therapy, thereby limiting the risk of AMR and improving clinical outcomes.

DS is highly relevant in settings that involve immunocompromised and critically ill patients. Diagnostic stewardship, which implies judicious selection, analysis, and timely reporting of diagnostic tests, holds promise in refining diagnostics and patient care.

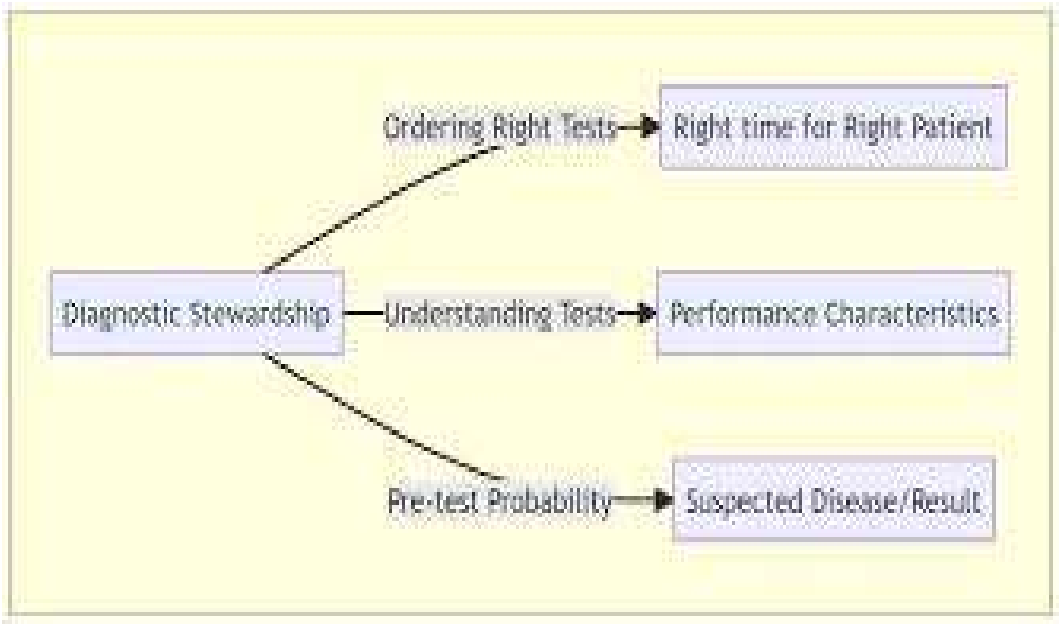
Ordering the right tests, for the right patient, at the right time, to provide the right treatment is called diagnostic stewardship.



Definition:

Global Antimicrobial Resistance and use Surveillance System (GLASS) manual is "Coordinated guidance and interventions to improve appropriate use of microbiological diagnostics to guide therapeutic decisions. It should promote appropriate, timely diagnostic testing, including specimen collection, and pathogen identification and accurate, timely reporting of results to guide patient treatment."

The three phases of the microbiological diagnostic cycle are pre-analytical, analytical, and post-analytical, each play a crucial role in ensuring the accuracy and reliability of microbiology reports.



In the first step of the pre-analytical phase, the appropriate test is chosen after careful consideration of the clinical signs and symptoms. In addition to rational considerations whether or not to test, healthcare professionals should use clinical practice guidelines to optimize the use of diagnostic tools.

Accuracy, availability, cost effectiveness, turnaround time and clinical impact are ideal characteristics that affect the choice of testing. However, the diagnostic performance of a test also depends on the type of specimen used and the time of collection.

It is important to avoid tests that are either low-yield or carry a high risk of false positivity. Similarly, diagnostics that are unlikely to affect patient management should be avoided. This is important when dealing with acute pharyngitis, for example, where requesting streptococcal antigen or throat culture should be guided by the pre-test probability of the likelihood of bacterial pharyngitis.

Once appropriate testing is decided, proper sampling is crucial to maximize yield. During the analytical phase, the microbiology lab plays an important role in reducing unnecessary testing. Sample rejection is an intervention that has consistently shown great potential to reduce unnecessary testing and treatment. For instance, laboratories may not perform urine culture in the absence of significant pyuria, except in neutropenic patients, or refuse Clostridioides difficile infection (CDI) testing for non-loose stools.

During the post-analytical phase, multidisciplinary interventions are necessary to enable correct interpretation of results. The integration of these interventions in the electronic medical record (EMR), whenever available, can improve communication and enable timely decision-making. It is important that results are reported quickly to reduce time to optimal antimicrobial therapy.

Antimicrobials act on bacteria in multiple ways. They can kill the cell (bactericidal) or retard its multiplication (bacteriostatic).
They can act by:

- Inhibition of cell wall or cell membrane synthesis in microbes.
- Disruption of essential processes such as protein synthesis of microbes.
- Disruption of nucleic acid synthesis in microbes.

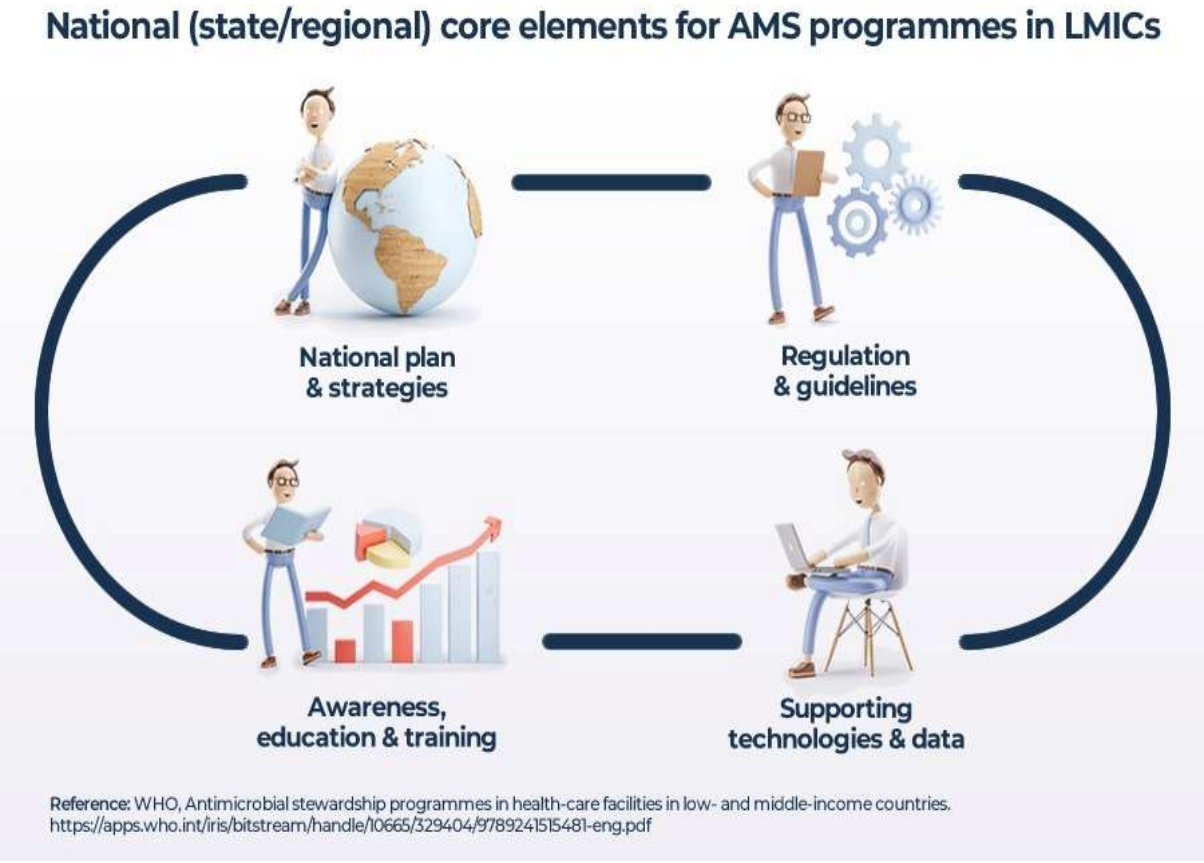


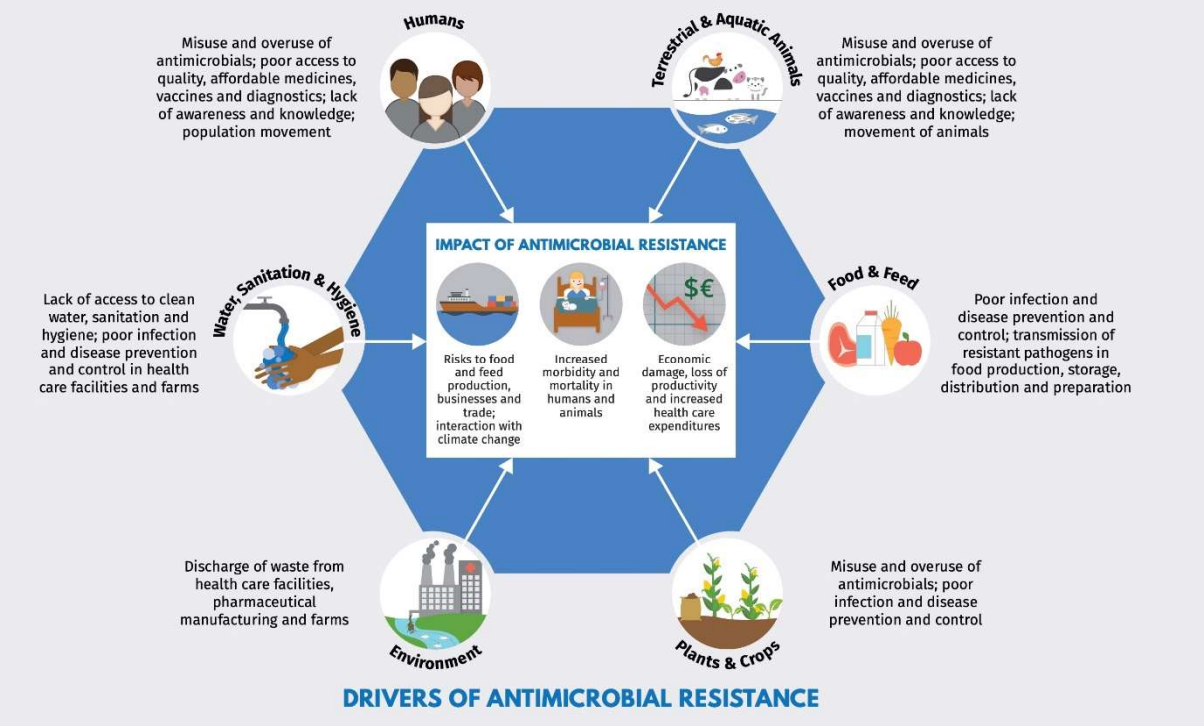
Figure 4: Steps in monitoring and evaluation of diagnostic stewardship at AMR surveillance sites

STEPS	EXAMPLES
PLANNING (baseline)	• Situation analysis, resources and needs assessment conducted
INPUT (needed resources)	• Funding for diagnostic stewardship activities in the surveillance site • Local guidelines and SOPs for diagnostic stewardship • Trained and capacitated staff on local diagnostic stewardship guidelines • Microbiological laboratory facilities with equipment and consumables • Communication protocols and facilities
PROCESS (activities)	• Mobilization and management of funds • Development or adaptation of SOPs • Development and implementation of training materials for diagnostic stewardship • Implementation of training courses • Internal and external quality assurance, regular procurement & maintenance of equipment and consumables • Agreed means and frequency of communication among clinical, laboratory and surveillance staff
OUTPUT (results)	• Sustainable financing and resources available on regular basis • Common understanding of protocols for diagnostic stewardship • Staff trained and capacitated leading to compliance with local diagnostic stewardship protocols and steps • Increase in specimens submitted to the laboratory according to SOPs • Good laboratory practices in place resulting in reliable and timely results • Patient treatment and surveillance actions are informed in a timely manner
OUTCOME	• Patient treatment guided by timely microbiological data resulting in safer and more efficient patient care • Accurate and representative AMR surveillance data to inform treatment guidelines and AMR control strategies

Drivers of Antimicrobial Resistance:

Bacteria have developed resistance against every class of antimicrobial drug. The development of AMR is multifactorial. The risk factors most commonly found to be associated with development of antimicrobial resistance are:

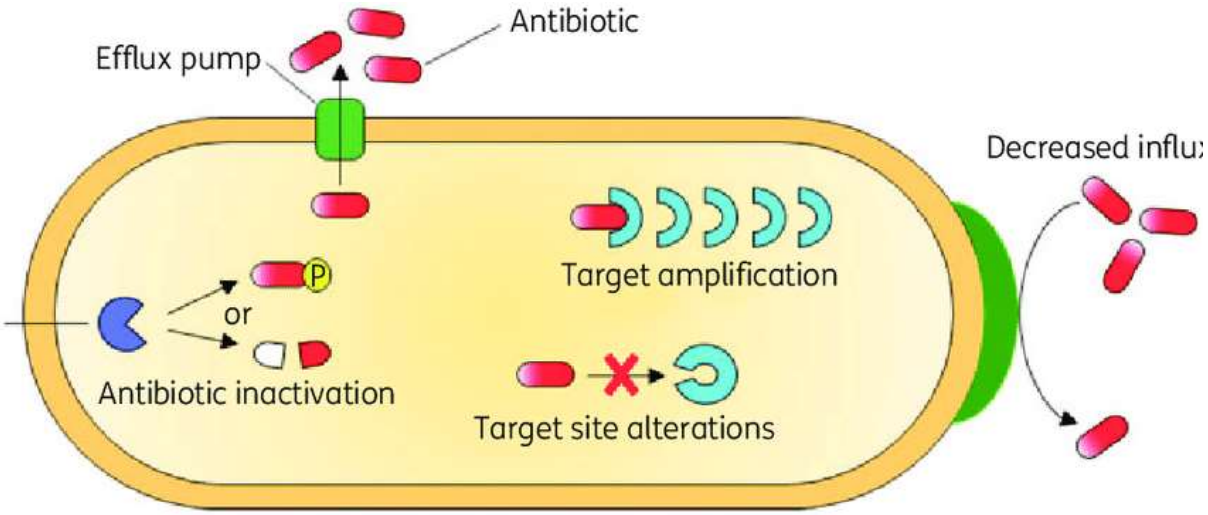
1. Excessive and irrational prescriptions of antimicrobials in community and hospitals
2. Increase in invasive procedures, transplants surgeries and immunosuppressive therapy.
3. Increase use of prosthetic devices amenable to super-infection and resistant bacteria.
4. Lack of effective preventive infection control measures such as hand hygiene, isolation procedures of patients with multi drug resistant organisms.
5. Lack of effective antimicrobial stewardship programs restricting antimicrobial usage in community and hospitals.
6. Use of antimicrobial in agriculture sector, animal husbandries and fisheries.



Key Antimicrobial Resistant Pathogens:

The World Health Organization (WHO) has identified a list of priority pathogens that pose a critical threat to human health due to their resistance to antimicrobials. These pathogens as per the recent 2024 list include:

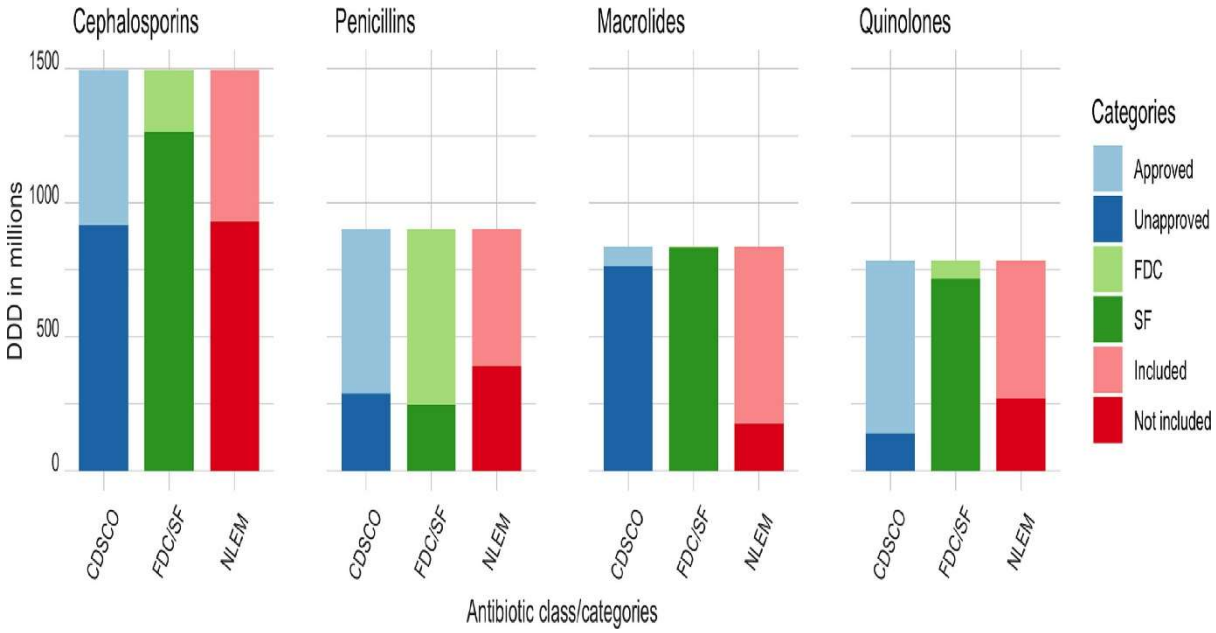
- Carbapenem resistant Enterobacterales (CRE) These include Klebsiella spp. and Escherichia coli that are resistant to carbapenems and are placed atop in the critical list of priority pathogens.
- Carbapenem resistant Acinetobacter baumannii (CRAB) The emergence of CRAB poses a formidable challenge due to limited treatment options particularly in ICU settings.
- Methicillin-resistant Staphylococcus aureus (MRSA) – S, aureus resistant to many common antibiotics, making it difficult to treat skin infections, pneumonia, and bloodstream infections.
- Vancomycin-resistant Enterococcus (VRE) - one of the last-resort antibiotics used to treat serious infections.
- Fluoroquinolone-resistant bacteria - This includes strains of E. coli, Salmonella and Campylobacter that are resistant to fluoroquinolones, commonly used to treat urinary tract infections, diarrhoea, and respiratory infections



Antimicrobial Consumption:

The overuse and misuse of antimicrobials are major drivers of AMR. This includes:

- Using antibiotics for viral infections, which are ineffective.
- Taking antibiotics for an incomplete course of treatment.
- Using antibiotics for non-medical purposes, such as promoting growth in livestock.



Impact of AMR:

The consequences of AMR include:

- Increased morbidity and mortality from infections.
- Longer hospital stays and higher healthcare costs.

- Limited treatment options for common infections.
- The emergence of untreatable "superbugs."

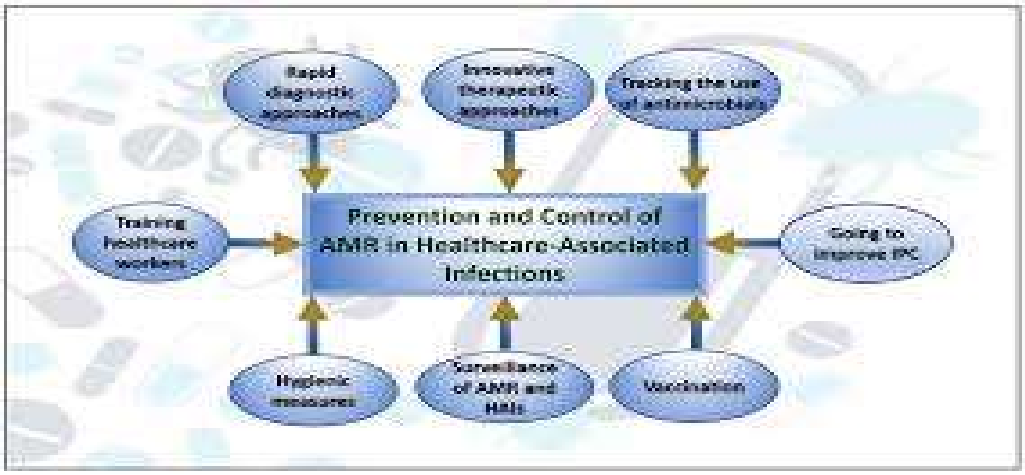
Clinical Impact:

Both drug resistant infections (DRIs) and hospital acquired infections (HAIs) pose significant challenges for patient care.

The clinical impact are-

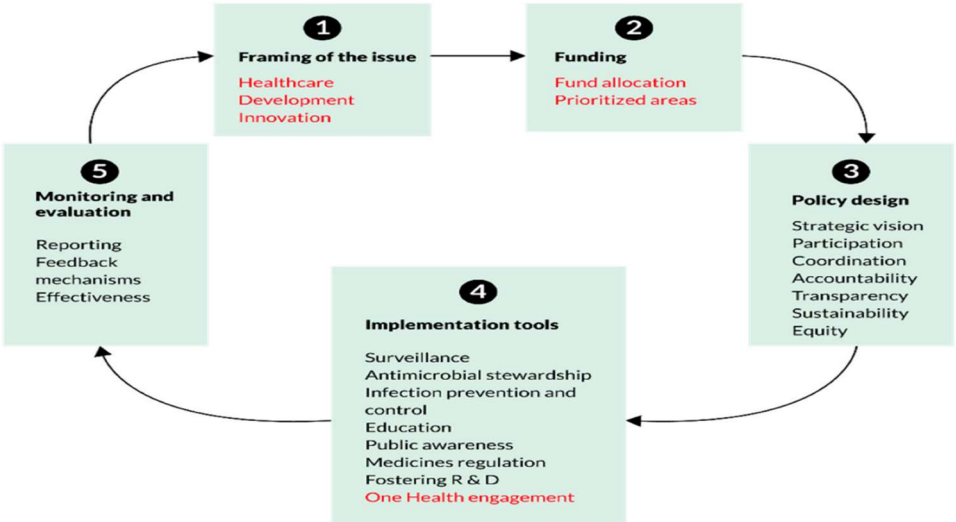
- Drug resistant infections
- Increased mortality: DRIs are associated with higher death rates, because effective treatment options become limited, and alternative drugs may have lower efficacy or more severe side effects.

- Treatment delays: due to delay in diagnosis worsen the course of the infection.
- Limited treatment options: for DRIs, effective antibiotics may be scarce or unavailable or having serious side effects.
- Increased length of hospital stay
- Hospital acquired infections
- Increased mortality compared to community-acquired infections due to compromised immune status of the patient making them more susceptible to severe complications.
- Antibiotic resistance: the frequent use of antibiotics in hospitals selects for resistant strains, making it harder to treat future infections.
- Increased complications like pneumonia, sepsis, and organ failure.
- Psychological impact in form of anxiety, stress, and depression in patients, impacting their overall well-being.



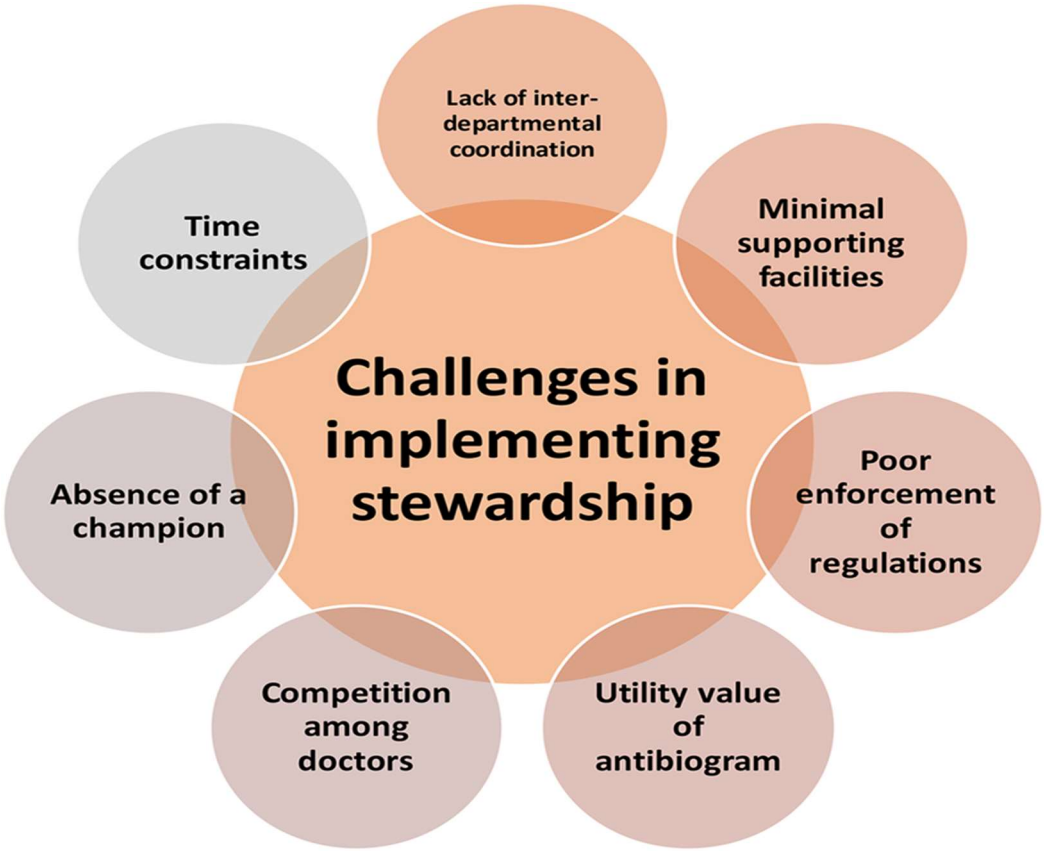
Developing Clinical Guidelines and Protocols

Establishing and updating clinical guidelines and protocols based on the latest diagnostic evidence can support effective stewardship. These guidelines should outline appropriate testing procedures, interpretation of results, and treatment recommendations.



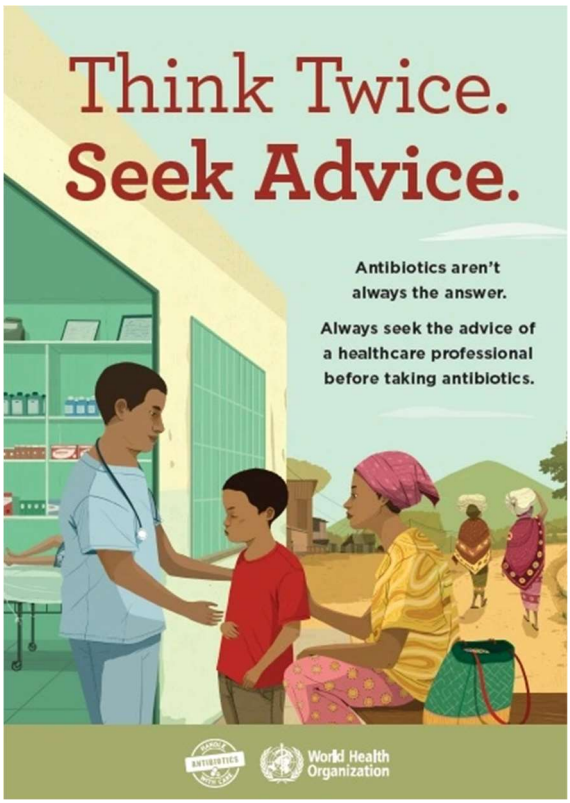
Implementing Stewardship Programs

These programs should include mechanisms for monitoring and evaluating diagnostic practices, providing feedback, and promoting adherence to best practices. Engaging multidisciplinary teams, including microbiologists, pharmacists, and clinicians, can enhance the effectiveness of stewardship programs.



Conclusion

Diagnostic stewardship plays a crucial role in combating antimicrobial resistance by improving the accuracy and appropriateness of diagnostic testing and subsequent treatment decisions. By reducing unnecessary antimicrobial use, enhancing targeted therapy, and preventing diagnostic errors, diagnostic stewardship contributes to better patient outcomes, cost savings, and overall public health. Through the promotion of rapid diagnostic technologies, education and training, development of clinical guidelines, and implementation of stewardship programs, healthcare systems can enhance their ability to combat AMR and protect global health.



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EMERGING RESISTANCE OF ANTIPARASITIC AGENTS

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Introduction

Parasitic diseases have significant health, social, and economic impacts, particularly in tropical countries, with malaria and schistosomiasis causing around 1.1 million deaths and leading to high disability-adjusted life years; the absence of effective vaccines and drugs, along with growing drug resistance, exacerbates this burden.

Table-1: Causes and treatment of parasitic infections and infestations

Intestinal protozoan infections			
CONDITION	COMMON PATHOGENS	PRIMARY DRUGS	ALTERNATIVE DRUGS
1)Ameobiasis, symptomatic With diarrhea, Dysentery, or hepatic abscess	Entamoeba histolytica	Tissue agents: Metronidazole or Tinidazole Followed by Lumional Agents: Paromomycin or iodoquinol	
2) Ameobiasis, asymptomatic Cyst passer	Entamoeba histolytica	Paromomycin or iodoquinol	Diloxanide
3) Dientamoeba infection	Dientamoeba fragilis	iodoquinol	Tetracycline Metronidazole or Paromomycin
4) Giardiasis	Giardia intestinalis	Tinidazole or nitazoxanide	Paromomycin or Metronidazole
5) Cryptosporidiosis	Cryptosporidium parvum	Nitazoxanide	Antidiarrheal medication
6) Balantidiasis	Balantidium coli	Tetracycline	Metronidazole

Extraintestinal protozoan infections			
CONDITION	COMMON PATHOGENS	PRIMARY DRUGS	ALTERNATIVE DRUGS
Amebic 1)meningoencephalitis	Naeglaria fowleri	Amphotericin B, RIF, Miconazole, Mitefosine	
2)Trichomoniasis	Trichomonas vaginalis	Metronidazole or tinidazole	
3)Leishmaniasis	Leishmania species	1)Lipid Amphotericin B 2)combination: Lipid Amphotericin B + Mitefosine, Lipid Amphotericin B + Paramomycin, 3) Amphotericin B deoxycholate 4)Miltefosine 5)Paramomycin 6)Pentavalent Antimonials	Amphotericin B, Fluconazole
4)Trypanosomiasis (African sleeping sickness)	Trypanosoma brucei	Pentamidine	Suramin
5) Trypanosomiasis, American (chagas disease)	Trypanosoma cruzi	Nifurtimox	Benznidazole

Cestode infection			
CONDITION	COMMON PATHOGENS	PRIMARY DRUGS	ALTERNATIVE DRUGS
Beef tapeworm	Taenia saginata	Praziquantel	Niclosamide
Pork tapeworm	Taenia solium	Praziquantel	Niclosamide
Dog tapeworm	Dipylidium caninum	Praziquantel	Niclosamide
Dwarf tapeworm	Hymenolepis nana	Praziquantel	
Fish tapeworm	Diphyllobothrium latum	Praziquantel	Niclosamide
Cysticerosis	Larval T.solium	Praziquantel + dexamethasone	
Echinococcosis	Echinococcus granulosus	Percutaneous aspiration+ albendazole	

Trematode infections			
CONDITION	COMMON PATHOGENS	PRIMARY DRUGS	ALTERNATIVE DRUGS
Schistosomiasis	Schistosoma species	Praziquantel	Oxamniquine for schistosoma mansoni
Chinese liver fluke	Clonorchis sinensis	Praziquantel	Albendazole
Sheep liver fluke	Fasciola hepatica	Triclabendazole	Bithionol
Lung fluke	Parsgonimus westermani	Praziquantel	Bithionol

Intestinal Nematode infections			
CONDITION	COMMON PATHOGENS	PRIMARY DRUGS	ALTERNATIVE DRUGS
Ascariasis	Ascaris lumbricodes	Albendazole or mebendazole	Ivermectin; nitazoxanide
Capillariasis	Capillaria philippinensis	mebendazole	Albendazole
Pinworm infection	Enterobius vermicularis	Albendazole or mebendazole	Pyrantel pamoate
Hookworm infection	Ancylostoma Duodenale and Necator americanus	Albendazole or mebendazole	Pyrantel pamoate
Strongyloidiasis	Strongyloides stercoralis	Ivermectin	Albendazole
Whipworm infection	Tichuris trichura	Albendazole	Mebendazole or ivermectin

Extraintestinal Nematode infections			
CONDITION	COMMON PATHOGENS	PRIMARY DRUGS	ALTERNATIVE DRUGS
Cutaneous larva migrans	Ancylostoma braziliense	Albendazole	Ivermectin
Filariasis, lymphatic	Wucheria bancrofti; Brugia malayi and Brugia timori	Doxycycline 6-8 week then add diethylcarbamazine	Albendazole or ivermectin
Guinea worm	Dracunculus medinensis	Surgical removal	
Filariasis, cutaneous	Loa loa	Diethylcarbamazine	Albendazole
River blindness	Onchocerca volvulus	Doxycycline followed by Ivermectin+prednisone	Suramin
Trichinosis	Trichinella spiralis	Albendazole + prednisone	Mebendazole+ prednisone

Ectoparasite infestations			
CONDITION	COMMON PATHOGENS	PRIMARY DRUGS	ALTERNATIVE DRUGS
Head lice, crabs	Pediculus humanus, Phthirus pubis	Permethrin, Spinosad (head lice)	Malathion,ivermectin
Scabies(mites)	Sarcoptes scabiei	Permethrin	Ivermectin

Antiparasitic Drugs and their Mechanisms of Action

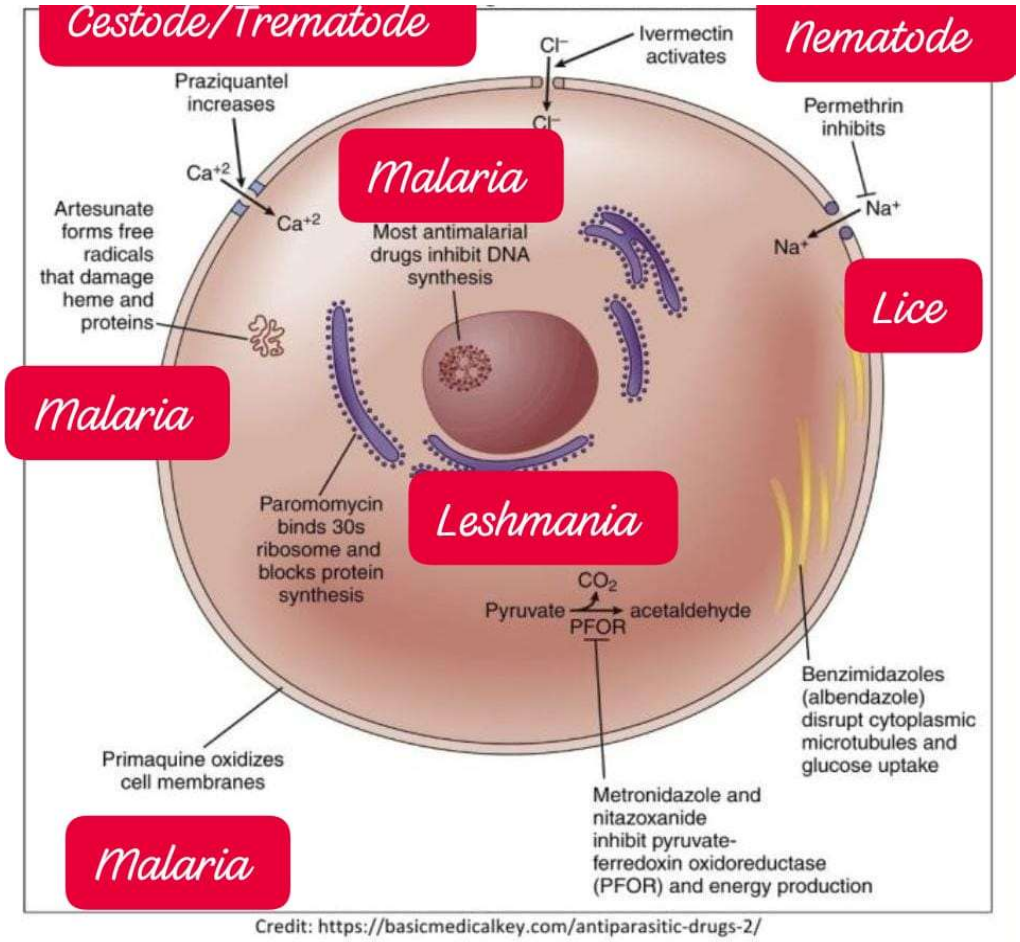

Figure-1: Antiparasitic Drugs and their Mechanisms of Action

How risky parasitic infections are?

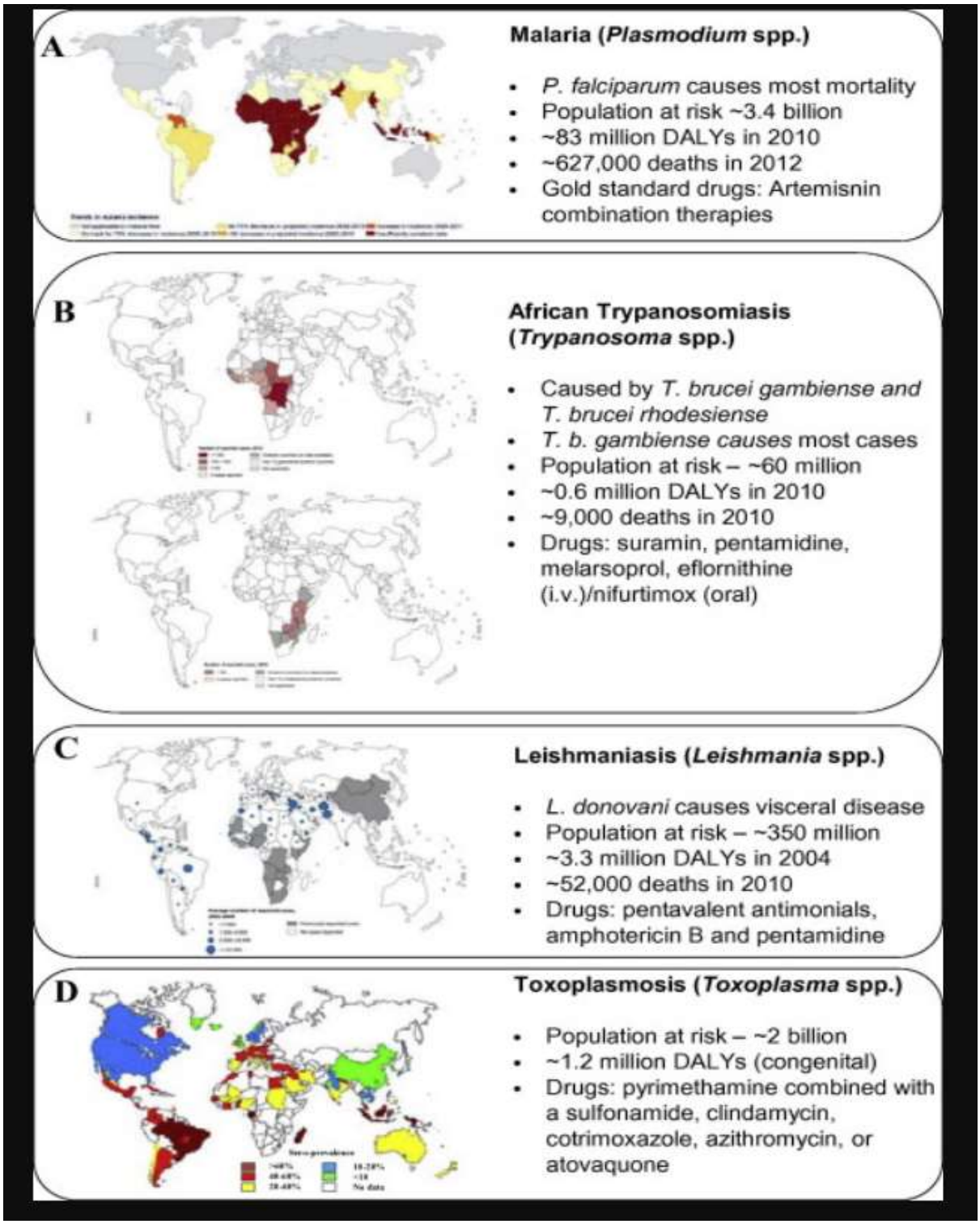

Figure-2: Andrews KT, Fisher G, Skinner-Adams TS. Drug repurposing and human parasitic protozoan diseases

Why do resistance emerge in antiparasitic agents?

- Frequent treatment
- Underdosing
- Genetics of parasite
- Target and timing of mass treatment in some parasites

What are the emerging antiparasitic drug resistances?

Malaria

Malaria, a serious protozoan infection, is caused by five Plasmodium species (P. falciparum, P. vivax, P. ovale, P. malariae and P. knowlesi) and affects billions worldwide. Antimalarial drugs are mainly divided into three groups according to their mechanisms of action: quinolone, antifolates, and artemisinin derivatives.

History of drug resistance

Global antimalarial drug resistance has affected P. falciparum, P. vivax, and P. malariae, with notable increases in resistance to chloroquine in Africa during the 1980s. This resistance, linked to mutations in the Pfcrt gene, led to a shift to sulphadoxine/pyrimethamine (SP), which also faced growing resistance by the early 2000s. To counteract this, combination therapies were introduced, and while artemisinin-based treatments are now effective, resistance in Southeast Asia poses a significant threat to global malaria control efforts.

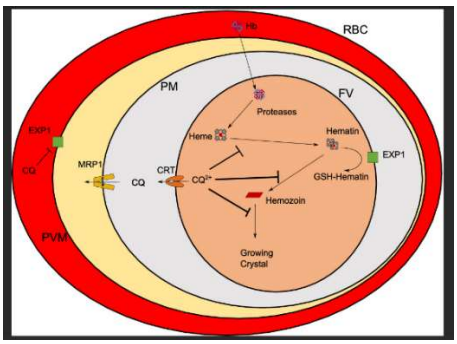

Figure 3. modes of action of chloroquine against Plasmodium falciparum parasites [3]

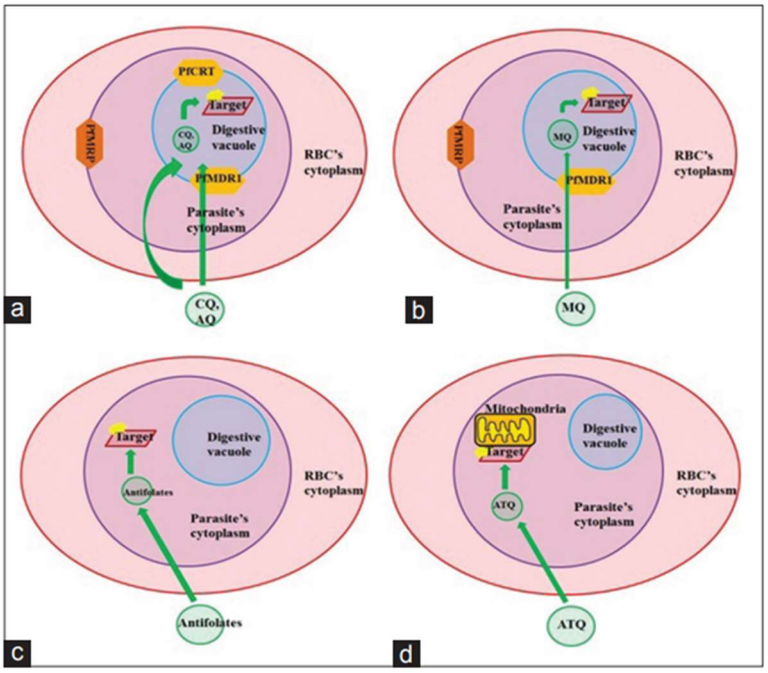

Figure 3: Proposed model for the mechanism and target localization of the antimalarial drugs (a and b) the 4-aminoquinolones such as chloroquine and amodiaquine and the amino alcohol derivatives such as mefloquine, quinine binds to the β-hematin molecule and inhibits the heme detoxification pathway in the parasiteæs digestive vacuole. (c) The antifolate derivatives target the *Phdhps* and *Plasmodium falciparum* bifunctional dihydrofolate reductase-thymidylate synthase gene involved in the folate biosynthesis in the cytoplasm of the parasite. (d) Atovaquone binds to the cytochrome b and interferes the electron transport mechanism in the mitochondria of the parasite

Figure 4: mechanism and target localization of the antimalarial drugs [9]

Antifolate drugs target the P. falciparum enzymes dihydropteroate synthase (Pfdhps) and dihydrofolate reductase-thymidylate synthase (Pf-dhfr-ts), crucial for folate synthesis, with mutations in these genes leading to reduced drug sensitivity. Artemisinin resistance has been linked to mutations in the kelch 13 (K13) protein, as revealed by whole-genome sequencing of resistant parasites. Additionally, the Pfmdr1 gene on chromosome 5, which encodes a protein similar to PfCRT and involved in ATP binding in the parasite's digestive vacuole, harbors mutations like N86Y, Y184F,

S1034C, N1042D, and D1246Y. Among these, N86Y and N1042D are specifically associated with resistance to drugs such as chloroquine, quinine, mefloquine, halofantrine, lumefantrine, and artemisinin.

Mutations in the Pfcrt gene (K76T and A220S) and Pfmdr1 gene (N86Y) are linked to high chloroquine resistance, while variations in Pfmdr1 copy number affect resistance to several drugs. The Pfmrp gene, also involved in drug transport, has mutations (Y191H and A437S) associated with chloroquine and quinine resistance. The Pfnhe1 gene on chromosome 13 is linked to quinine resistance, and mutations in the Pfdhfr-ts and Pfdhps genes are associated with resistance to pyrimethamine and sulfadoxine, respectively.

Atovaquone resistance in P. falciparum is linked to mutations in the Y268N/S/C codon of the cytb gene, while increased chloroquine sensitivity in P. vivax is associated with the Y976F mutation in the Pvmdr1 gene. P. vivax resistance to mefloquine is related to the amplification of the Pvmdr1 gene, and mutations in the Pvdhfr gene are linked to pyrimethamine resistance.
How to reduce it?

Earlier detection of drug-resistant parasites in clinical isolates using molecular markers of drug resistance.

Assisting an effective national drug policy for malaria treatment.

Toxoplasmosis:

Toxoplasma gondii, the parasite causing toxoplasmosis, infects a wide range of vertebrates, with severe outcomes in immunocompromised individuals and congenitally infected fetuses. The three main T. gondii strains are Type I (highly virulent), Type II (avirulent), and Type III (avirulent).

Drug of choice

Treatment typically involves sulfonamide and pyrimethamine, which inhibit key enzymes necessary for parasite replication.

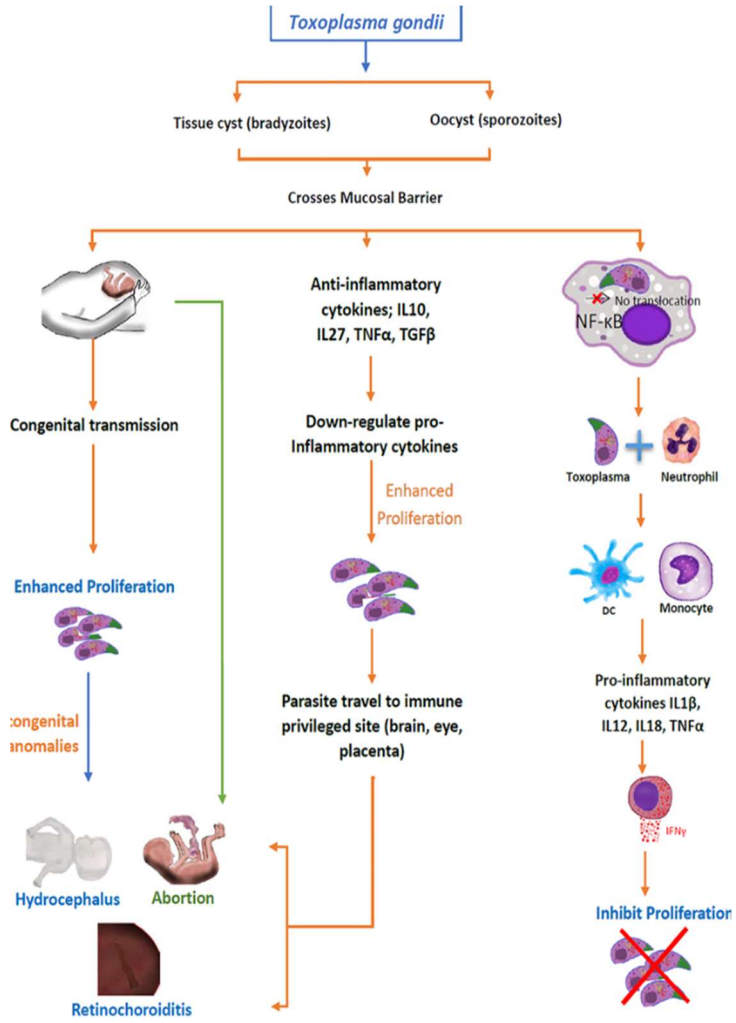

Figure 5: Outcome of toxoplasmosis [5]

Reason for treatment failures?

- Drug intolerance
- Malabsorption
- Resistance.

In vitro studies show no resistance to pyrimethamine or atovaquone, but some strains exhibit resistance to sulfonamide. Resistance mechanisms involve mutations in the dihydropteroate synthase (dhps) and dihydrofolate reductase (dhfr) genes, similar to patterns observed in Plasmodium species. Specific mutations in T. gondii's dhps gene, such as at position 407, have been linked to sulfonamide resistance, but no clear correlation was found between SDZ susceptibility and gene polymorphism.

Additionally, a 2017 study identified a mitochondrial protein, TgPRELID, associated with multiple drug resistance, highlighting the need for further investigation into its resistance mechanisms.

How to reduce drug resistance?

- Understanding mechanisms of drug resistance is essential for controlling the disease and it helps identify targets that are crucial to the parasite and predicts which combinations of drugs should act synergistically.
- Establishing a more effective therapeutic scheme in the treatment of toxoplasmosis, particularly among high-risk individuals is critically needed.
- Monitoring the presence of resistant parasites, particularly in food products, would thus seem a prudent public health measure

Leishmaniasis

Leishmaniasis, a vector-borne disease found worldwide except Antarctica, has various forms, including cutaneous, mucocutaneous, and visceral leishmaniasis. Sodium stibogluconate and meglumine antimoniate have been the primary treatments for leishmaniasis for over 50 years, but resistance has emerged in South America, Europe, the Middle East, and India. In Bihar, India, treatment with pentavalent antimony compounds has failed in 60% of cases. Alternative treatments include amphotericin B, pentamidine, and oral miltefosine, though reduced efficacy of miltefosine and resistance to meglumine antimoniate for cutaneous leishmaniasis have been reported in the Middle East.

Reason for resistance?

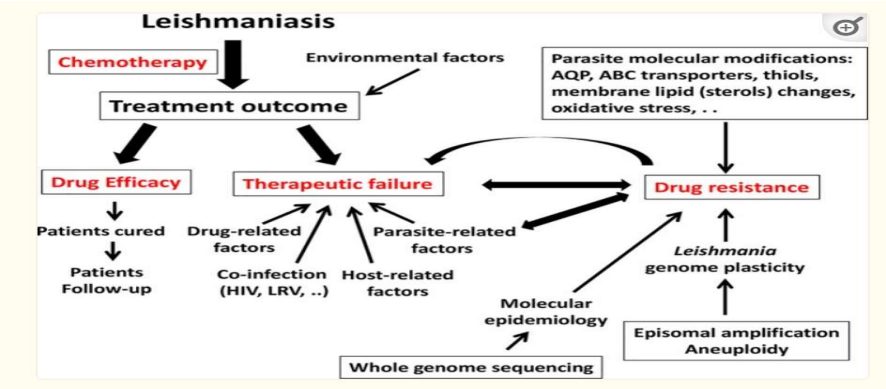

Figure 6: Factors that influence Therapeutic failure and Drug resistance [6]

Recent molecular studies have clarified resistance mechanisms in leishmaniasis, identifying key genes involved in drug resistance.

In L. tropica, genes such as ubiquitin and amino acid permease (AAP3) are implicated.

Other studies highlight five important genes:

- Aquaglyceroporin (AQP1) and
- ATP-binding cassette transporter (abc-3) for drug release
- Phosphoglycerate kinase (PGK) for metabolism
- Mitogen-activated protein kinase (MAPK) and
- protein tyrosine phosphatase (PTP) for phosphorylation.

In resistant isolates, multidrug resistance protein A, PTP, and PGK are upregulated, while AQP1 and MAPK are downregulated. Specifically, MAPK1 is linked to antimony resistance in L. donovani, and L. major MAP2 resistance is influenced by the AQ120 influx pump. Laboratory studies on L. panamanensis show increased abc-3 and decreased AQP1 expression in antimony-resistant strains, though this pattern varies in clinical isolates. Gene expression related to drug transport and metabolism plays a crucial role in resistance and susceptibility, making drug efficacy studies challenging due to the parasite's intracellular location and complex interactions with host cell compartments.

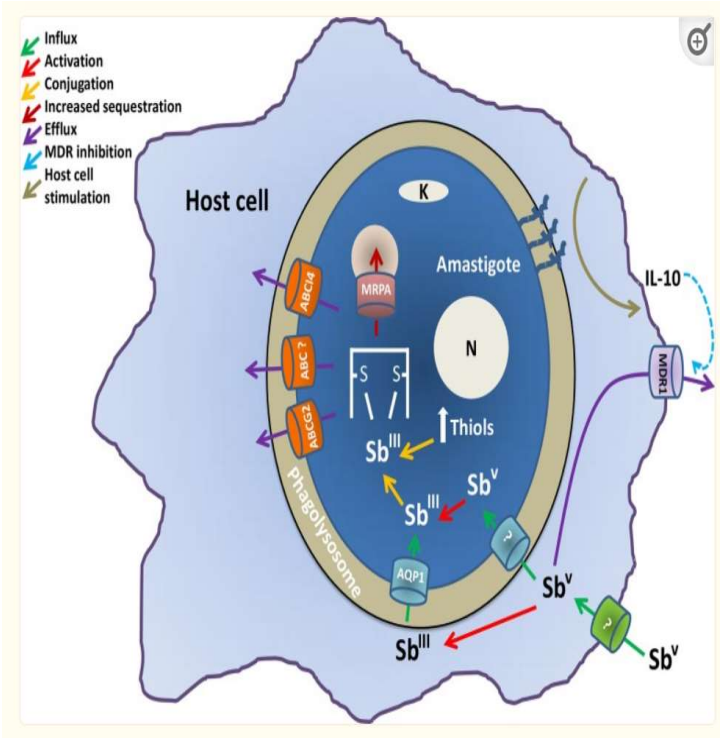

Figure 7: Molecular mechanisms of antimonial Drug resistance in Leishmania. [6]

Giardiasis

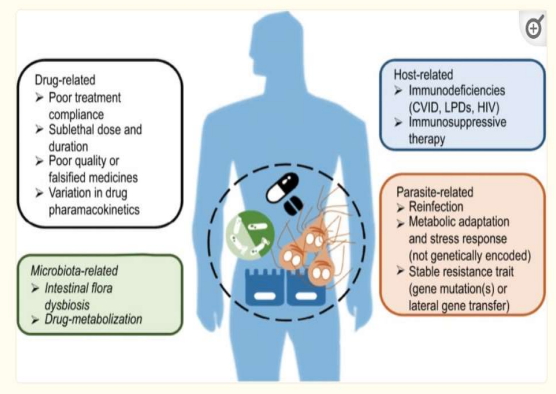

Figure 8: Overview of known and potential factors associated with clinical drug treatment failure of giardiasis. [7]

Giardia intestinalis can cause steatorrhea and symptoms like diarrhea and nausea, with resistance to metronidazole (MTZ) linked to mutations in the ferredoxin oxidoreductase gene.

Factors contributing to resistance

In amoebiasis caused by Entamoeba histolytica, MTZ is commonly used. Resistance involves altered reduction efficiency and decreased drug uptake. Trichomoniasis, caused by Trichomonas vaginalis, is often treated with MTZ, but resistance has emerged. recent studies are investigating genetic markers related to resistance.

Alternative treatment

Tinidazole and nitazoxanide

Nematode infections

Parasitic helminth infections, such as those caused by Ascaris lumbricoides, affect millions globally.

Underlying factors for resistance

Albendazole and mebendazole, used to treat these infections, may face resistance due to changes in β-tubulin genes. Resistance is common in isotype 1 β-tubulins but rare in isotype 2, and may also involve other mechanisms like drug metabolism. For Trichuris trichiura, increased frequency of a specific β-tubulin gene suggests it could be related to resistance development.

Ectoparasites

Head lice (Pediculus humanus var. capitis), body lice (P. humanus var. corporis), and pubic lice (Phthirus pubis) are blood-feeding ectoparasites treated with topical insecticides like pyrethrins, pyrethroids, organophosphates, and carbamates.

Reason for resistance?

Resistance to these treatments is increasing, with genetic mutations in the voltage-sensitive sodium channel (VSSC) gene, such as M815I, T917I, and L920F. For scabies caused by Sarcoptes scabiei, permethrin is commonly used, but resistance has developed due to prolonged use, with SNPs in the VSSC gene, especially at codon 733, linked to decreased susceptibility.

Conclusion

Earlier detection of drug-resistant parasites in clinical isolates, will aid in employing immediate and appropriate treatment that in turn reduces treatment failure and thereby mortality, and also prevents the spread of resistance.

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DIAGNOSTIC CHALLENGES DUE TO EMERGING ANTIFUNGAL RESISTANCE

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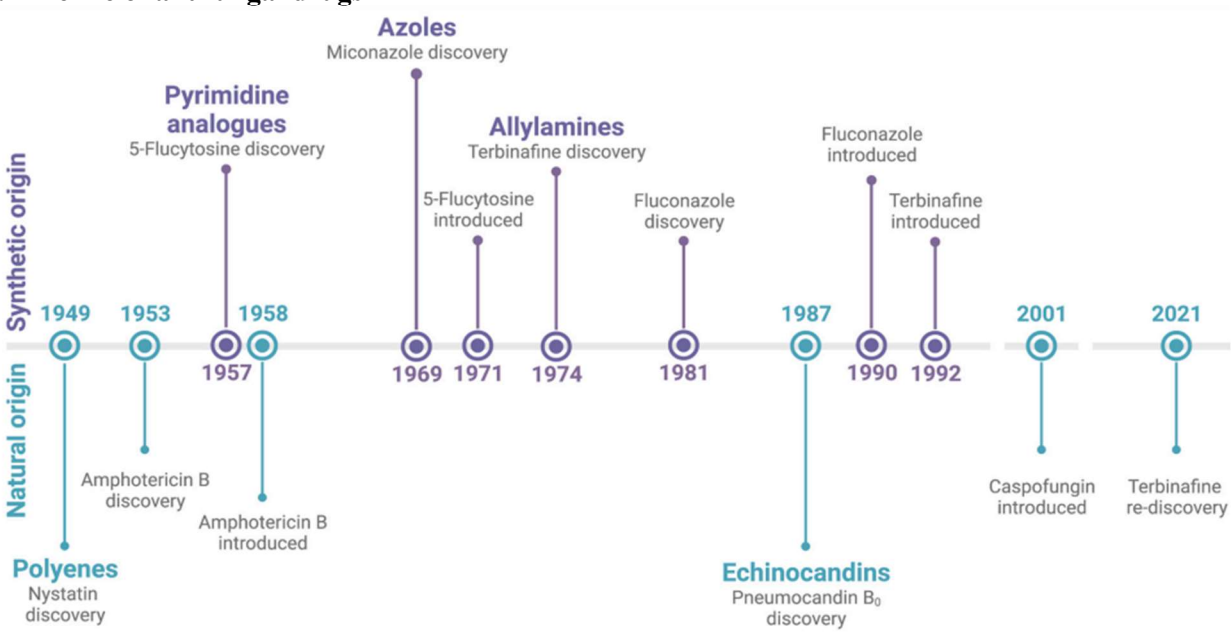
1.Introduction

Fungi encapsulates wide-range of diversity. Diagnosing fungal infections remains challenging due to the unique characteristics of fungi and their relative rarity compared to bacterial infections. Reports on emerging pathogens, in addition to the antifungal drug resistance amongst emerged fungal pathogen are on increase due to altered pathogenicity and availability of new biological niches. The Global Antimicrobial Resistance Surveillance System (GLASS-FUNGI) highlighting the growing concern of antifungal-resistant infections is crucial for gathering and consolidating global data on antifungal resistance.

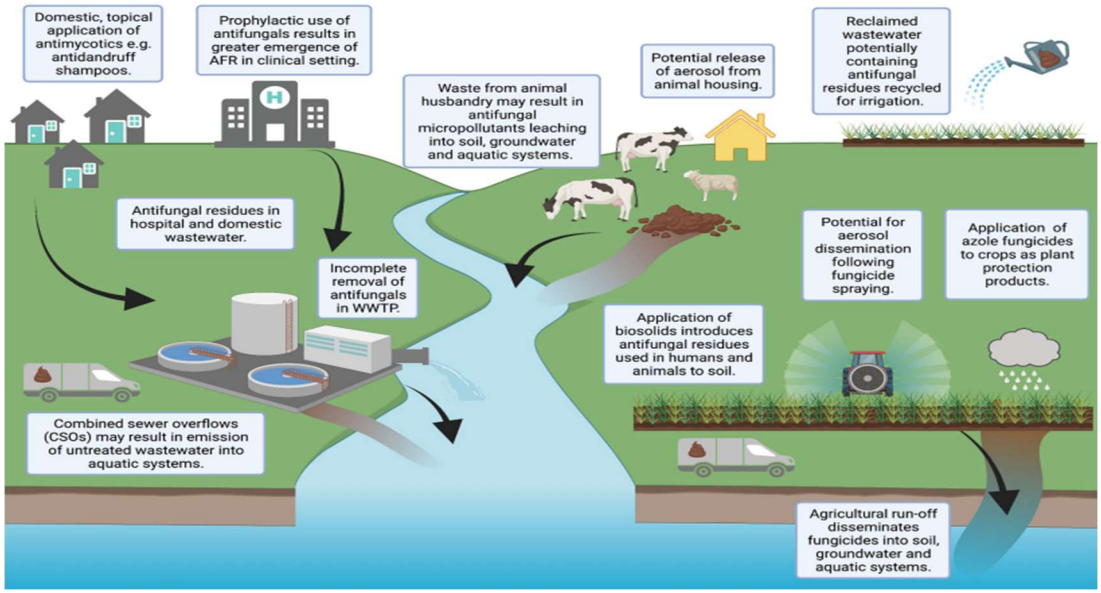
2.Timeline of antifungal drugs



Essential antifungal agents as assessed by WHO are Griseofulvin, Fluconazole, Amphotericin B, Flucytosine, Itraconazole, Voriconazole, Natamycin 5% and Echinocandins. Though fungal disease is fought with four antifungal drug classes- azoles, echinocandins, polyenes and pyrimidine analogues , yet clinicians are losing the battle because of rising drug resistance and tolerance.

3.Antifungals usage in the clinic and the field

The global movement of people, urbanization and global trade have hastened the free flow of fungal pathogens from country to country. The 'Plastisphere'- might favor intraspecies interactions and increase natural resistance evolutive pressure.



4.Emerging Antifungal Drug Resistance in Previously Susceptible Pathogens

Species	Azoles	Echinocandins	Polyenes	5-flucytosine	Allylamines
Aspergillus fumigatus	*Cyp51A* amino acid substitutions (L98H, M220, G54, Y121F/T289A) *Cyp51A* promoter tandem repeats (TR_{34}, TR_{46}, TR_{53}, TR_{120})	*FKS1* amino acid substitution (S678P)	*ERG3* amino acid substitutions	pH-dependent down-regulation of *FCY1*	
Candida auris	*ERG11* amino acid substitutions (Y132F, K143R, F126L)	*FKS1* amino acid substitutions (S639F/P/Y, F635C)	*ERG6* indel mutation	*FUR1* amino acid substitutions (F211I) *FUR1* partial deletion *FCY1*/*FCY2* amino acid substitutions (S70R, M128fs)	
Trichophyton indotineae	*ERG11* promoter tandem repeats (hypothesised) *Cyp51B* overexpression *ERG1* amino acid substitution (A448T)				*ERG1* amino acid substitutions (F397L, L393F, F397L/A448T)

Table-1: Known drug resistance mechanisms for emerged and emerging fungal pathogens.
Azoles were effective drugs against Aspergillus fumigatus. However, in last 2 decades there is rise in prevalence of azole- resistance. Surprisingly two-thirds of patients have no history of azole intake suggesting environmental route of acquisition.

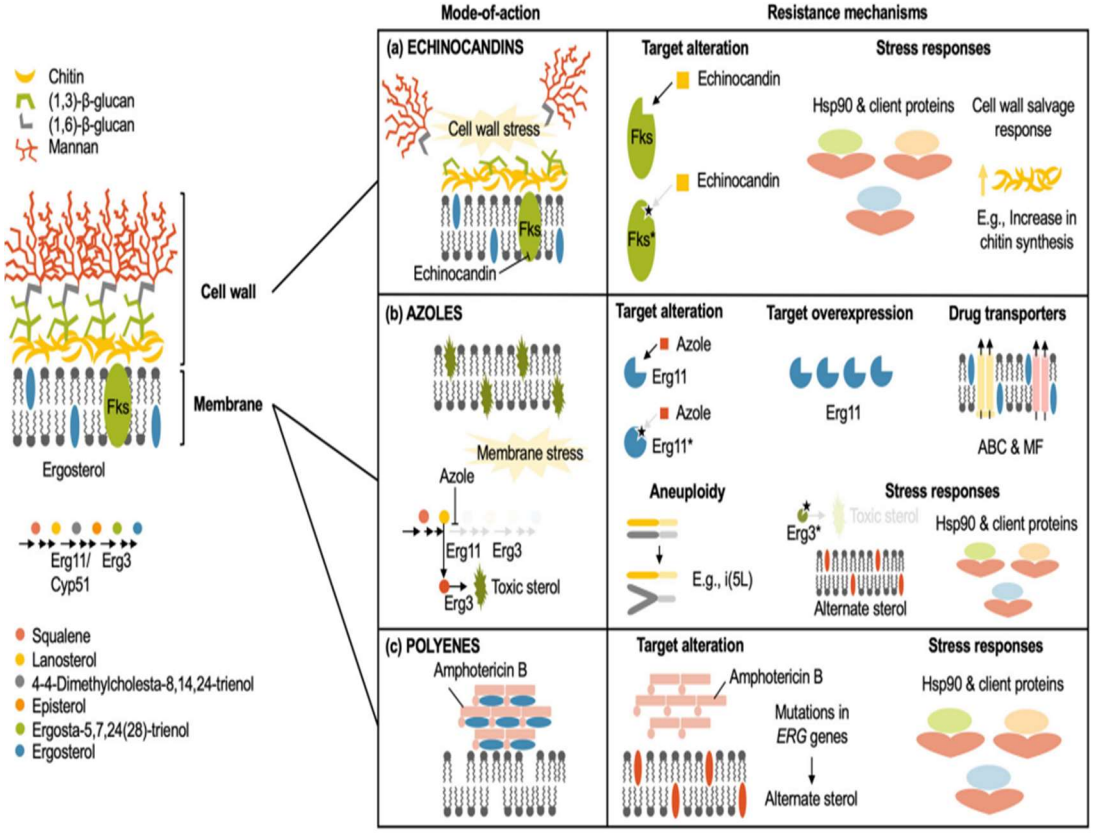
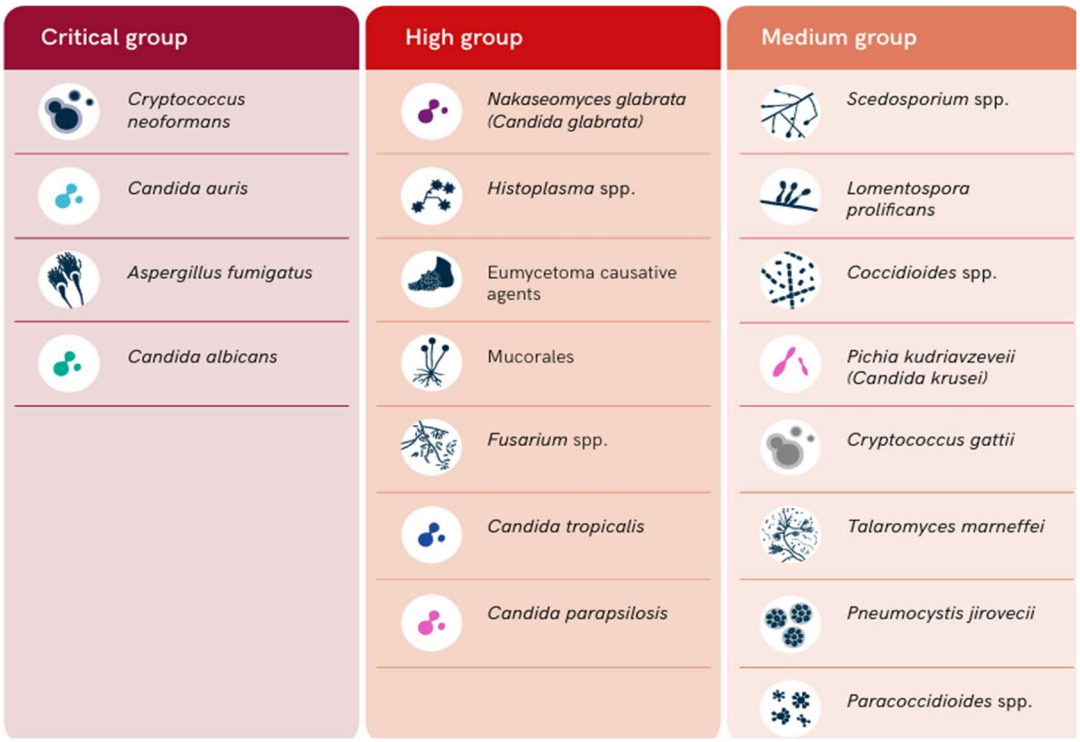


Figure- Antifungal mode-of-action and mechanisms of resistance.

5.Emerging Drug-Resistant Fungal Pathogens

First described in Japan (2009), C.auris has since been reported in over 40 countries, showing high level fluconazole (FLC) resistance and varying levels of other antifungal resistance.
Trichophyton indotineae, has become a significant health concern, especially in India, due to its high infection rates and shows high resistance to terbinafine (TERB).
Candida africana, an emerging pathogen associated with vulvovaginal candidiasis, shows low current resistance but has reported changes in drug susceptibility.

6. WHO Fungal Priority Pathogen List (FPPL)- 2020



7.Diagnostic Challenges

Microscopic examination and cultures have lower accuracy and reproducibility compared to newer methods like molecular and immunological assays. However, information and resources for these newer techniques are limited, with suboptimal sensitivity and specificity.

AFST for Pneumocystis jirovecii is impractical due to its inability to grow in culture.
While conventional sequencing methods like Sanger sequencing and real-time PCR are limited in scope, mass spectrometry (MS MALDI-TOF) offers quick results but lacks universal standards.
Identifying Candida auris is challenging due to its genetic similarity to Candida haemulonii, leading to frequent misdiagnosis.

Patient variability in symptoms, infection severity, and underlying health conditions complicates diagnosis and treatment.

7.1 In Vitro and In Vivo correlation of drug resistance

Evaluating fungal resistance in vitro is challenging due to conditions that don't accurately mimic in vivo environments. Opportunistic mold infections, especially in the lung, often involves low inoculum, poor substrates, and semi anaerobic conditions, and frequently form biofilms.

7.2 Are known antifungal resistance determinants identifiable through genome sequencing?

Whole-genome sequencing (WGS) offers valuable insights for routine analysis.

Illumina NGS technology can effectively detect point mutations, small indels, premature stop codons, and gene duplications.

Tools like OVarFlow and SnpEff assist in analyzing these variations, while fungal-specific tools like YMAP are also available.

Large chromosomal changes are more challenging but can be evaluated using tools like PerSVade and Assemblytics.

7.3 Antifungal resistome predictors from research to clinical use

Cost Efficiency: Optimizing the number of samples per NGS run and ensuring high genome coverage with minimal contigs are crucial. Cost-effective options like nanopore flongle cells or multiplexing in NGS-TGS hybrid approaches, and targeted resequencing, can help manage expenses.

Turnaround Time: In-house sequencing facilities can reduce delays. Nanopore technologies, which are relatively inexpensive and allow simultaneous sequencing of up to 48 cells.

Bioinformatics: High bioinformatics expertise is required. Training for laboratory staff and user-friendly online resources like Pathogenwatch2, Microreact, and Galaxy can improve analysis.

7.4 Challenges and Future Directions in Fungal Resistome Prediction:

Incomplete Understanding: The understanding of antifungal resistance mechanisms is limited, with current knowledge largely focused on *C. albicans*. Other pathogens like *A.fumigatus* are less studied.

Genomic Complexity: Fungal resistance often involves polygenic traits, chromosomal changes, and complex pathways, requiring advanced algorithms and high-quality genomic data.

Technological Limitations: While NGS (Next generation sequencing) and TGS (Third generation sequencing) provide valuable data, their cost and turnaround time can be prohibitive. Nanopore technologies offer a cost-effective solution but require careful calibration.

Data Integration: Effective predictors need to integrate diverse data types (raw reads, assemblies, long reads) and handle various genetic events. Standardized frameworks and comprehensive databases are essential.

Operational and Financial Constraints: Routine incorporation of these predictors into clinical settings may be uneven due to high costs and the need for specialized bioinformatics expertise.

Roadmap for Progress:
Literature Review: Utilize resources like FunResDb and MARDy to identify known resistance determinants.
Laboratory Screenings: Map mutations associated with resistance.
Database Updates: Maintain and expand resistance databases.
Pipeline Development: Build and refine prediction algorithms.

7.5 Diagnostic Methods

Name	Measuring principle	Assay time	Advantages	Disadvantages
		Standard methods		
Broth microdilution (BMD)	Visual or spectrophotometric measurement of turbidity in a liquid medium	24 h for *Candida* species, 46–50 h for *Aspergillus* species and up to 72 h for *Cryptococci*	Standardized by EUCAST and CLSI Available for yeasts and molds Cheap when prepared in the laboratory	Laborious and lengthy Subjective when read visually
Disk diffusion	Zone of inhibition around antifungal disks on agar plates	24 h for *Candida* and *Aspergillus* species	Standardized by CLSI Available for yeasts and molds Easy to perform and cheap	Lengthy No MIC values
Agar screening	Visual detection of growth in a liquid medium	48 h	Standardized by EUCAST Cheap, easy to perform, and read	Lengthy No MIC values Only screening of *Aspergillus* species
		Commercial adaptations		
Vitek2 (bioMérieux, France)	Turbidity in a liquid medium	Usually 12–18 h	Automated Objective results Accelerates AFST compared to BMD	High investment costs Only available for yeast
Sensititre YeastOne (Thermo Fisher Scientific, USA)	Colorimetric detection in a liquid medium	24 h	Easy to perform Less subjective than BMD	Lengthy Not validated for molds
Etest (bioMérieux, France)	Zone of inhibition around a strip with an antifungal gradient	24–48 h for yeast (up to 72 h for *Cryptococcus* species) and 16–72 h for molds	Easy to use Available for yeast and molds An agar-based method that provides MICs	Lengthy Sometimes subjective

Table-2: Summary of standard AFST methods and their commercial adaptations

7.6 Bottlenecks in Molecular Detection

Rather than detecting, we should uncover gene mutations first.
Different mechanisms involving different mutations are responsible for similar phenotypes.
Must evaluate overexpression rather than just diagnosing presence of mutations.
No universal primers, so must identify etiological agent before detecting resistance mechanisms.
Can detect only known mechanisms, but in phenotypic methods all mechanisms can be detected.

Name	Measuring principle	Assay time	Advantages	Disadvantages
Mass spectrometry	Shifts within mass spectral profiles	15 h incubation for minimal profile changing concentrations (MPPC) 3 h for differentiation between susceptible and resistant isolates	Rapid differentiation of susceptible and resistant isolates Different approaches possible (e.g., whole-cell profiles, antibiotic degradation analysis)	High investment costs for instrumentation Arduous workflow
Flow cytometry	Fluorescence signal to differentiate dead and viable cells	1–9 h exposure of fungal cells to antifungals	Rapid AFST Demonstrated for yeast and molds	Labor-intensive workflow and required technical expertise
Calorimetry	Heat flow related to fungal metabolism	24 h for *Candida* species and 48 for *Aspergillus* species	Available for yeast and molds	Does not accelerate AFST compared to reference methods
Fluorescence microscopy	Fluorescence microscopy analyzes microcolony area on porous aluminum oxide	3.5–7 h for various *Candida* species and 14 h for *Aspergillus* species	Rapid AFST	Arduous workflow including culturing, staining, and microscopy
On-chip optical assays	Intensity changes of light reflected from a silicon diffraction grating	10–12 h for *Aspergillus niger*	Rapid compared to reference BMD Label-free and real-time monitoring	Only demonstrated for *Aspergillus niger*

Table-3: Overview of new AFST methods

7.7 Xtreme Challenges

Template preparation-
Fungal diagnostic assays face significant challenges in template preparation due to rigid cell walls. In clinical samples, fungal elements are often sparse and can be overwhelmed by host material like tissue or body fluids. Moreover, fungal cell wall components, can inhibit PCR . Avoiding contamination is challenging due to the presence of environmental molds which could be pathogens too.

Assay scope-
Focusing on common pathogens would cover most human systemic fungal infections but might be less useful in regions where these fungi are rare. Conversely, rare but life-threatening infections, like mucormycosis, require rapid identification but may not justify the financial investment due to their rarity.

Fungal taxonomy and Nomenclature-
Shift to "one fungus, one name" system in fungal nomenclature simplified fungal naming. However, rapid advancements in molecular taxonomy has led to new species designations, thus complicating fungal identification.

Colonizer versus invader discrimination-
Many pathogenic fungi are also part of normal human flora. Distinguishing between normal colonization and invasive infection often requires quantitative assays.

Administrative Challenges-
Limited mycology training, a lack of suitable diagnostic alternatives, and difficulties in standardizing new tests challenges fungal diagnosis. Labs must consider factors such as personnel needs, cost, and geographic relevance, as certain fungi are regionally specific.

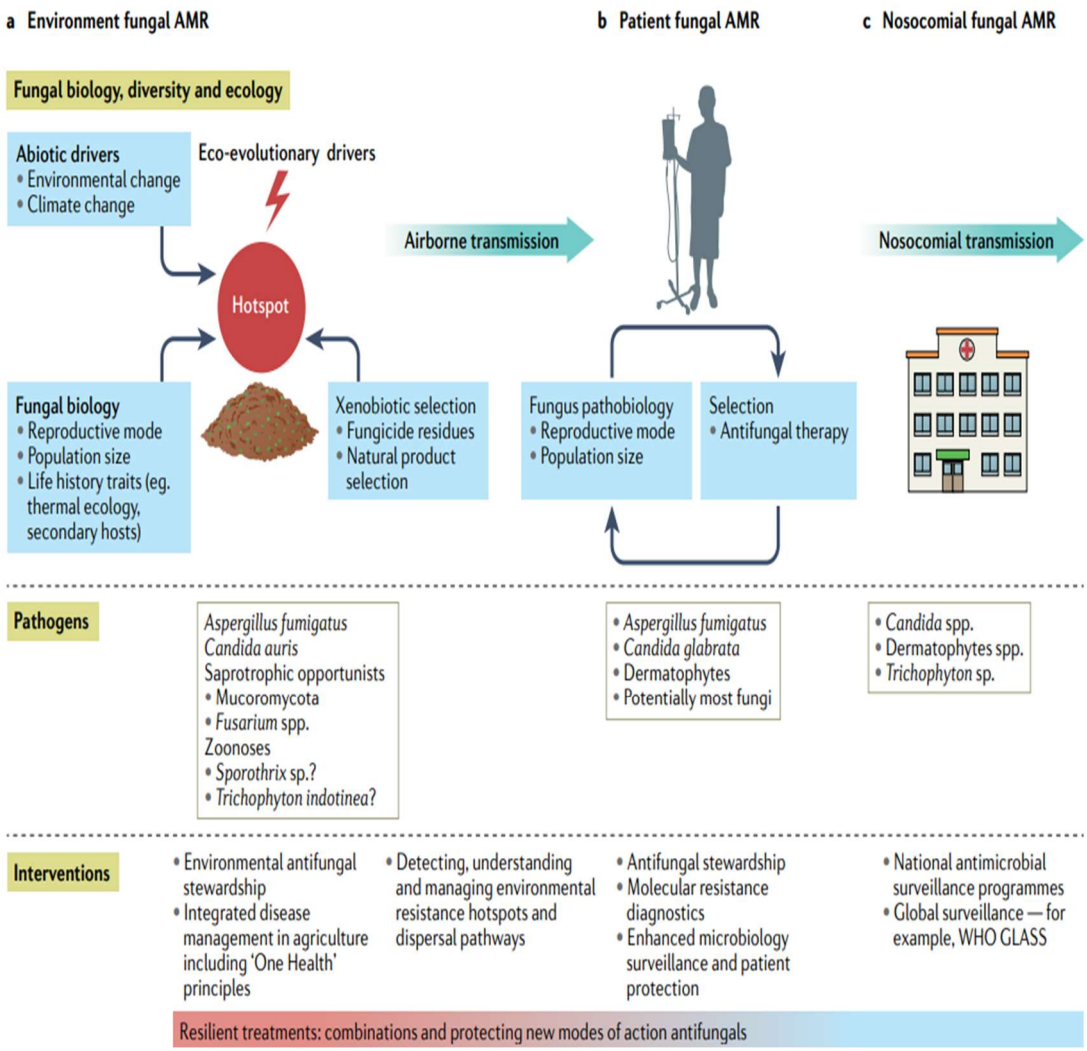
S.No	Format	Detected Mechanism	Points to consider
1.	PCR-RFLP	TR34-L98H [133] and TR34-L98H and TR46-Y121F-T289A	Good correlation with MIC but false negative (isolates harboring other mechanisms)
2.	Loop-mediated isothermal amplification (LAMP)	Detection of TR34 alone	Rapid (<25 min) High sensitivity.
3.	Classical PCR using astringent conditions	Detection of R65K mutation and TR34.	Low-cost detection of a triazole-cross resistance (TR34) and pan azole resistance (R65K)
4.	Whole-genome sequencing	P216L	Complex to perform in a clinical setting.
5.	AsperGenius multiplex real-time PCR assay	Detects intrinsic resistant species (A. terreus and cryptic species of the Fumigatii section) and the following secondary resistance markers separately: TR34, L98H, Y121F, and T289A.	Some cross-reactivity (false-positive results) were obtained when R. oryzae (R. arrhizus) and P. chrysogenum DNA is present in high concentrations.
6.	MycoGENIE multiplex real-time PCR assay	-	Possible false positive when aspergillosis is caused by non-Aspergillus fumigatus species.

Table-4:Molecular tools for detection of mechanisms of triazole resistance in Aspergillus spp.

S.No	Organism	Format	Detected Mechanism	Points to consider
1.	Candida glabrata	Asymmetric real-time PCR coupled with melting curve analysis	Echinocandin resistance. Melting curve analysis can differentiate different nucleotide substitutions in the same position (8 at FKS1 and 7 at FKS2).	100% concordance with sequencing.
2.	Candida albicans	Classical PCR with astringent	Echinocandin resistance.	96% sensitivity. It can detect all the homozygous mutants included. Heterozygous mutants give false susceptibility.
3.	Candida albicans, Candida glabrata and Candida parapsilosis	NGS	Azole and echinocandin resistance.	It gave the proof that it is possible to use NGS for extensive assessment of mutations responsible for antifungal resistance
4.	Candida auris	WGS	ERG11 mutations	Worldwide strains were divided into 5 clades and each clade showed differential FLC susceptibility

Table-5: Molecular tools for detection of azole and echinocandin resistance in Candida spp.

8. Strategies to combat:



9. Conclusion

Significant progress has been made in addressing antifungal resistance , but challenges persist. Overcoming these obstacles will require advancements in technology and innovative applications of existing methods. A One Health approach—integrating human, animal, and environmental health—is essential for effective management.

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