MICROBIOLOGY DIVISION, COE-NPT
Department of Biotechnology & Bioinformatics
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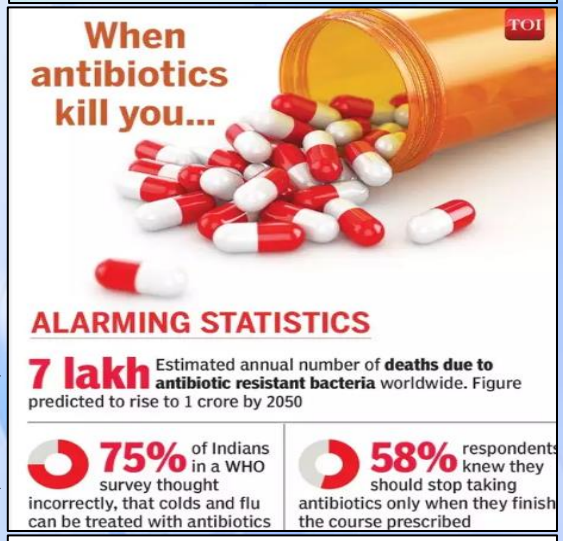
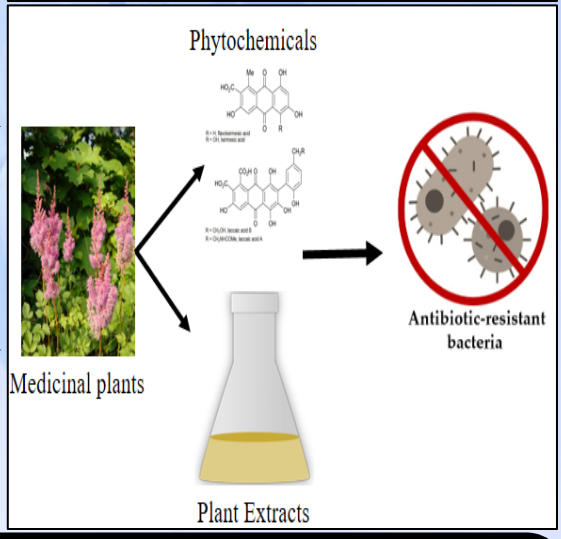
BACKGROUND

- Antibiotic discovery to treat chronic microbial infections has revolutionized the conventional therapeutic regimens.
- However, irrational and indiscriminate use of antibiotics leads to the emergence of antibiotic resistant bacterial pathogens.
- Among the priority pathogens with **multidrug resistance (MDR)** and **extensive drug resistance (XDR)** as enlisted by **World Health Organization (WHO)**, the ESKAPE group of pathogens (***Enterococcus faecium***, ***Staphylococcus aureus***, ***Klebsiella pneumoniae***, ***Acinetobacter baumannii***, ***Pseudomonas aeruginosa***, and ***Enterobacter* sp.**) exhibit catastrophic influence on antibiotic resistance by activation of **biofilms** and **efflux transporters**.
- Natural products in particular plant-derived phytochemicals have received considerable scientific attention due to their widespread **folkloric** & **pharmacological relevance** and thus could be instrumental in the management of antibiotic resistance in the post-antibiotic era.

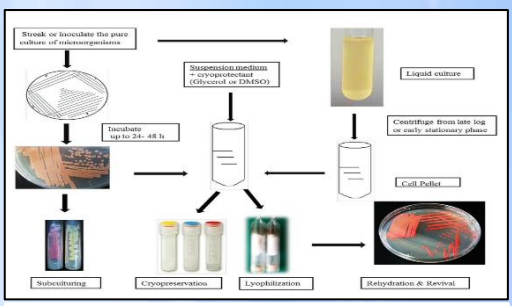




Microbiology Division
Supported by:
COE-NPT, DBT-BUILDER, DST-SERB

RATIONALE and OBJECTIVES

- To maintain and preserve the clinically isolated pathogenic bacterial strains for therapeutic purposes (**Biosafety Level-2**).
- To screen the **antimicrobial efficacy** of different extracts (extracted in different solvent medium) of medicinal plants collected from the **Gandhamardan Hills**, Odisha against the pathogenic bacterial strains (**12 Clinical strains**; **5 MTCC strains**).
- To evaluate the role of selected plant extracts in the management of drug resistance by targeting the pathophysiological phenomena such as **quorum sensing (QS)**, **biofilm dynamics**, and **efflux pump mechanisms** in pathogenic bacterial strains.
- To determine the **anti-infective** potential of selected phytochemicals derived from selected medicinal plants against **bacterial biofilms** and **efflux machinery**.
- To determine the **synergistic** potential of selected phytocompounds with antibiotics for improved **antibacterial** and **antibiofilm** activities against **ESKAPE** pathogens.



Maintenance of bacterial culture

Media Preparation and Sterilization

Culture Room (BSL-2 Facility)

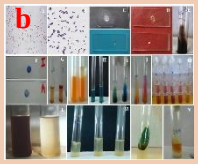
Bacterial Culture Plates

List of Clinical Bacterial strains		List of MTCC (Microbial Type Culture Collection) strains
Esherichia coli	*P. aeruginosa* PAO1	MTCC 740 (*Staphylococcus aureus*)
Enterococcus faecium	*Shigella* sp.	MTCC 2488 (*Pseudomonas aeruginosa*)
Klebsiella pneumoniae	*Salmonella typhi*	MTCC 109 (*Klebsiella pneumoniae*)
Pseudomonas aeruginosa,	*Chromobacterium violaceum*	MTCC 439 (*Enterococcus faecalis*)
Staphylococcus aureus	*Salmonella paratyphi A*	MTCC 736 (*Bacillus subtilis*)
Salmonella paratyphi B	*Shigella* sp.	

WORK PLAN and PROGRESS

1. Bacterial Characterization

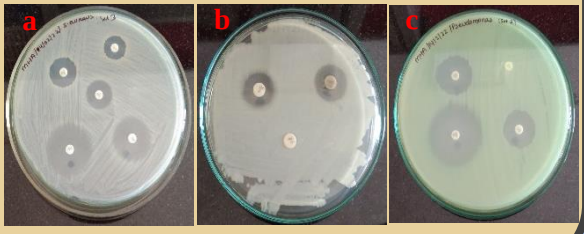
a. Morphological characterization
b. Biochemical characterization
c. Molecular characterization



2. Antibiotic susceptibility Assay (Disc Diffusion Test)

a. Gram Positive Antibiotics
b. Gram Negative Antibiotics
c. Common Antibiotics

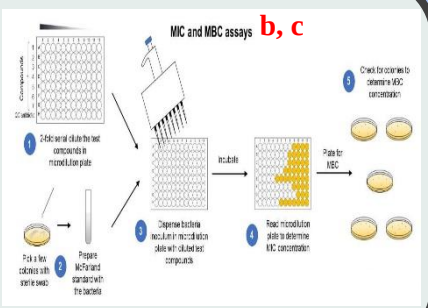
(0.5 McFarland Standard)



3. Antimicrobial Screening

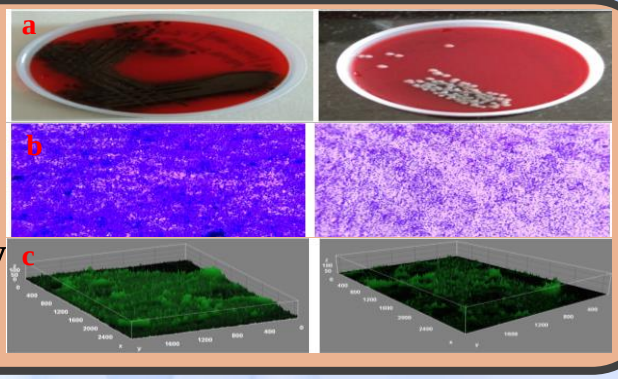
a. Agar well diffusion Test
b. Determination of MIC
c. Determination of MBC

(As per CLSI Guidelines)



4. Antibiofilm Activities

a. Congo Red Agar Assay
b. Light Microscopy
c. Fluorescent Microscopy



- Till date more than **180 medicinal plant extracts** were screened for their prospective antimicrobial activities against pathogenic bacteria.
- More than **15 medicinal plants** exhibited promising **antimicrobial** activities against MDR pathogenic bacteria.
- More than **10 medicinal plants** showed characteristic inhibitory potential against recalcitrant **bacterial biofilms**.

IN VIVO STUDIES and Urinary Tract Infections (UTIs) MODEL DEVELOPMENT

Plant extracts

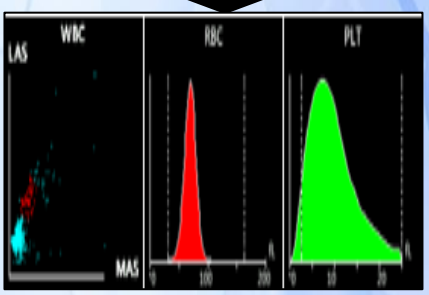
Acute Oral Toxicity of Plant Extracts (As per **OECD Guidelines**)

UTI Model Development (As per **Standard Guidelines**)

2000 mg/kg body weight of Plant extracts given orally, Single limit dose (***Cordia* sp. & *Homalium* sp.**)

No Death in 14 Days observations, **LD_{50}>Limit dose**

- **1/10^{th} of Limit dose** of Plant extracts given orally, everyday for 14 days infected with UTI causing bacteria (***Escherichia coli***) (**1.5×10^{8} CFU/ml**).
- Significant reduction in **CFU/ml** in Treatment group (***Cordia* sp. & *Homalium* sp**. treated) as compared to Negative control.



Haematological Analysis (CBC)

Histopathological Evaluation

- **No Acute toxicity** of Plant extracts given orally, was observed.
- Significant reduction in the bacterial load (i.e. **CFU/ml**) in Treatment group (***Cordia* sp. & *Homalium* sp**. treated) as compared to Negative control (UTI induction only).
- CBC & Biochemical parameters, Histopathological evaluation inferred the mitigation of UTI when treated with ***Cordia* sp. & *Homalium* sp**.