Emerging applications of probiotics: Antimicrobial resistance

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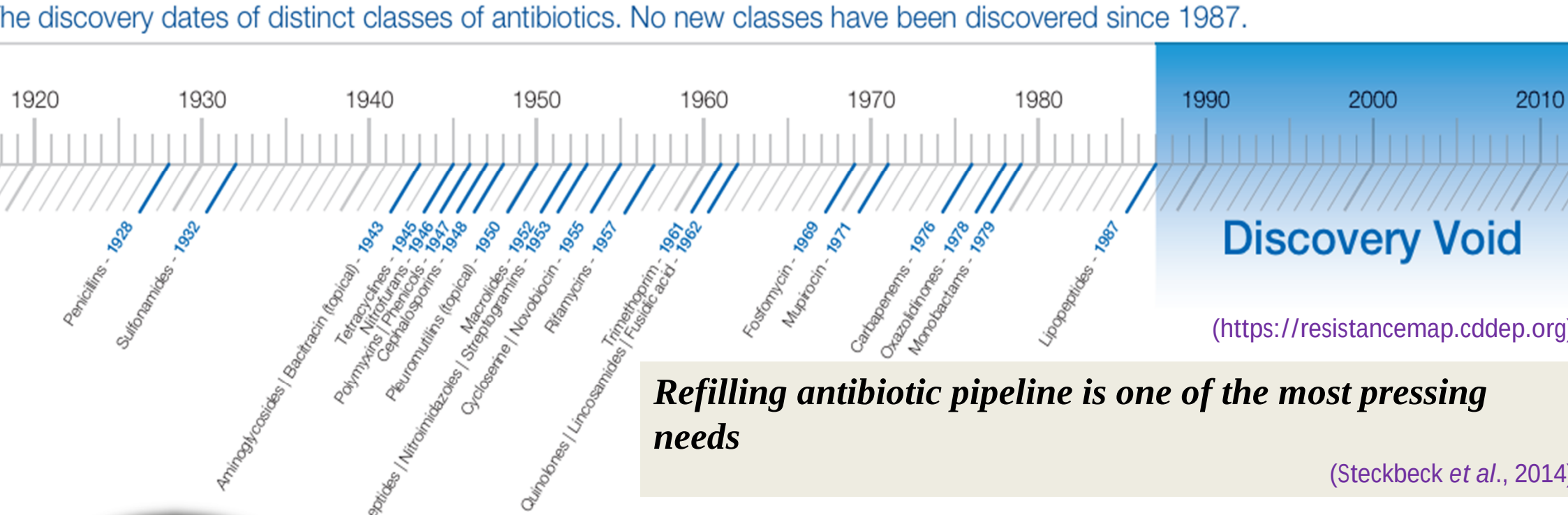
Antibiotic resistance: A lost clue may be a solution!



(https://resistancemap.cddep.org)

Refilling antibiotic pipeline is one of the most pressing needs

(Steckbeck *et al*., 2014)

A Visionary accurately predicted today's calamity !!

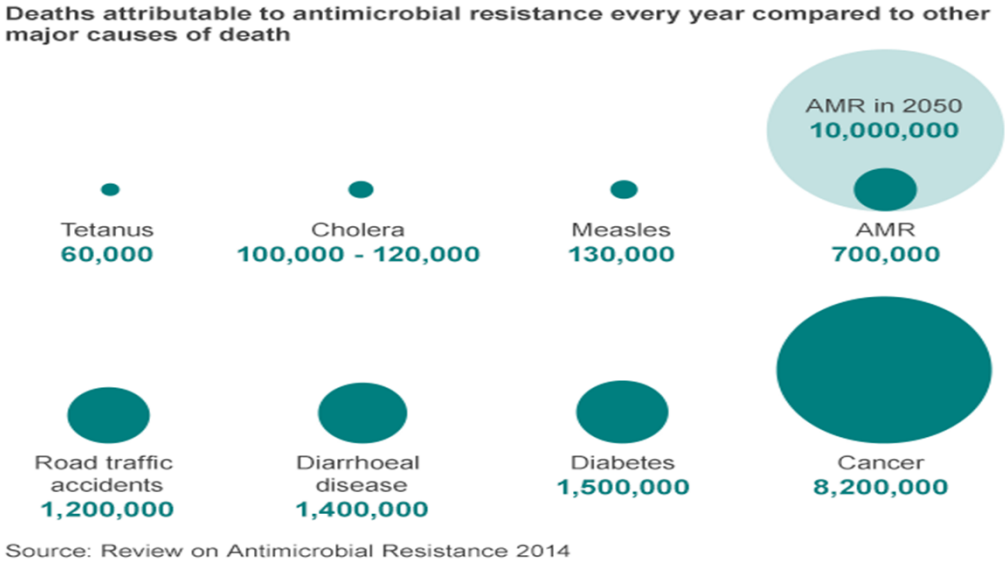
The time may come when penicillin can be bought by anyone in the shops. Then there is the danger that the ignorant man may easily under dose himself and by exposing his microbes to non- lethal quantities of the drug make them ***resistant .....***

(Sir Alexander Fleming's Nobel Prize Lecture, 11.12.1945)

Facts and Figures of AMR

- ✓ Worldwide antimicrobial resistance (AMR) is a major health concern **(Hay *et al.*, 2018)**
- ✓ Whole human and animal might face new **MDR epidemics at 2050**
- ✓ **GLASS** –suspect antibiotic resistance infection among **5,00,000** people across 22 countries **(WHO, 2018)**
- ✓ In **USA and Europe,** over 50,000 people die every year by antibiotic resistance infection **(Elena Villanueva , 2017)**
- ✓ In **INDIA** almost 60,000 newborn babies die every year by antibiotic resistance infection **(Laxminarayan *et al.*, 2016)**
- ✓ **Global antibiotic consumption in livestock** – In 2010: 63,200 tons ↑ 105,600 tons by 2030 (van Boeckel *et al.*, 2015)
- ✓ **"Antimicrobial resistance is a global health emergency that will seriously threaten progress in modern medicine" - Tedros A. Ghebreyesus, DG., WHO**

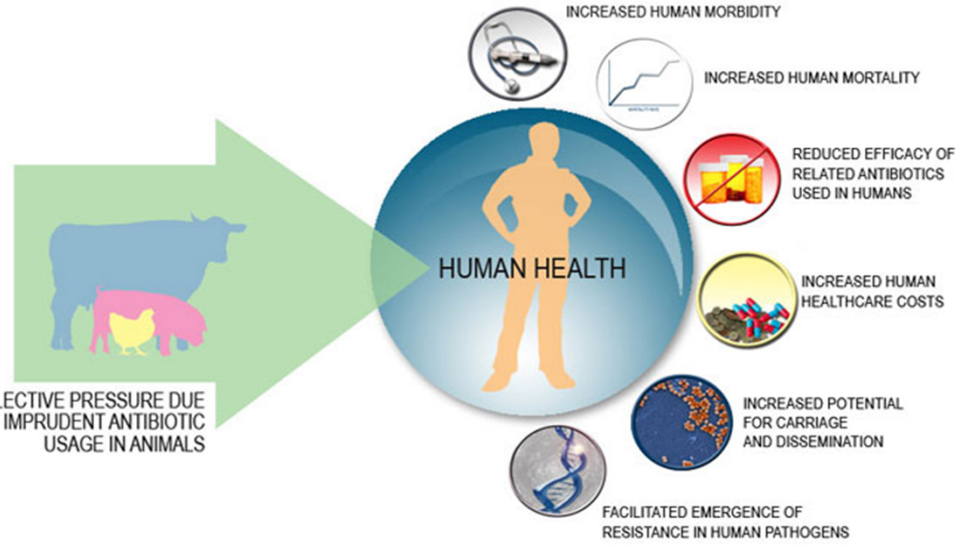
AMR: Effects on Public Health



- ✓ Increased morbidity
- ✓ Prolonged illness
- ✓ Higher mortality rates

Greater risk of complications

- **Antibiotic induced diarrhoea**
- **Mottled teeth**
- **Gastritis**



AMR: Effects on Animals

- ✓ Loss of effectiveness of antimicrobials
- ✓ Increased ***mortality and morbidity***
- ✓ ***Decrease in productivity and economy*** in food animals
- ✓ Spread of resistance - ***food security problem***– negative effects on public health
- ✓ Potential spread of bacteria and resistance genes from animals-humans

Factors Driving Antibiotic Resistance in India

Antibiotic consumption in humans

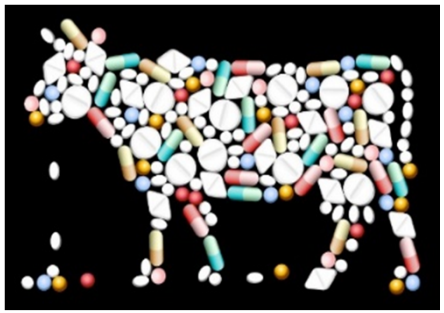
- **India**
- China and the United States

Social factors

- Self-medication,
- Antibiotics without prescription,
- Informal healthcare,
- Cultural events (Mass pilgrims)

Antibiotic consumption in food animals

- **4th** largest consumer of antibiotics in animals by 2030



Environmental Sanitation

World Bank **> 50%** of the Indian population not access to sanitation facilities

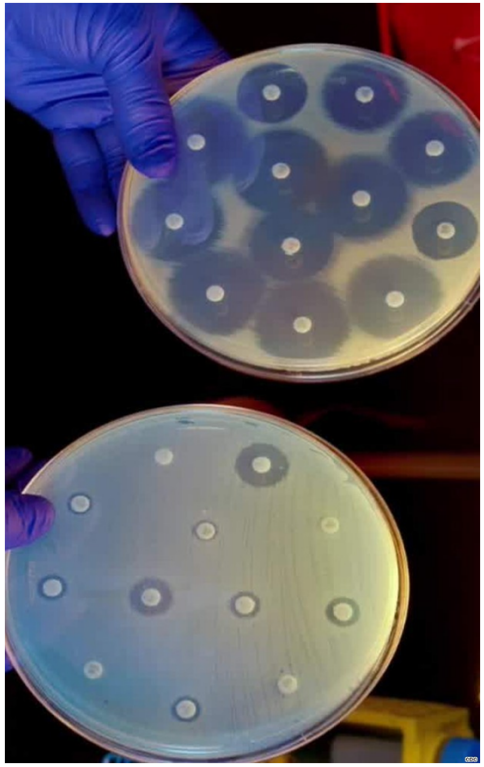
Health care Settings

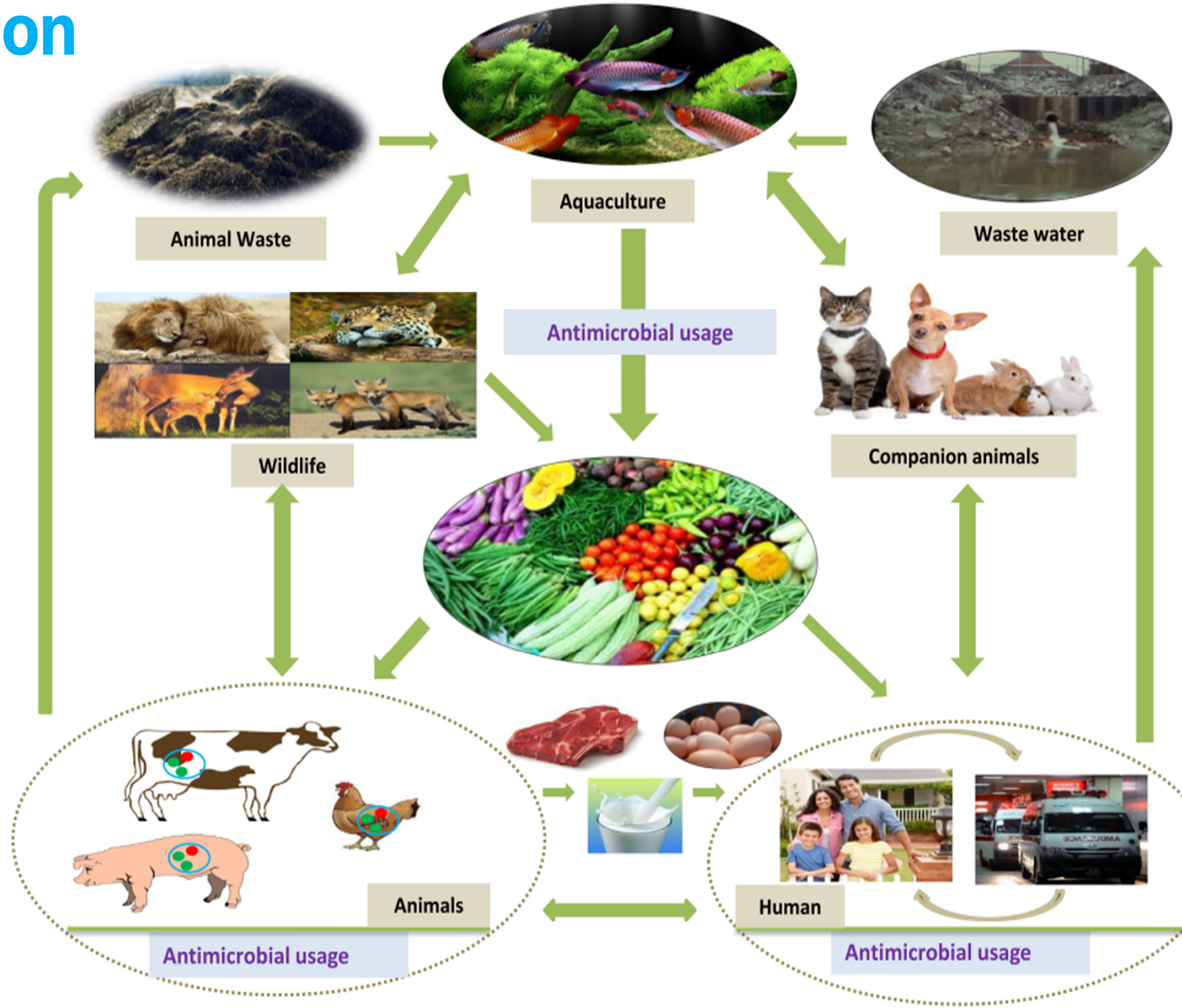
HAI burden

- Global: **7%** to **12%** of the
- India- Ranges from **11%** to **83%**

(DBT Scoping Report on Antimicrobial Resistance in India, 2017)

Antibiotic resistance:
Transmission





STATUS OF AMR IN INDIA

ANTIBIOTIC RESISTANCE THREAT IS REAL AND LOOMING

TOI

WHAT IS IT?

Misuse of antibiotics has sped up the natural process of resistance. As a result some antibiotics are now useless. And we have created "superbugs" that aren't deterred by any drug

TOI

INDIA UNDER SUPERBUG THREAT

Out of every **10 patients** with infection in ICU, 4 have drug resistant bacteria

Out of every **10 patients**, **2-3** have drug resistant bacteria, making recovery difficult, and increase treatment costs

Why India needs to worry more

In 2010 India was world's **largest consumer** of antibiotics

Antibiotic units consumed:
India 12.9x 109 units
China 10x109 units
US 6.8x109 units

India already has cases resistant to **Colistin**, a drug used when all antibiotics fail

HOW IT CAN AFFECT YOU

- Consuming antibiotic resistance livestock can affect bacterial flora in the human body and create a bacterial strain that is resistant to available antibiotics.
- Mild infections will need stronger dosage, and treating diseases like tuberculosis (TB), pneumonia and sepsis are getting tougher.
- Steroids can lead to unnecessary weight gain and suppress the human immune system.
- Bacteria that have become resistant to antibiotics can cause urinary tract infections, eye and ear infections.

WHY THE DANGER?

TOI

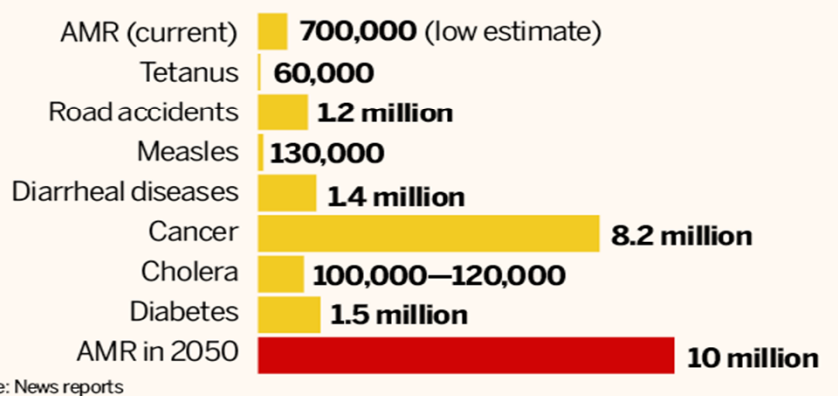
Overuse of antibiotic kills the good bacteria in people's bodies, weakening immune systems.
This means more people get sick and **stay sick for longer**

Experts warn we are at the "dawn of a post-antibiotic era", a "catastrophic threat" on a par with terrorism

When antibiotics stop working certain procedures will be extremely dangerous, some **diseases will become incurable...**

TOI

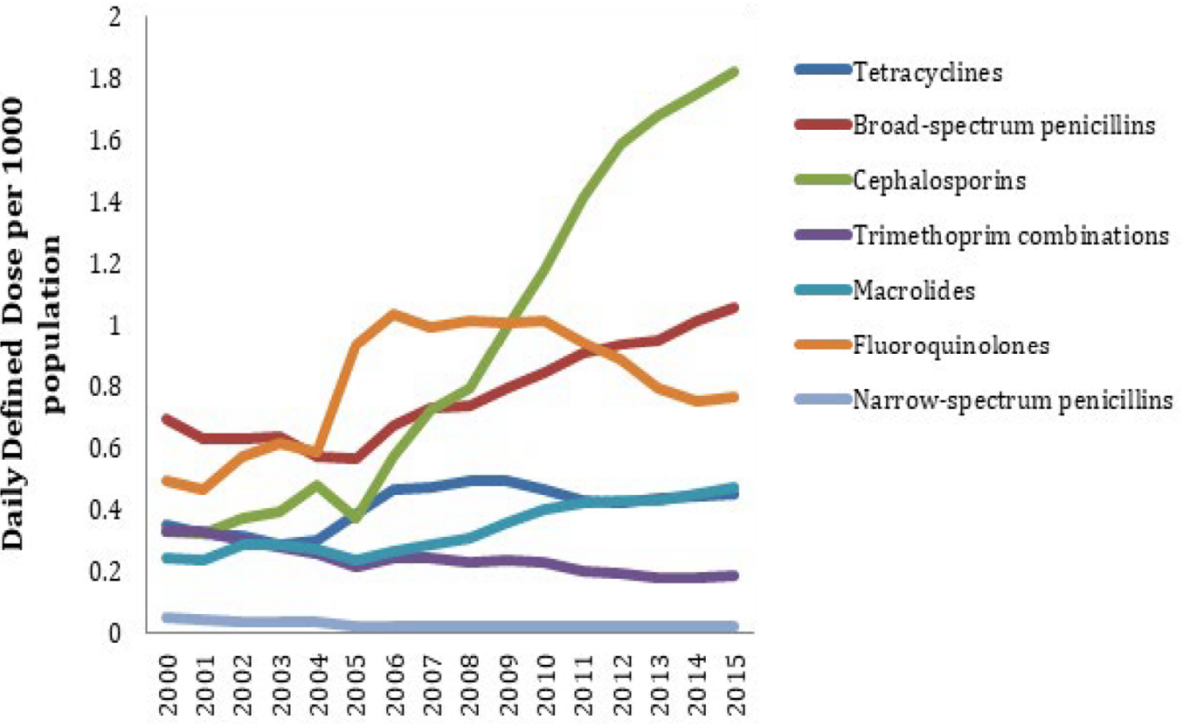
Antibiotic resistance (AMR) to be a leading cause of death by 2050

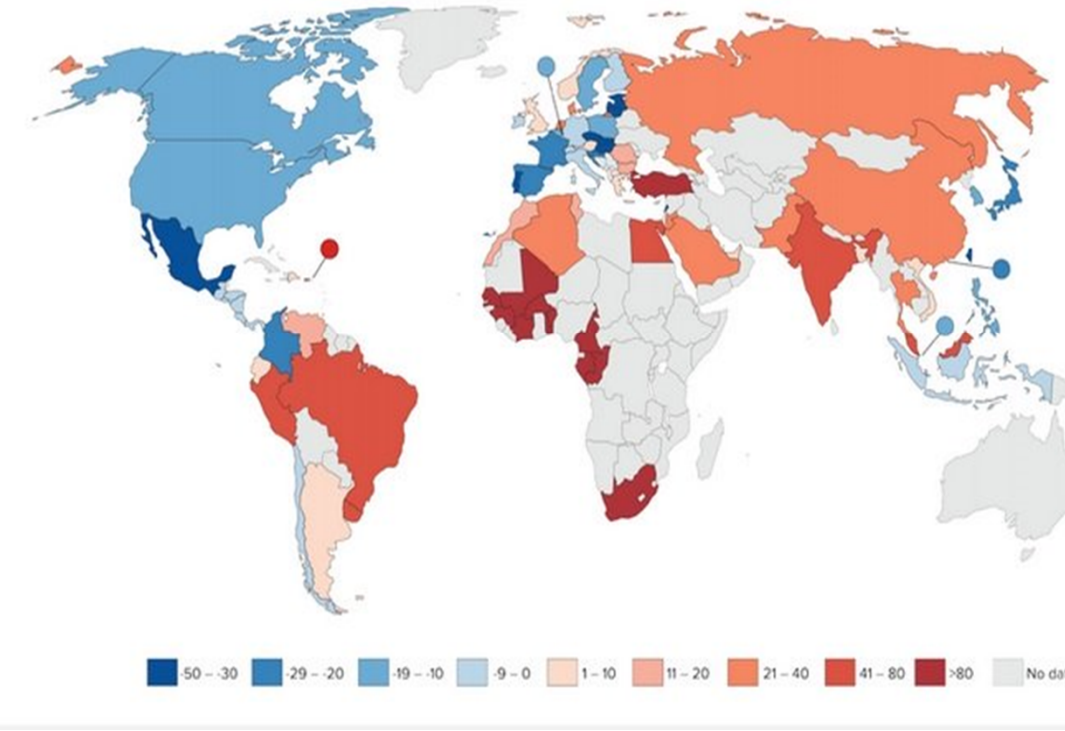


Source: News reports

Antibiotic consumption… Trends in India

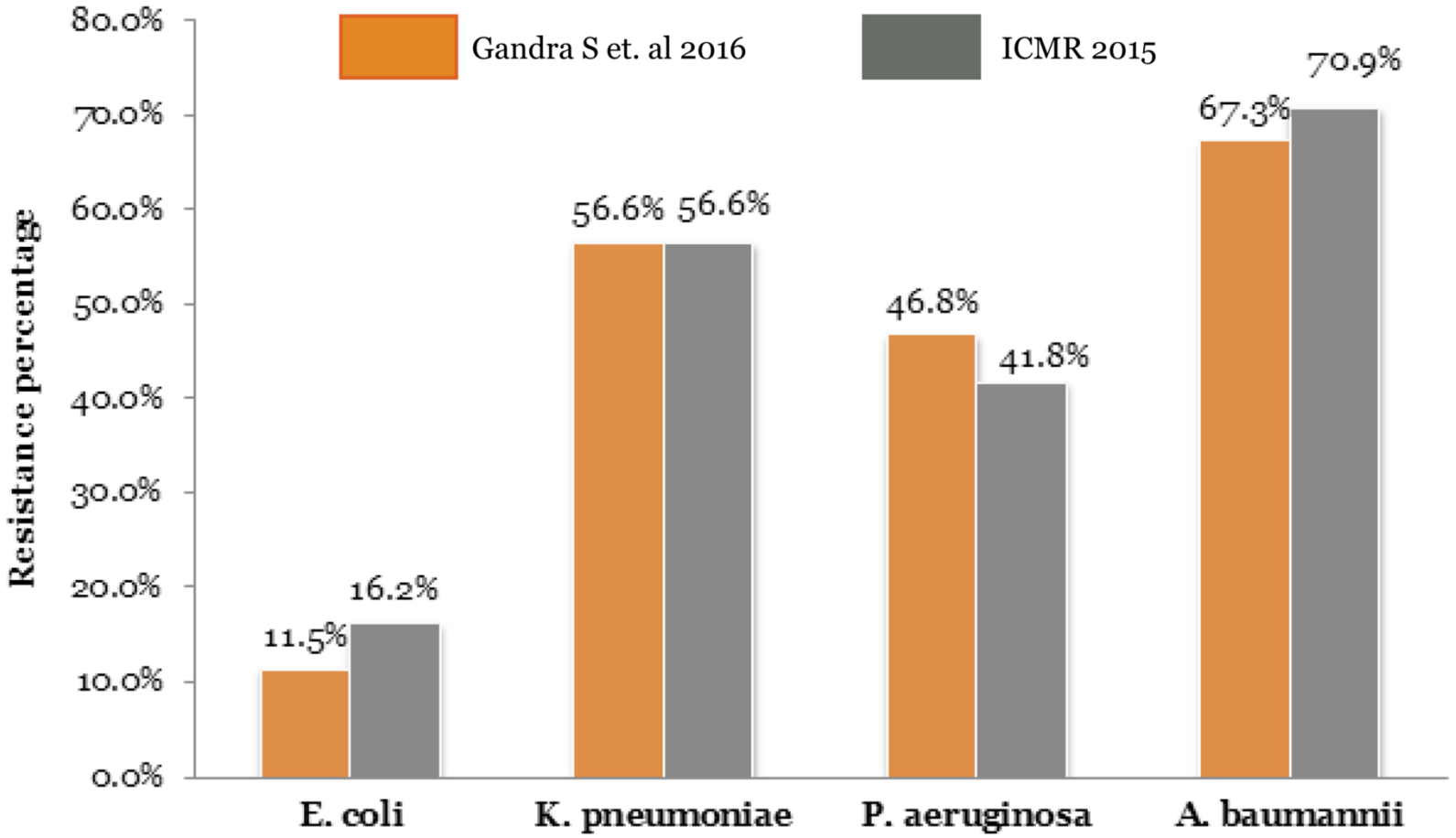
(2000–2015)





(DBT Scoping Report on AMR in India, 2017)

Antimicrobial resistance in India: Humans



Carbapenem (meropenem/imipenem) resistance among various bacteria isolated from blood culture

(DBT Scoping Report on AMR in India, 2017)

Antimicrobial resistance in India: Animals

- **Mastitic Cattle**
  - ❖ NDM-1 and ESBL producing Gram-negative bacteria (Ghatak *et al.*, 2013; Das *et al.*, 2017)
  - ❖ Vancomycin-resistant *Staphylococcus aureus* (VRSA) (Bhattacharyya *et al.*, 2016)
- **Pig faecal samples**
  - ❖ ESBL-producing *E. coli* (Lalzampuia *et al.*, 2013; Samanta *et al.*, 2015)
- **Chicken meat samples**
  - ❖ Multi-drug resistant *Salmonella* (Naik *et al.*, 2015)

Antimicrobial resistance in India: Aquaculture

- **ESBL producing *Enterobacteriaceae* isolates** (42%) from the gut of tilapia fish (Marathe *et al.*, 2016)
- ***Vibrio* species** from retail markets in Kerala were ***100% resistant to ampicillin*** (Sudha *et al.*, 2014)

Antibiotic alternatives: What we need ?

WHO:- Global Action Plan

(Adopted World Health Assembly, 2015)

AAT compounds

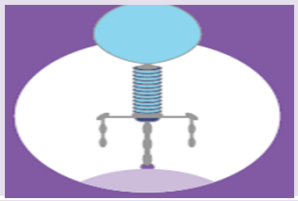
- Act alone/synergistically with antibiotics
- Less chance of developing resistance
- Prevent pathogen colonization in GIT
- Enhance antimicrobial protection
- Prevent infection caused by viruses & bacteria
- Maintaining/improving commensal gut bacterial population.
- Target specific

(Allen *et al.*, 2014; Bomko *et al.*, 2017)

Alternatives to antibiotics

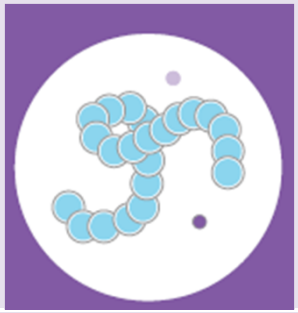
Phage therapy

- Natural/engineered viruses attack & kills bacteria



Lysins

- Enzymes directly & quickly act on bacteria

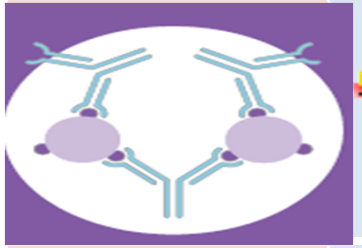


Antimicrobial peptides

- Small biological molecules, broad spectrum of activity

Probiotics

- Prevent colonization of pathogenic bacteria



Vaccine/antibody

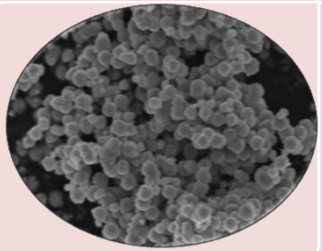
- Binds to MO and kill them

Predatory bacteria

- Bacteria eat others of their kind

Herbs

- Act synergistically with drugs, inactivate enzymes & inhibit efflux pumps



Nanoparticles

- Act synergistically with AMC

Probiotics

"Live bacteria which when administered in adequate amounts confers a health benefit to the host" - WHO

Characteristic of probiotic microbes:

- Gastric acid and bile resistant
- Adhere to intestinal epithelial cells
- Grow fast & colonize the intestinal tract
- Stabilize the intestinal microflora
- Non-pathogenicity
- Maintain viability in food & pharmacopoeia drugs manufacturing

(Gibson & Roberfroid, 1995; Bomko *et al.*, 2016)

Natural sources of probiotics

Curd

Sauerkraut

Kimchi

Coconut Kefir

Natto

Yogurt

Kvass

Miso

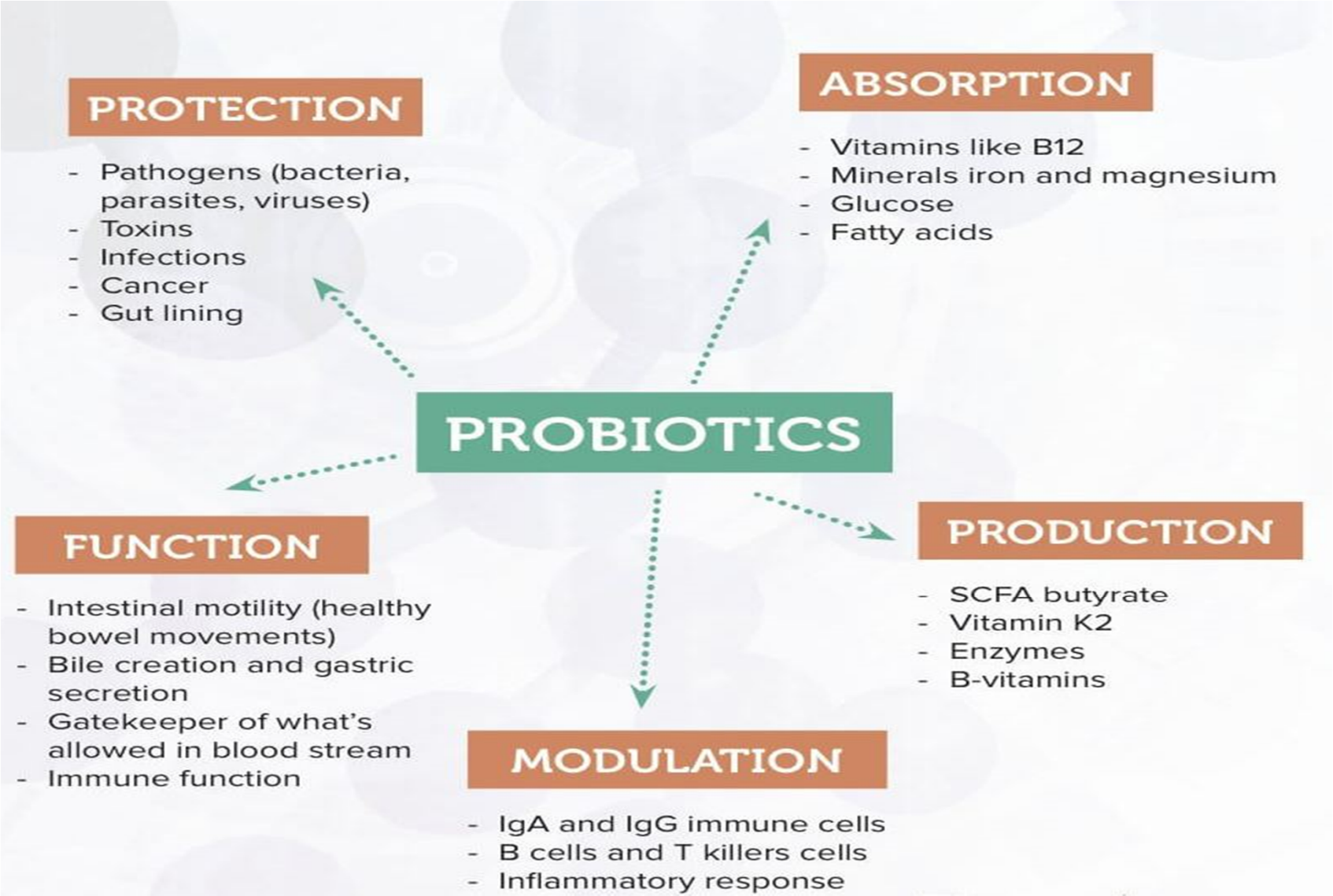
kombucha

Raw cheese

Kefir

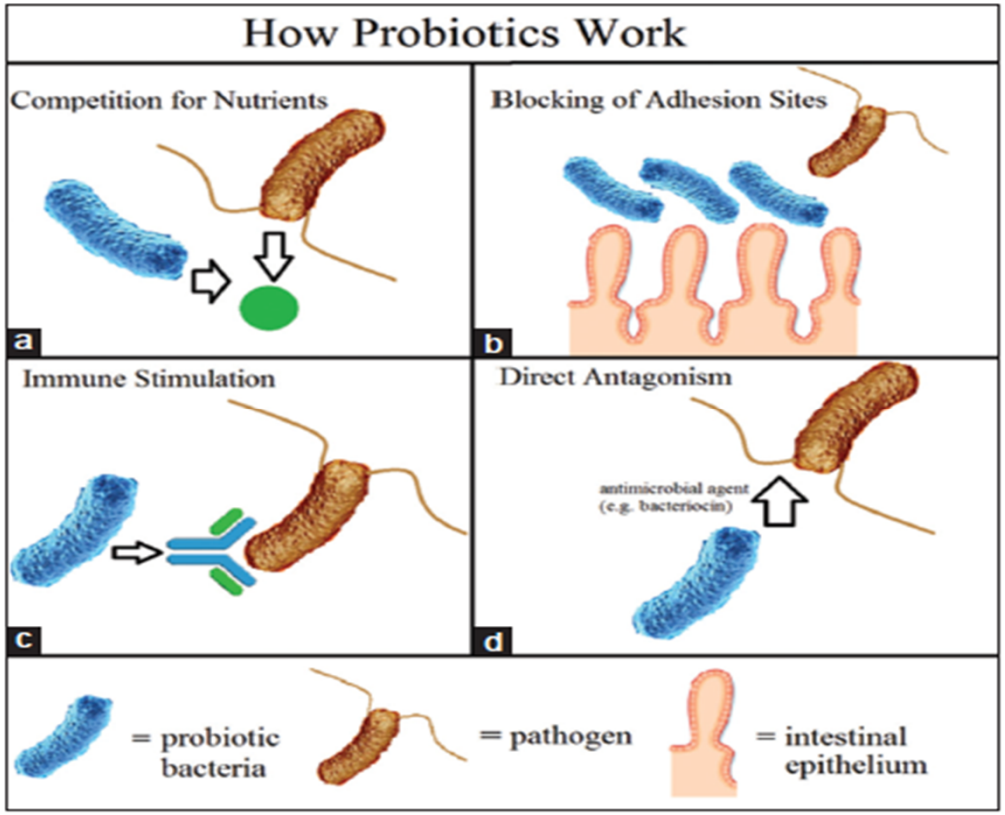
(https://draxe.com/probiotics-benefits-foods-supplements)

Health benefits of probiotics



(Goldin & Gorbach, 2008; Haggar & Boushey, 2009)

Mechanism of action of probiotics



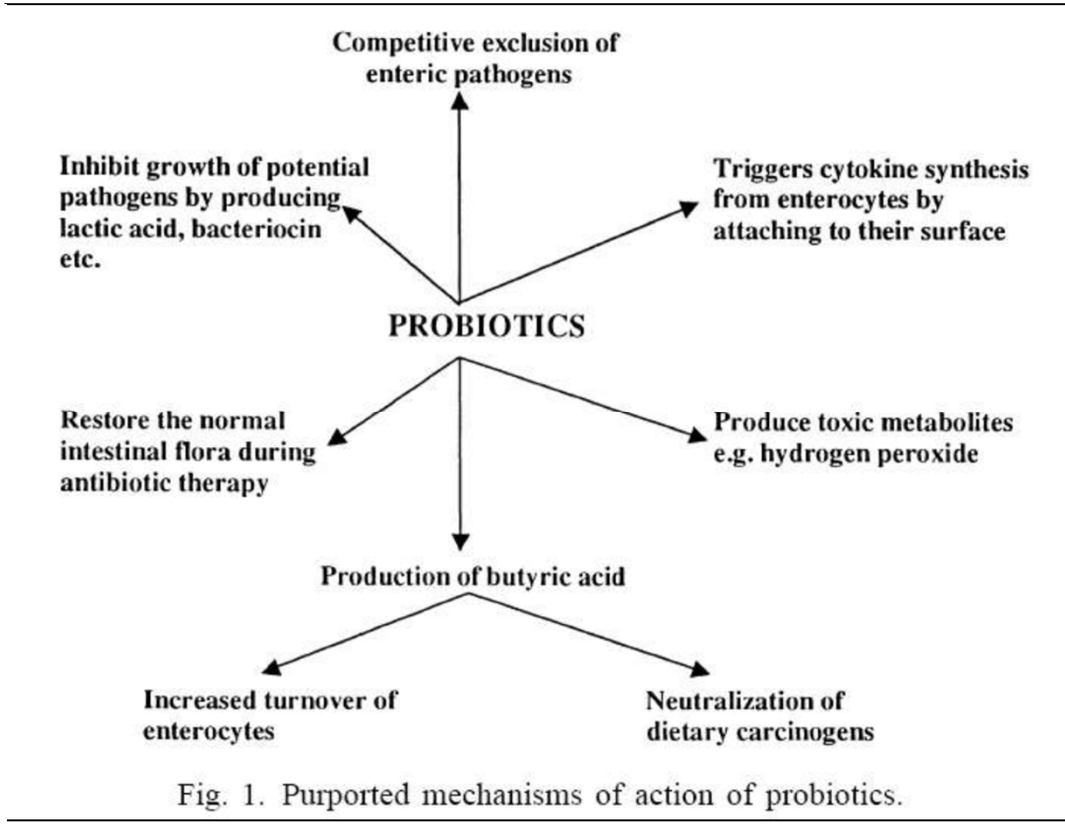


Fig. 1. Purported mechanisms of action of probiotics.

Four different methods of protecting probiotics from the intestines against known diseases

Probiotics compete against pathogens for essential nutrients and are less likely to be used for pathogens **(a)**; They attach to adhesion sites and reduce pathogen dependence by reducing the available ground surface for pathogenic colonization **(b)**; Signaling of immune cells by probiotics leads to secretion of cytokines and targeting the pathogen for destruction **(c)**; Ultimately, probiotics with direct bacterial release of bacteriocins **(d)**

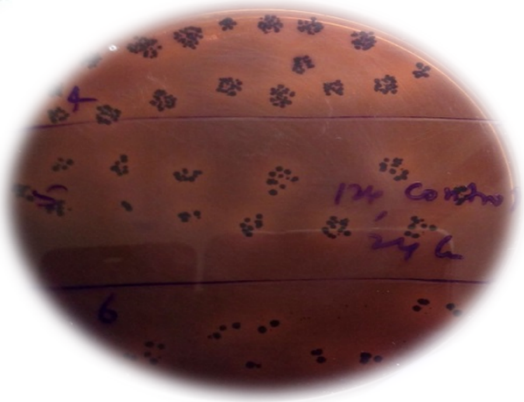
Growth inhibitory product produced by probiotic bacteria and Mechanism of inhibition on target microorganism

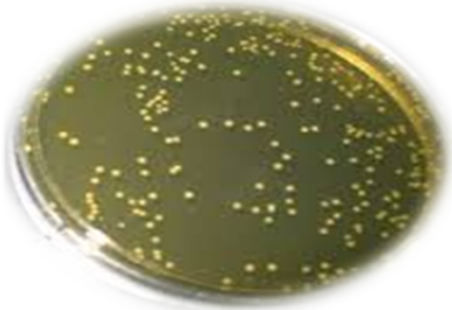
Inhibitory Product	Mechanism of action
Lactic acid, propionic acid, Butyric acid	Disruption of metabolism
Hydrogen peroxide	Inactivation of essential biomolecules, Induce lactoperoxidase system
Carbon dioxide	Creates anaerobic environment, inhibit decarboxylation
Diacetyl	Interferers with arginine utilization
Bacteriocins, Nicin, Pediocins, Pediocins Ach, Leucocin, helveticin, Carnobacteriocin, reuterin, Subtilicin, Colicin etc.	Broad and narrow spectrum activity against membrane and membrane structures; membrane lysis, disruption of receptors.

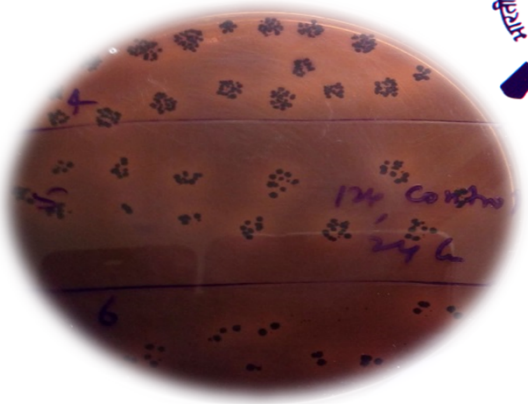
(Mishra and Lambert, 1996)

Medical applications in humans for different classes of probiotics

Medical condition	Class(es) of probiotic	Reference(s)
Lactose maldigestion	LAB and ***Streptococcus salivarius* subsp. *thermophilus***	(Savaiano *et al.*, 1984; Kolars *et al.*, 1984)
Gastroenteritis Acute diarrhea	LAB, ***Bifidobacterium* species, or *Saccharomyces boulardii***	Allen *et al.*, 2003
Antibiotic-associated diarrhea	LAB or ***S. boulardii***	(Cremonini *et al.*, 2002)
Traveler's diarrhea	LAB	(Hilton *et al.*, 1997)
Allergies	LAB	(Rautava *et al.*, 2002)
***Clostridium difficile*–induced colitis**	LAB	(Bennett *et al.*, 1996)
Dental caries	LAB	(Nase *et al.*, 2001)
Intestinal inflammation in children with cystic fibrosis	LAB	(Bruzzese *et al.*, 2004)
Respiratory infection in children	LAB	(Hatakka *et al.*, 2001)
Nasal colonization with pathogens	LAB	(Gluck and Gebbers, 2003)
Inflammatory bowel disease or irritable bowel syndrome	LAB and ***Bifidobacterium* spp., *S. boulardii* and drug, *S. boulardii* alone, or LAB alone**	(Guslandi *et al.*, 2000; Brigdi *et al.*, 2001)







Master Programme study

Our own experience on probiotics against MDR-EAEC

Objective:

To study *antimicrobial effects of probiotics* on multi-drug resistant Enteroaggregative *Escherichia coli* (MDR-EAEC) field isolates.

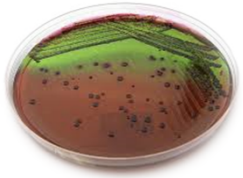
Probiotics evaluated were:

Lactobacillus plantarum *Lactobacillus acidophilus*

Work Plan

In vitro effects of *Lactobacillus plantarum* / *Lactobacillus acidophilus* on MDR- EAEC

Single pure colony of MDR-EAEC



↓

Inoculate in 5 ml TSGY broth

(Incubation: 37°C)

↓

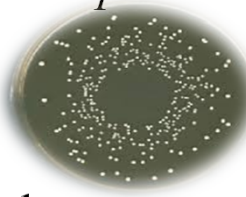
Pelleting of log broth cultures

(4000rpm @ 2 min)

↓

Re-suspension in sterile Normal Saline Solution

Single pure colony of *L. plantarum/L. acidophilus*



↓

Inoculate in 5 ml TSGY broth

(Incubation: 37°C)

↓

Pelleting of log broth cultures

(4000rpm @ 2 min)

↓

Re-suspension in sterile Normal Saline Solution

(Contd..)

MDR-EAEC
and
L. plantarum/L.acdiophilus

Add both bacterial strains as mentioned below in
5 ml TSGY (Tryptic soy glucose yeast) broth @ pH = 8.0

Group 1

1×10^7 cfu of MDR- EAEC
+
1×10^8 cfu of *L. plantarum*

Group 2

1×10^7 cfu of MDR- EAEC
+
1×10^9 cfu of *L. plantarum*

Group 3

1×10^7 cfu of MDR- EAEC
+
1×10^{10} cfu of *L. plantarum*

Group 4

1×10^7 cfu MDR-EAEC
(Control)

Incubate @ 37^0C
for 96 hours

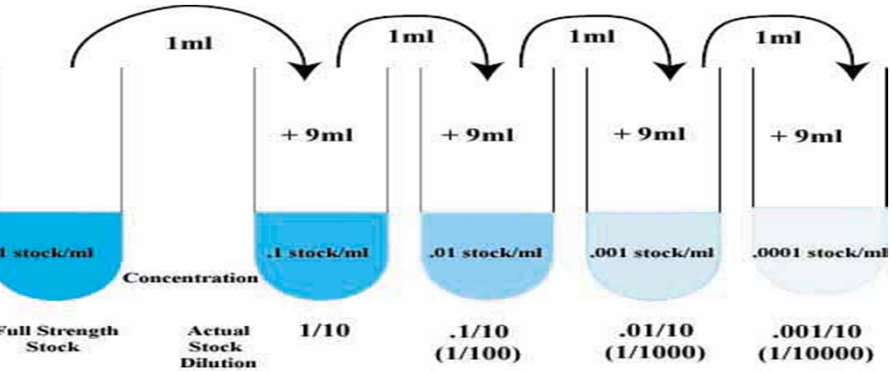
Post inoculation aliquots – for enumeration of MDR-EAEC at specified time periods

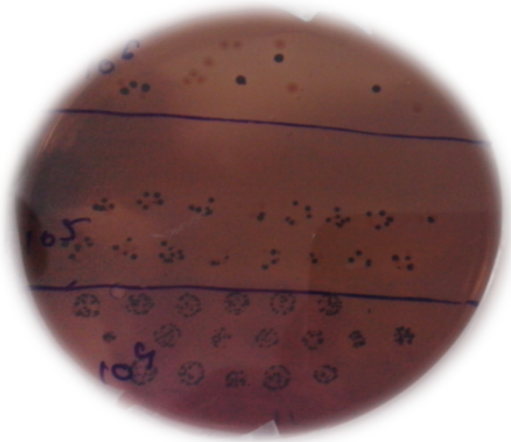
TECHNICAL PROGRAMME

(Contd..)

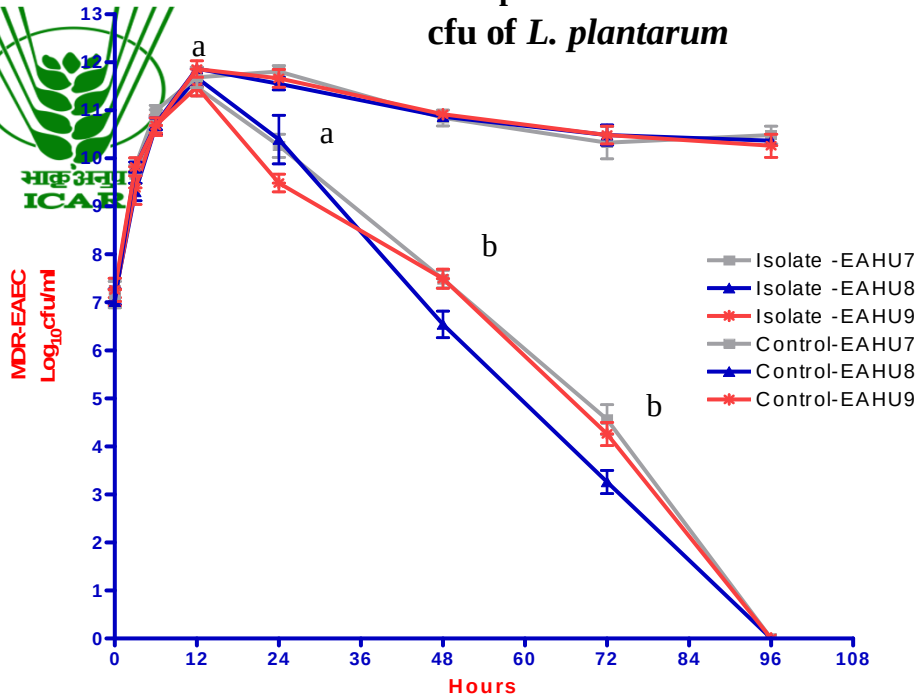
❖ Bacterial enumeration:

- The aliquots of test and control cultures were drawn at 0 h, 3 h, 6 h, 12 h, 24 h, 48 h, 72 h and 96 h post inoculation
- EMB agar plates will be used as selective media for enumerating the total count of MDR-EAEC at each time point (Miles and Misra,1938)
- The bacterial count will be expressed in cfu/ml.

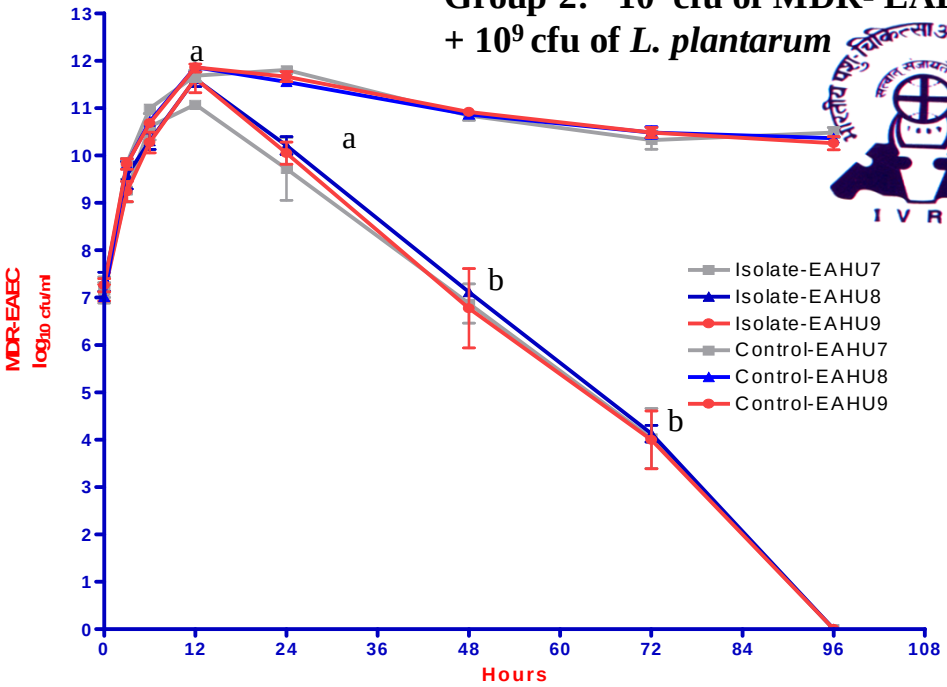




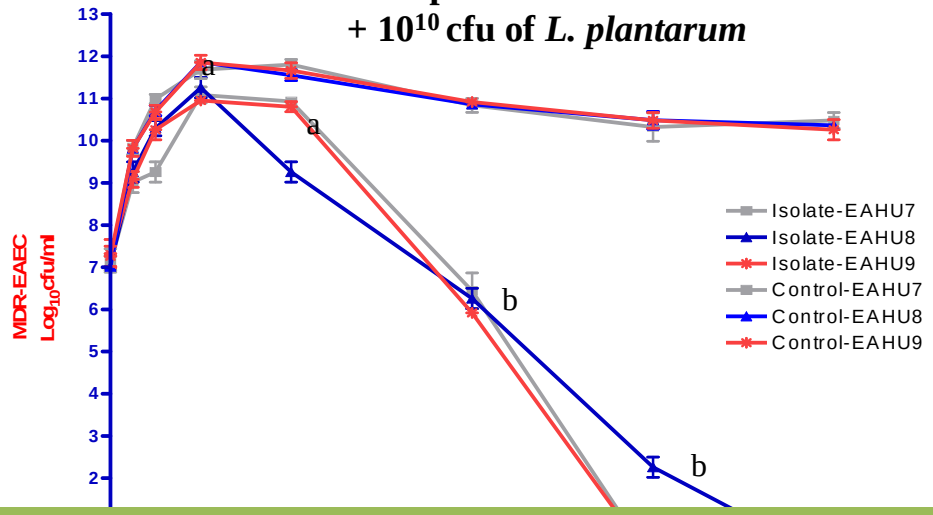
Group 1: 10^7 cfu of MDR- EAEC + 10^8 cfu of *L. plantarum*



Group 2: 10^7 cfu of MDR- EAEC + 10^9 cfu of *L. plantarum*



Group 3: 10^7 cfu of MDR- EAEC + 10^{10} cfu of *L. plantarum*

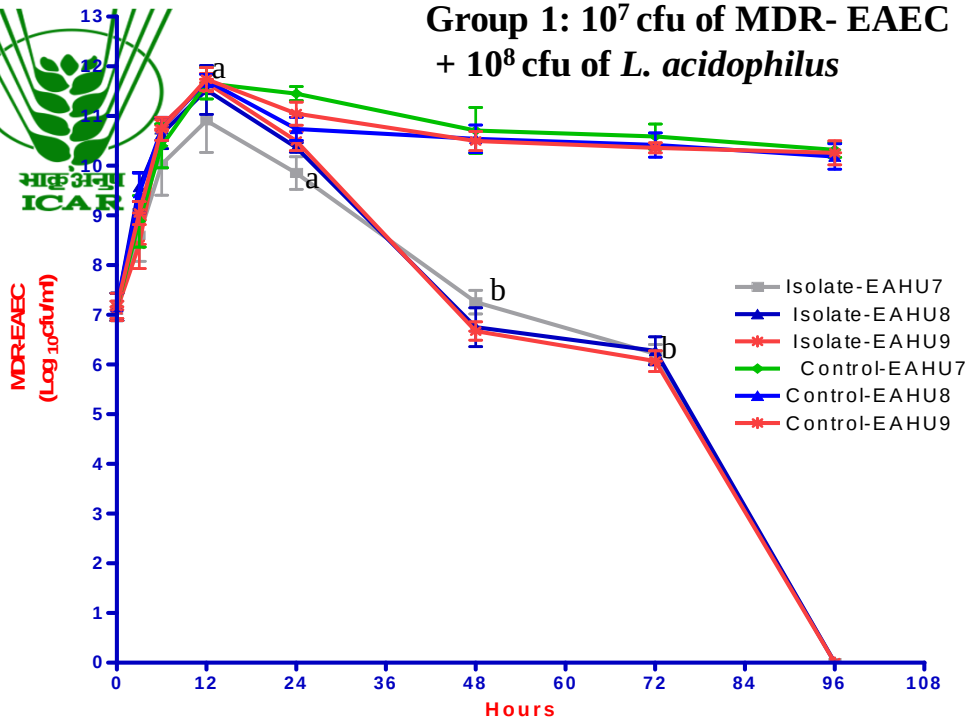


a ($p<0.05$)

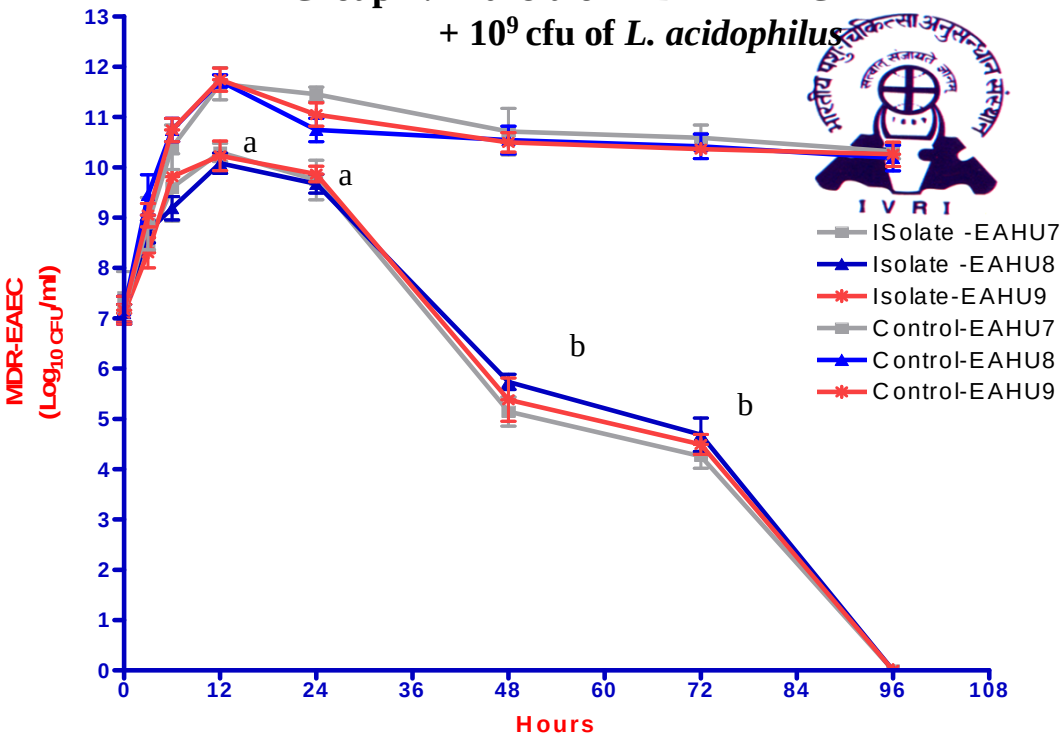
b ($p<0.01$)

***L. plantarum* @10^{10} cfu was most effective in inhibiting the growth of two MDR-EAEC isolates in 72 h**

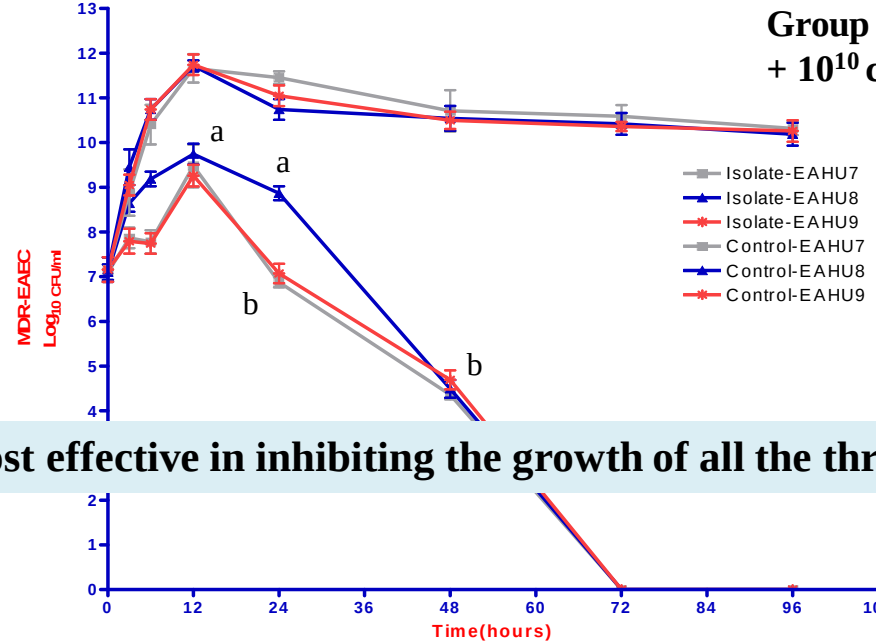
Group 1: 10^7 cfu of MDR- EAEC + 10^8 cfu of *L. acidophilus*



Group 2: 10^7 cfu of MDR- EAEC + 10^9 cfu of *L. acidophilus*



Group 3: 10^7 cfu of MDR- EAEC + 10^{10} cfu of *L. acidophilus*



a ($p<0.05$)
b ($p<0.01$)

***L. acidophilus* @10^{10} cfu was most effective in inhibiting the growth of all the three MDR-EAEC isolates in 72 h**

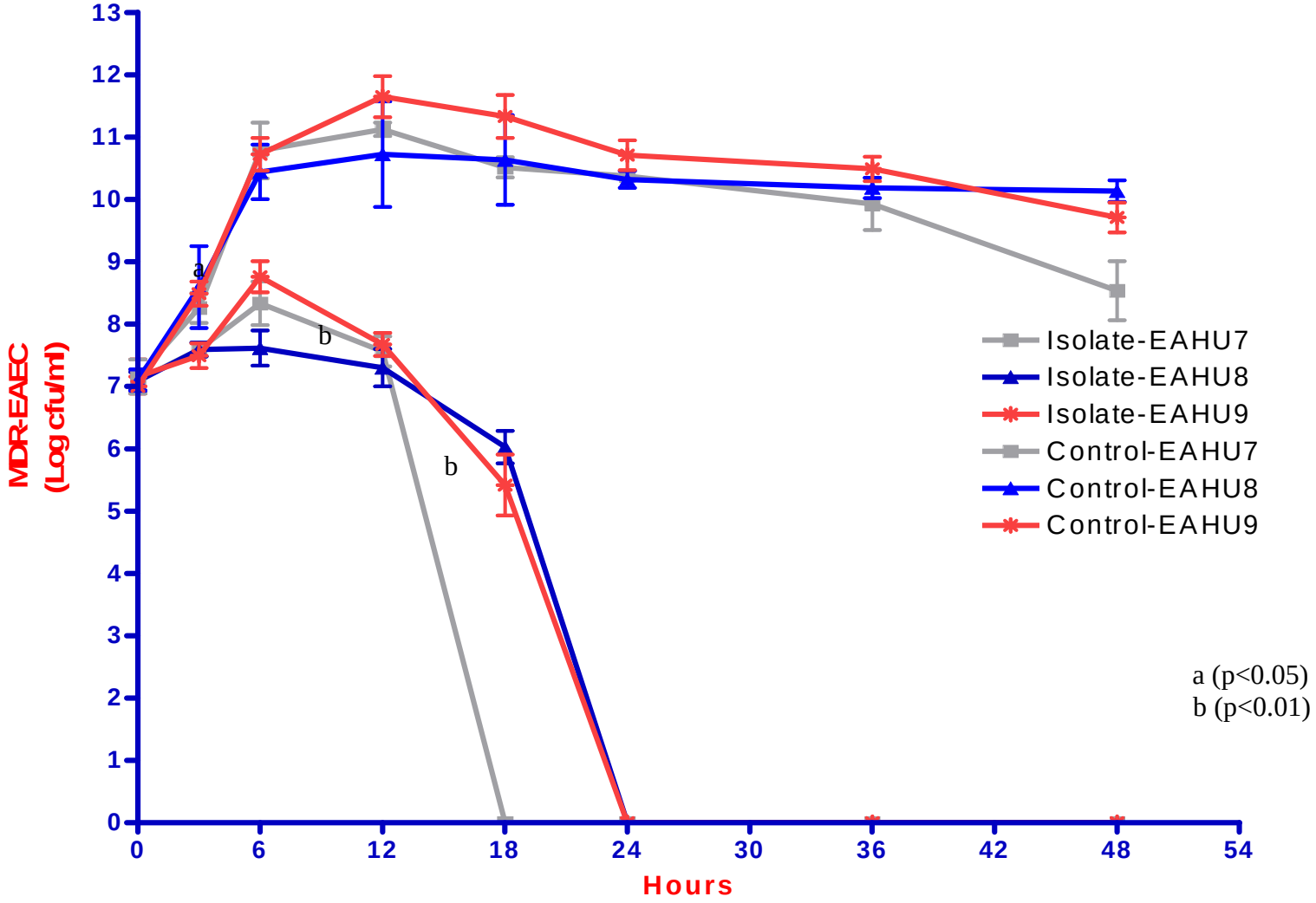
(Contd..)

(c) Synergistic antimicrobial effects of *Lactobacillus* spp. (*L. plantarum* and *L. acidophilus*) against MDR-EAEC

Group 1: 1 x 10^7 cfu of MDR-EAEC + *L. plantarum* (1x 10^{10} cfu)
+
L. acidophilus (1x 10^{10} cfu)

Group 2: 1 x 10^7 cfu of MDR-EAEC Control

Enumeration of MDR-EAEC was performed as described in earlier experiments



Growth profile of MDR- EAEC co-incubated with *L. plantarum* (1 x 10^{10} cfu) and *L. acidophilus* (1 x 10^{10} cfu)

In vivo antimicrobial efficacy of probiotics in mice model

Procurement of 32 Weaned Swiss albino mice (4-6 weeks of age) from LAR, IVRI

Grouping of mice into four groups

Group 1 (n=12)

Infected

Oral inoculation of MDR-EAEC @ 1x 10^7 cfu/mice in Group 1 & Group 2

Group 2 (n=12)

Treatment

Oral inoculation of MDR-EAEC @ 1x 10^7 cfu/mice in Group 1 & Group 2

Group 3 (n=4)

Control

Oral injection of PBS

Group 4 (n=4)

Probiotic control

Oral injection of Best Treatment group from *In vitro* Experiments

Monitoring of Body weight, behavior changes and faecal consistency of mice of each group & collection of faecal sample and enumeration of MDR-EAEC

3 days post infection, Group 2 mice were fed orally with 1 x 10^{10} cfu of *L. plantarum* and 1 x 10^{10} cfu of *L. acidophilus*

Monitoring of mice for faecal shedding of MDR-EAEC 2 days Post- treatment

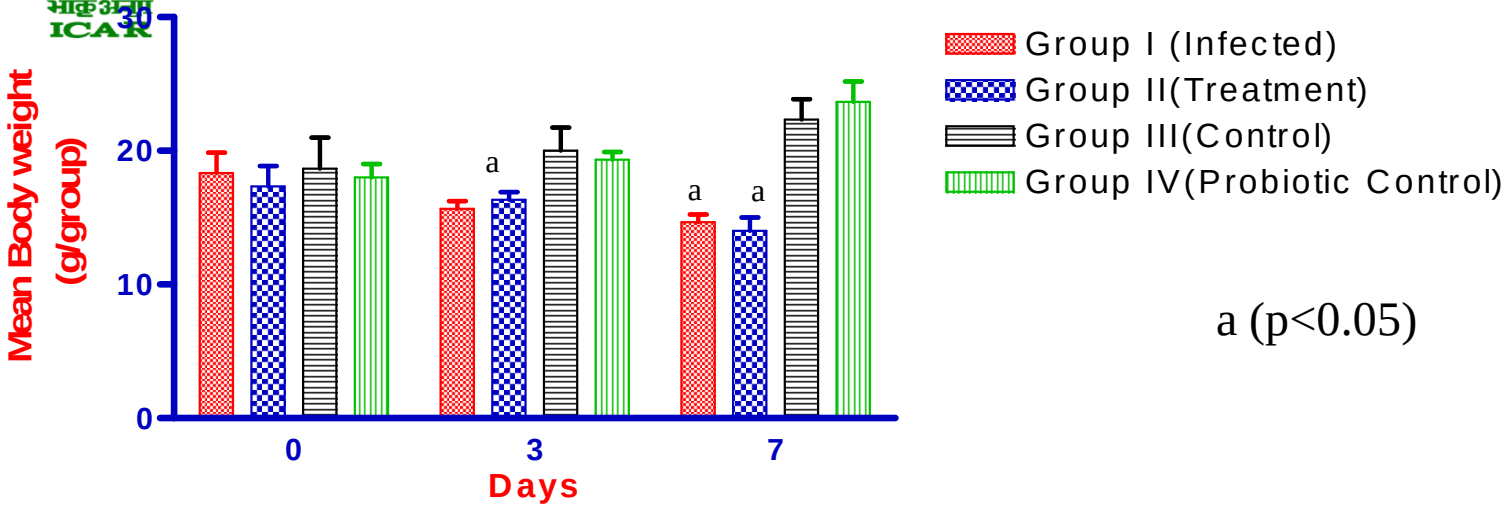
3^{rd}, 4^{th} , 5^{th} and 6^{th} day post-treatment, three mice from Group I and II and one mice from Group III and IV, respectively were euthanized

Collection of intestinal tissue (Ileum & colon)

Enumeration of MDR-EAEC in intestinal tissue on Ampicillin EMB agar plates and by Realtime PCR

Histopathological examination

Mean Body weight changes in mice



a ($p<0.05$)

Behaviour

Control

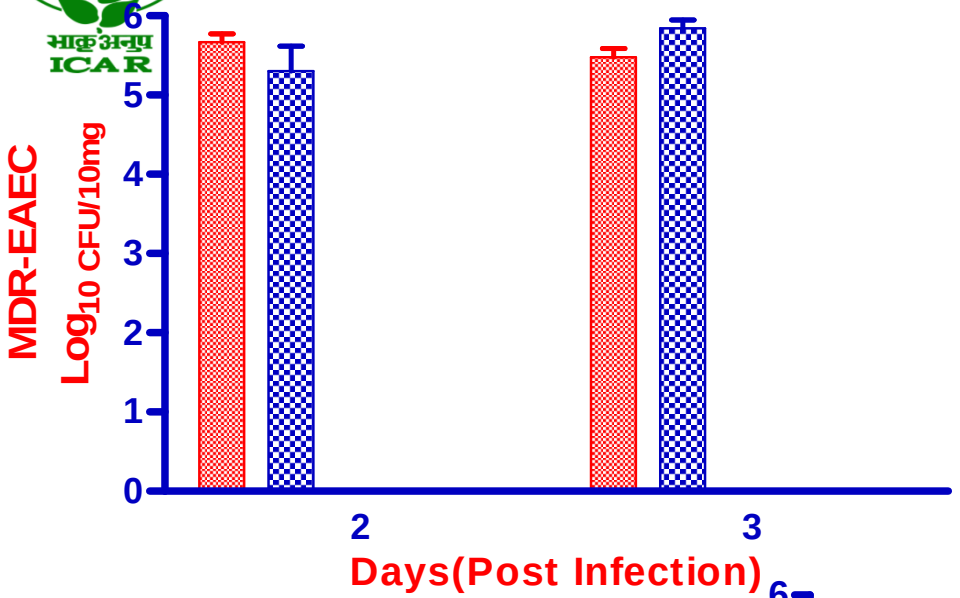
Infected

Less active

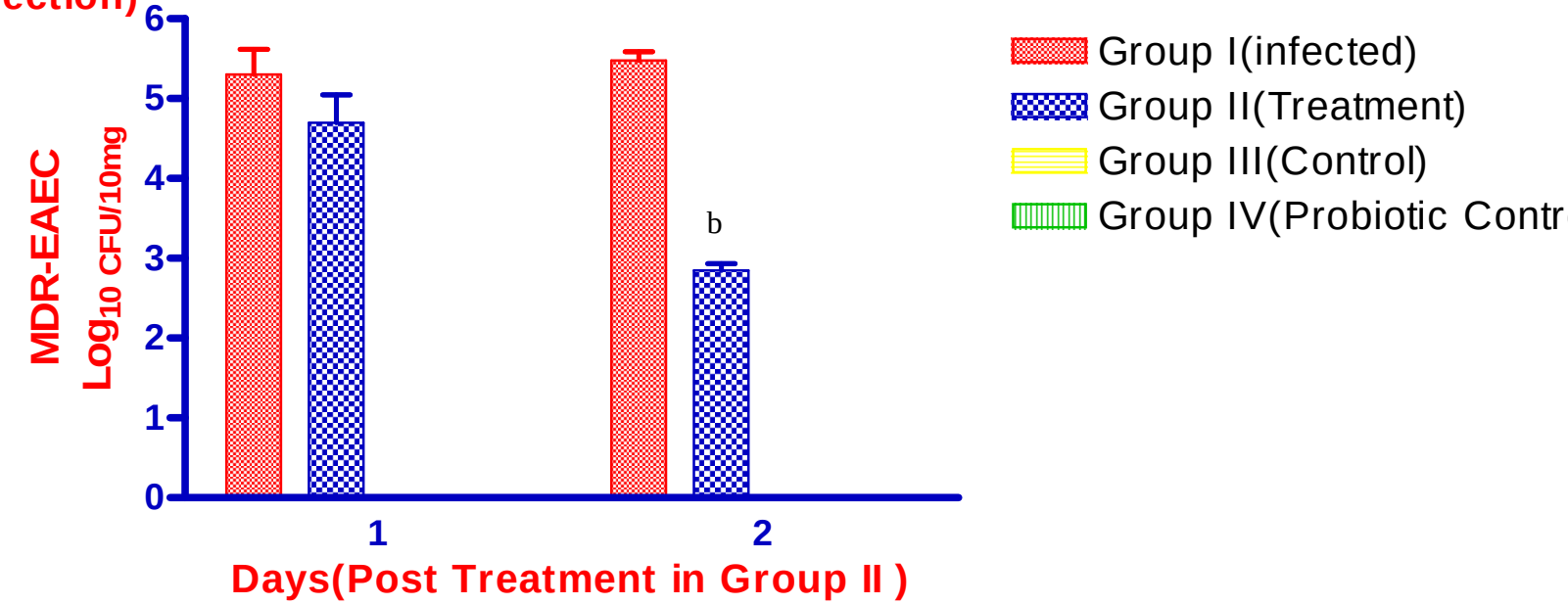
Less active

Active

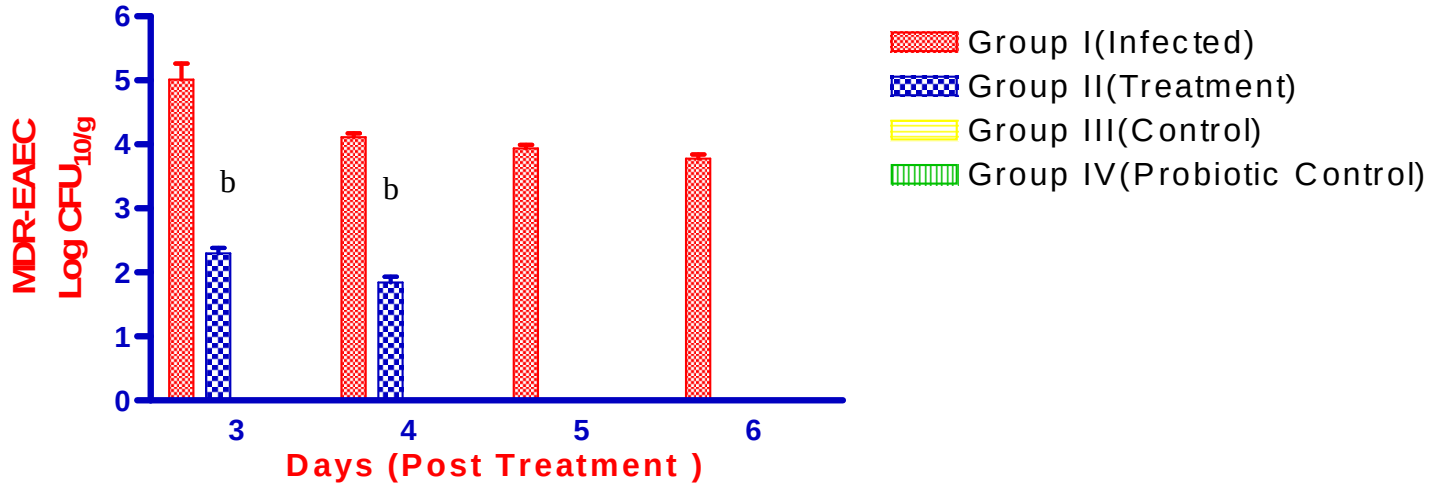
MDR-EAEC counts in faeces using plate count method supplemented with 100 µl of Ampicillin per plate



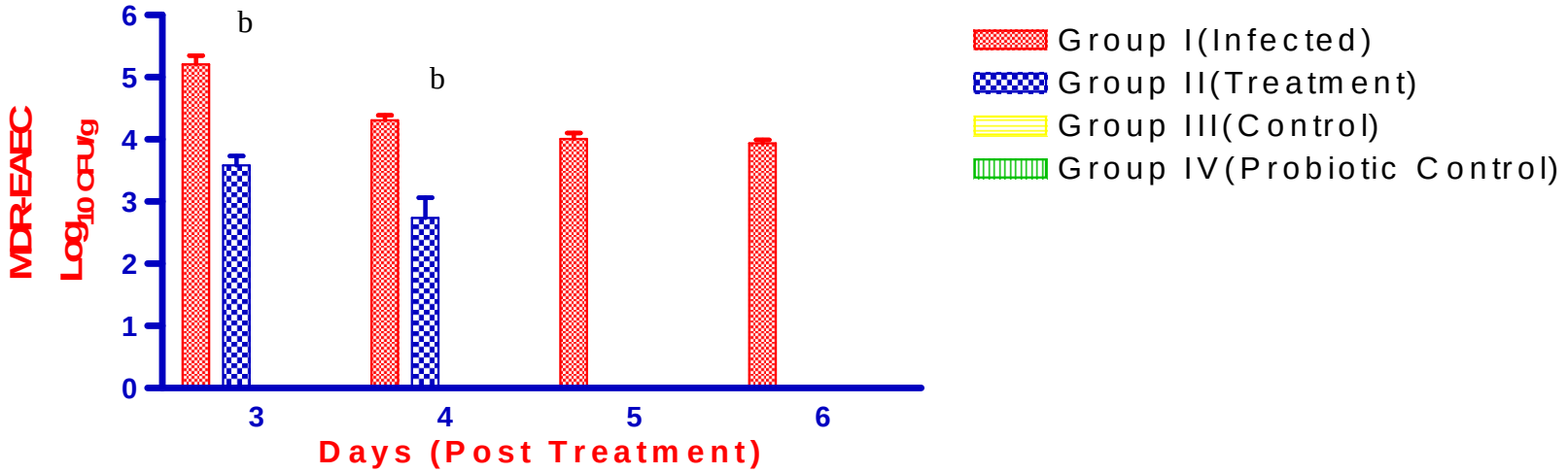
a ($p<0.05$)
b ($p<0.01$)



MDR-EAEC counts in intestine (ileum and colon) using plate count method



MDR-EAEC counts in intestine (ileum and colon) by Real Time PCR



Our contribution

International Journal of Antimicrobial Agents 48 (2016) 265–270

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: www.elsevier.com/locate/ijantimicag

Antimicrobial effects of *Lactobacillus plantarum* and *Lactobacillus acidophilus* against multidrug-resistant enteroaggregative *Escherichia coli*

Manesh Kumar [a], Pankaj Dhaka [a], Deepthi Vijay [a], Jess Vergis [a], Vysakh Mohan [a], Ashok Kumar [a], Nitin V. Kurkure [b], Sukhadeo B. Barbuddhe [c], S.V.S. Malik [a], Deepak B. Rawool [a,*]

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[c] National Institute of Biotic Stress Management, Baronda, Raipur 493225, India

More studies on use of probiotics - MDR pathogens

Multi Drug Resistant Pathogen (MDR)	Probiotics	Reference
E. coli, MRSA, *S. agalactiae*	*L. fermentum*, *L. plantarum*, *L. acidophilus*, *L. rhamnosus*, *L. gasseri*	Pradhan *et al*., 2011
VRE	*L. rhamnosus* GG	Szachta *et al*., 2011
Pseudomonas aeruginosa, *Acinetobacter baumannii*	*Bifidobacterium adolescentis*, *B. longum*, *B. pseudocatenulatum*	Lee *et al*., 2013
A. baumannii, *E. coli* and *S. aureus*	*L. jensenii* and *L. rhamnosus*	Sambanthamoorthy *et al*., 2014
P. aeruginosa	*Lactobacillus*, *Saccharomyces*, *Bifidobacterium*	Machairas *et al*., 2015
Acinetobacter baumannii	*B. breve* strain Yakult (BbY)	Asahara *et al*., 2016
E. coli	*B. longum*, *L. plantarum*, *L. helveticus*, *L. rhamnosus*	Abdelhamid *et al*., 2018

More studies on use of probiotics - MDR pathogens

Multi Drug Resistant Pathogen (MDR)	Probiotics	Reference
E. hormaechei, *K. pneumoniae*, *A. baumannii*	*Lactobacillus reuteri*	Chan *et al*., 2018
S. Enterica Serovar Heidelberg	*Propionibacterium freudenreichii*	Nair and Johny, 2018
Methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE)	Marine actinomycetes	Norouzi *et al*., 2018
Methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE)	Marine actinomycetes	Sikorska and Smoragiewicz, 2013; Norouzi *et al*., 2018
P. aeruginosa	*Lactobacillus plantarum* 299v	Moghadam *et al*., 2018

Advantages and Limitations of Probiotics

Advantages	Limitations	Reference
✓ Growth promoter and Immune modulation ✓ Effective against antibiotic-associated *Clostridium difficile*-associated and traveler's diarrhea), lactose intolerance, vaginal infections ✓ Produces vitamin B_{12}, vitamin K_2, butyrate, various enzymes that destroy harmful bacteria ✓ Stimulating secretion of IgA and T-cells ✓ Maintain or improve commensal gut bacterial population, lowering pH and improving mucosal immunity ✓ Promoting digestion, nutrient absorption and bioavailability ✓ Prevent pathogen colonization in GIT ✓ Have direct antagonistic activity against varied number of resistant strains. ✓ Prevent relapse of Crohn's disease in human	✓ Complex FDA regulatory process ✓ May be harmful when consumed in large quantity ✓ May be harmful to immunocompromised humans ✓ Chance of acquiring mobile elements responsible for spread of antimicrobial resistance	Trafalska and Grzybowska, 2004; Oyetayo and Oyetayo, 2005; Besselink *et al*., 2008; Callaway *et al*., 2008; Gill and Prasad, 2008; Rahimi *et al*., 2008; Reid *et al*., 2009; Gaggìa *et al*., 2010; Iannitti and Palmieri, 2010; Jamalifar *et al*., 2011; Brandt, 2012; Allen *et al*., 2014; Nami *et al*., 2015; Varankovich *et al*., 2015; Bomko *et al*., 2017; Sharma *et al*., 2018


Thank you very much for your
kind attention !!