Standing Up Against Antibiotic Resistance With Synergistic Approach

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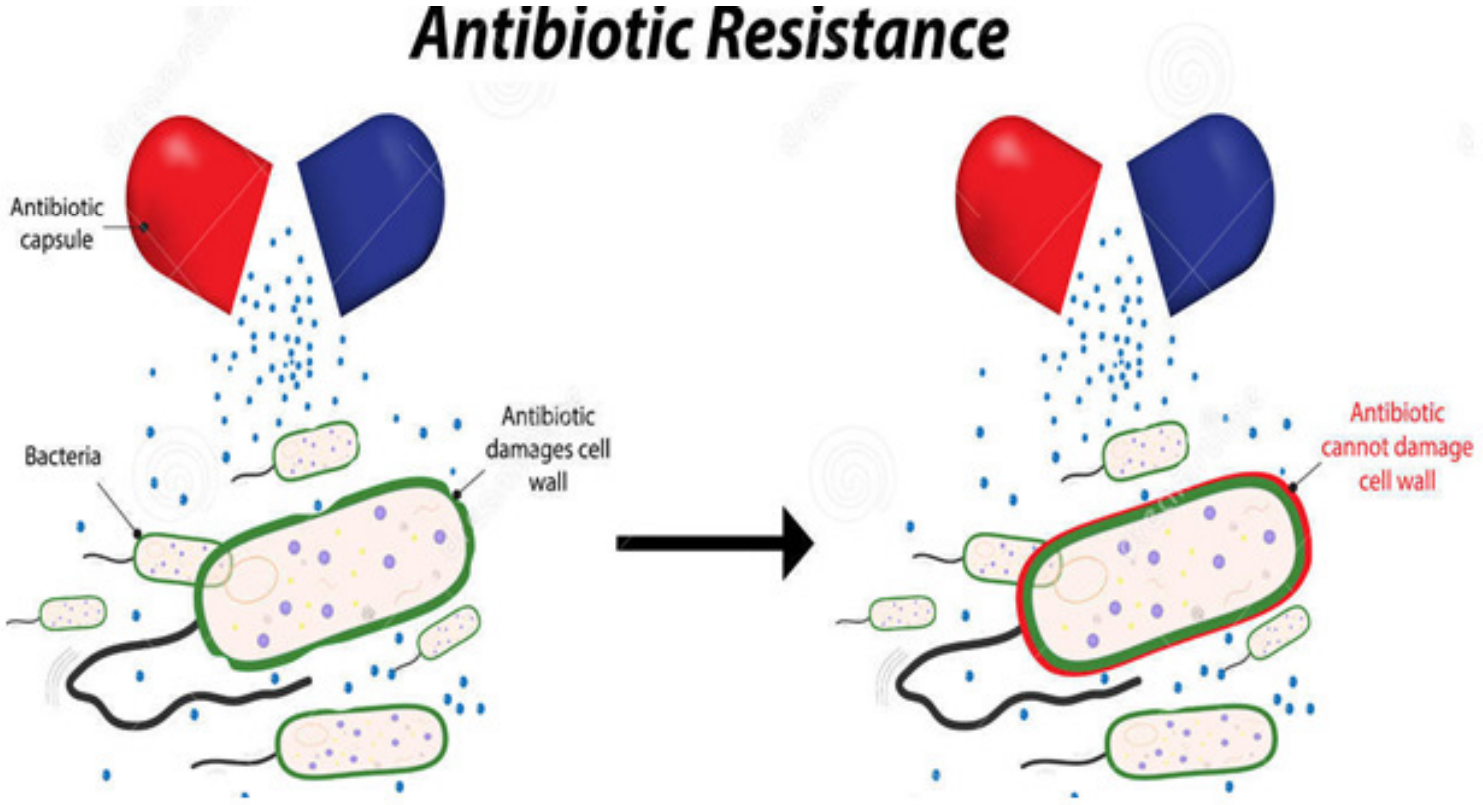
What is antibiotic resistance?

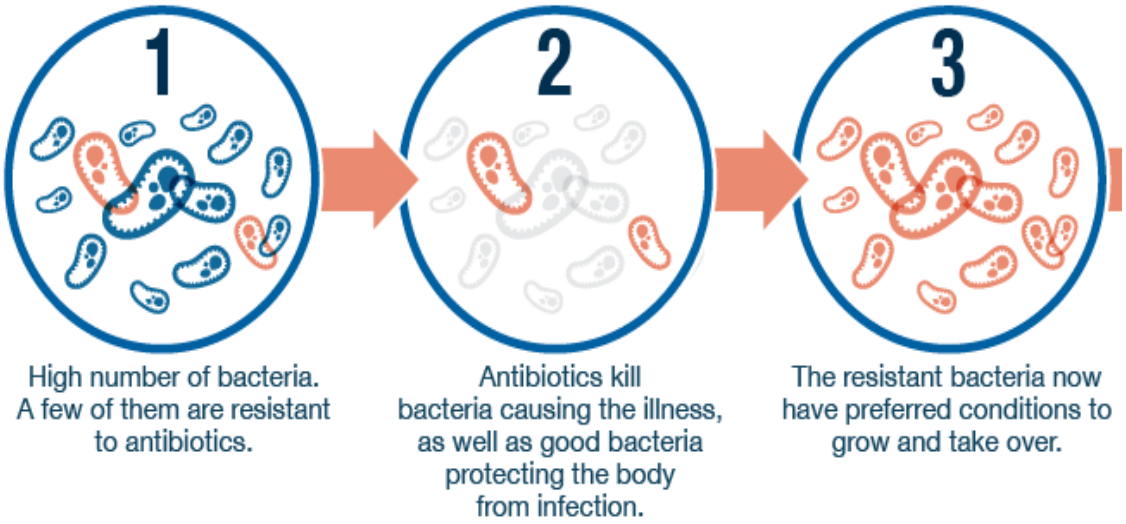
Antibiotic resistance occurs when an antibiotic has lost its ability to effectively control or kill bacte
growth



What are the causes of antibiotic resistance?

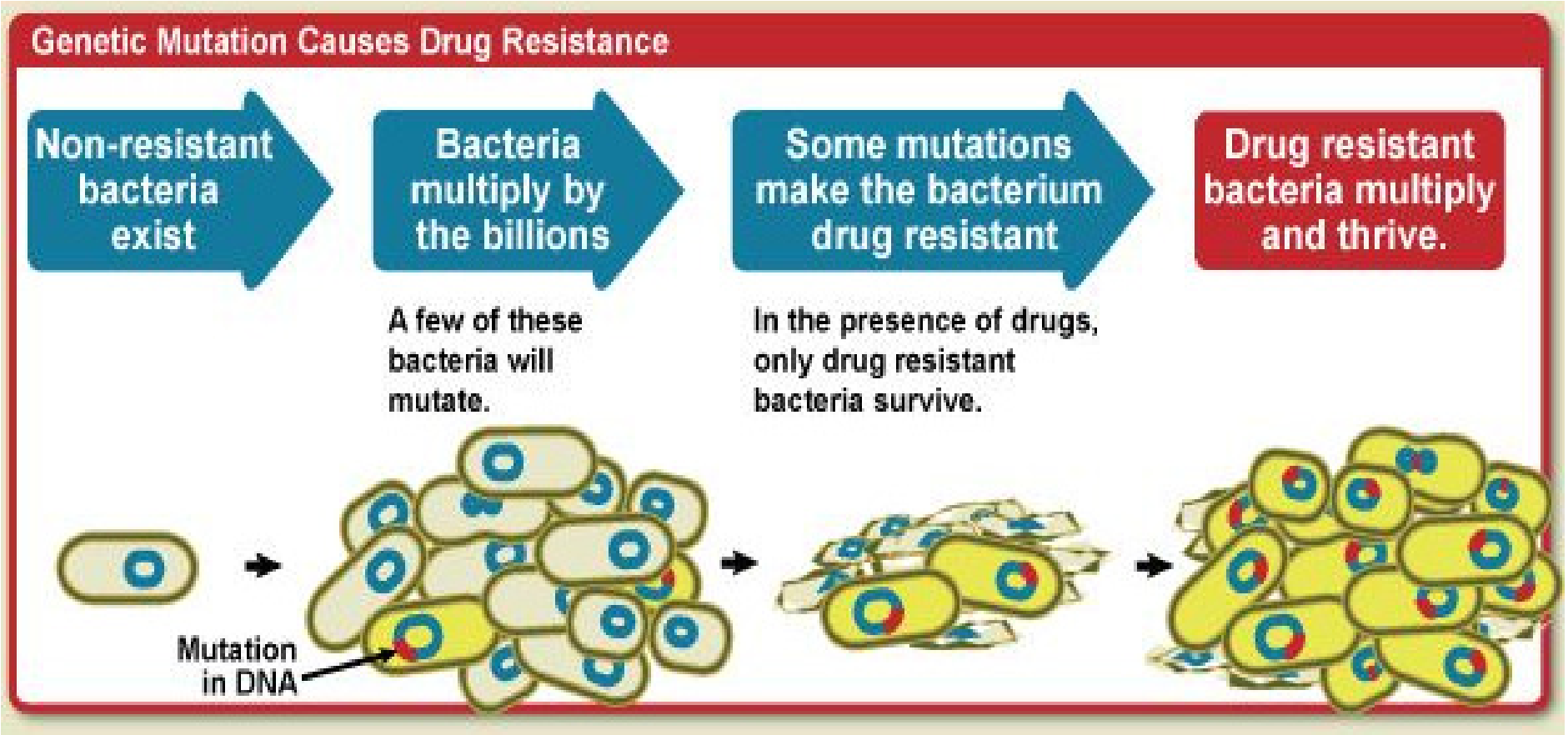
Selective Pressure

In the presence of an antimicrobial, microbes are either killed or, if they carry resistance genes, survive. These survivors will replicate, and their progeny will quickly become the dominant type throughout the microbial population.



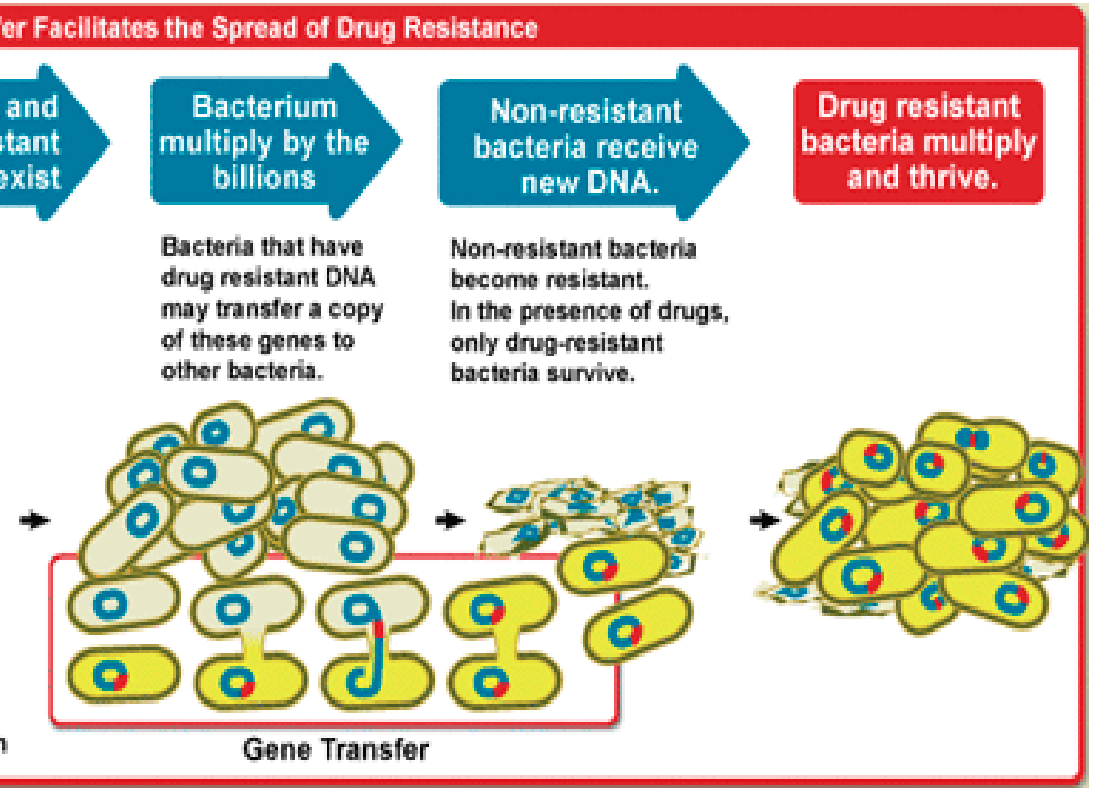
ation

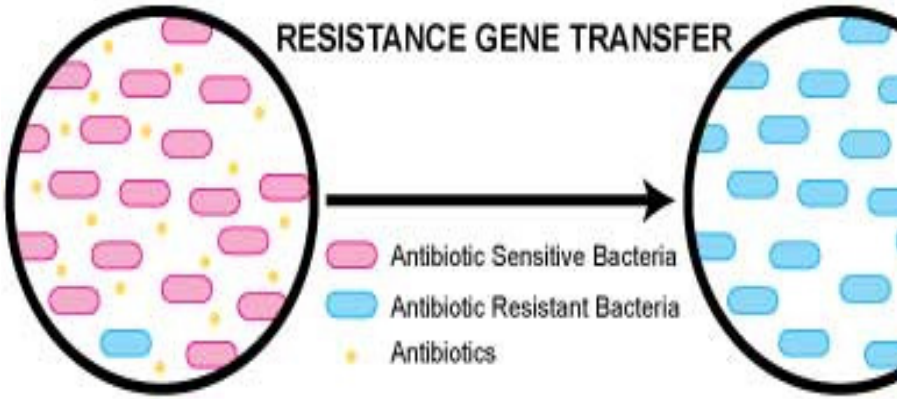
g replication, mutations arise and some of these mutations may help an individual microbe survive exposure t
crobial.



e Transfer

es also may get genes from each other, including genes that make the microbe drug resistant





ropriate Use

nes healthcare providers will prescribe robials inappropriately, wishing to pacify an insistent who has a viral infection or an as yet undiagnosed n.

quate Diagnostics

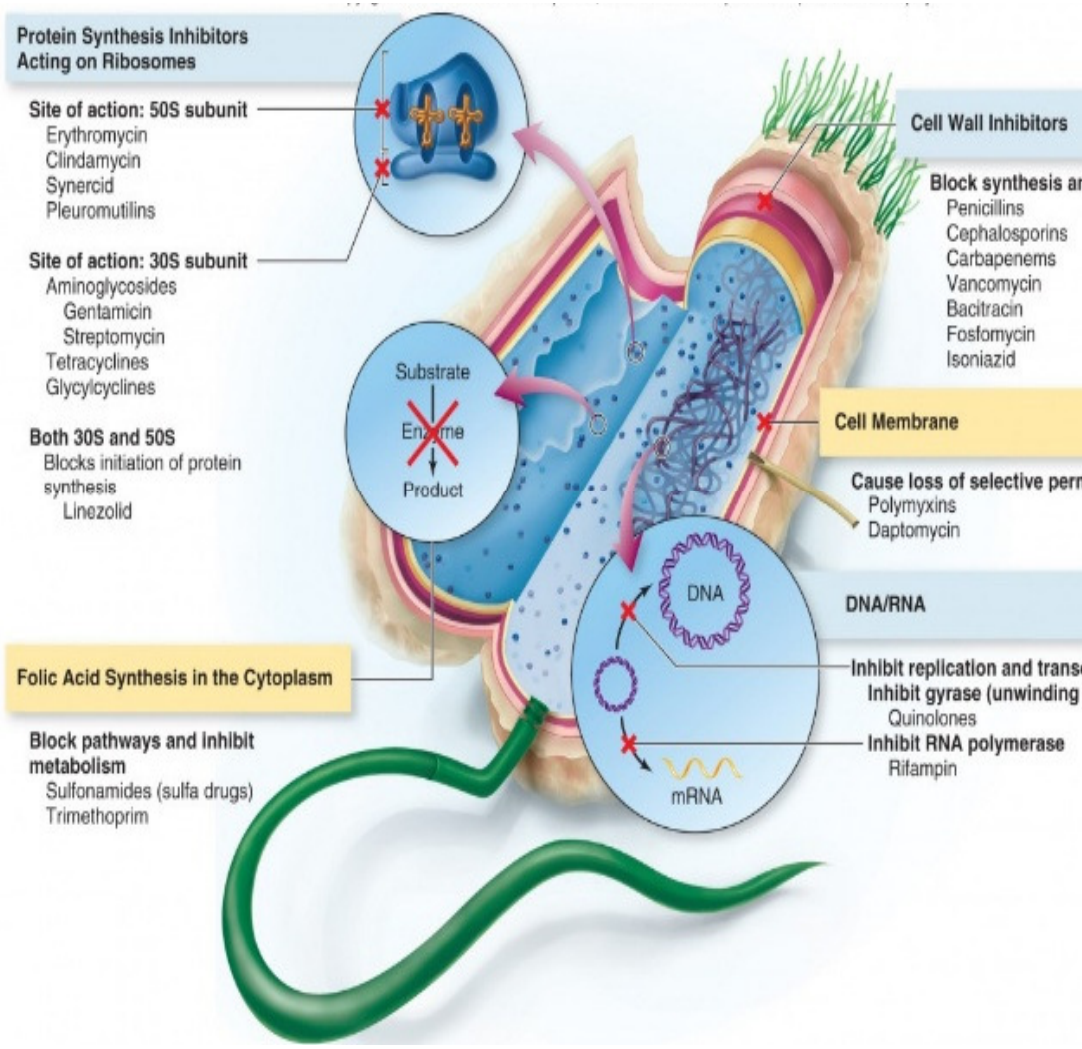
often, healthcare providers use incomplete or ct information to diagnose an infection and e a broad spectrum antimicrobial when a specific ic might be better. **These situations contribute to e pressure and accelerate antimicrobial resistance.**

tal Use

y ill patients are more susceptible to infections and, thus, often require the aid of antimicrobials. However, use of antimicrobials in these patients can worsen the problem by selecting for antimicrobial resis ganisms.

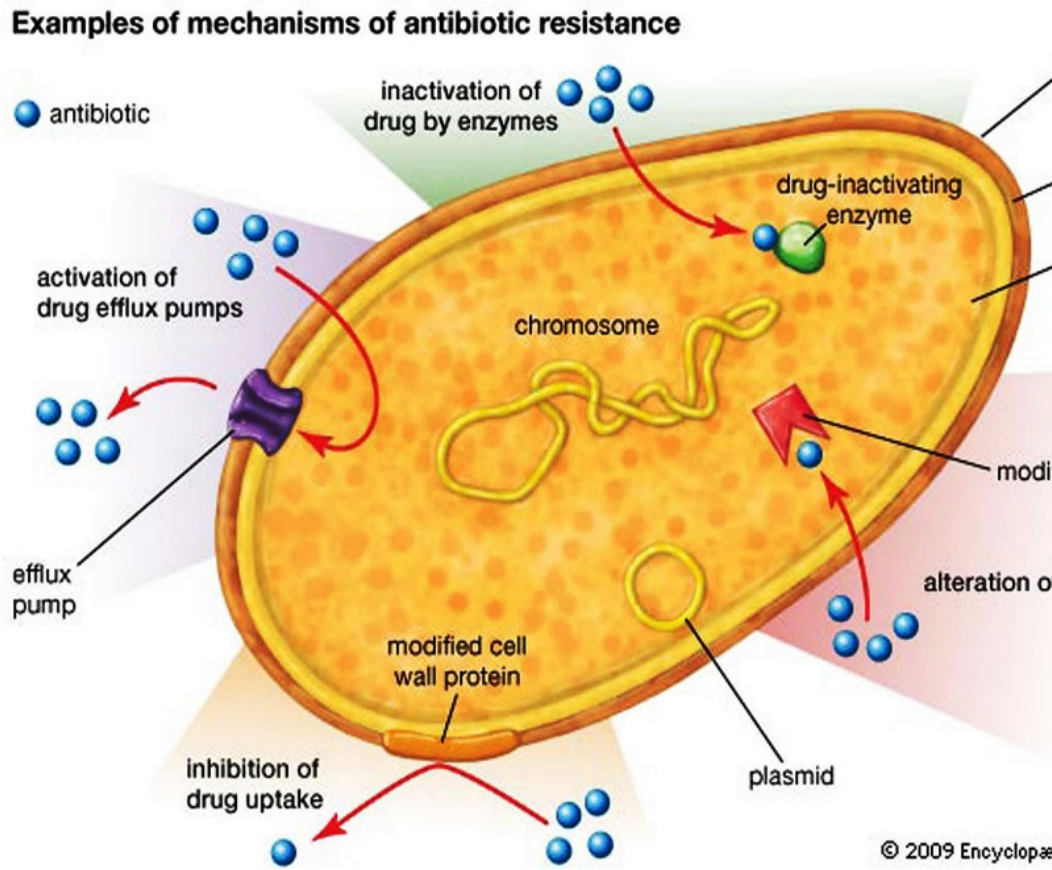
Antibiotics: Mechanism of action

1. Inhibition of cell wall synthesis
2. Inhibition of cell membrane function
3. Inhibition of protein synthesis
4. Inhibition of nucleic acid synthesis
5. Inhibition of bacterial enzymes

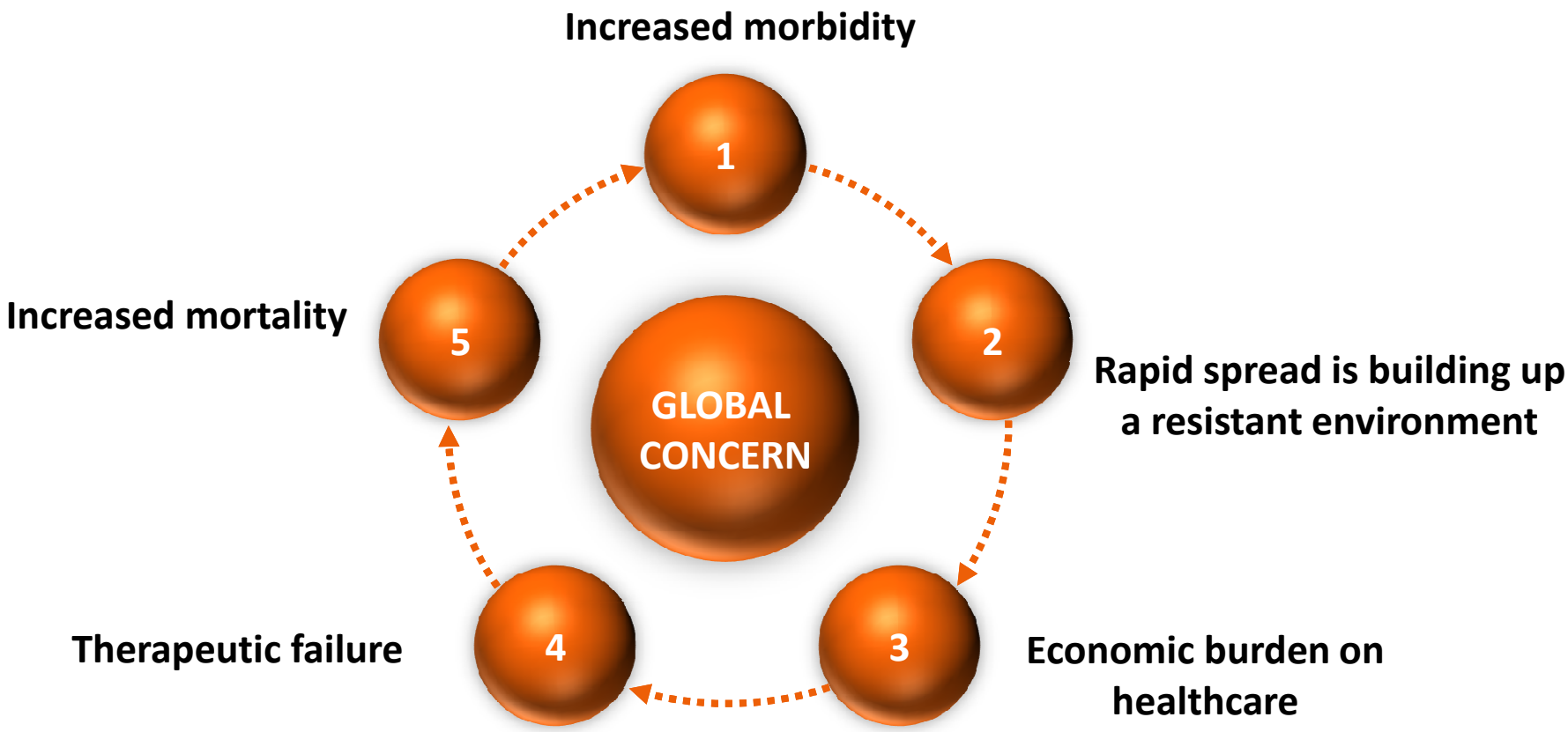


Bacteria: Mechanism of Resistance

- Activation of efflux pumps
- Modification of cell wall proteins (Porins)
- Alteration of target or binding sites
- Enzymatic inactivation of drugs (β- Lactamases)



Why is antibiotic resistance a global concern?



emergence of multidrug-resistant Gram-ve bacteria often present themselves as infections that are associated with high rates rtality.

penems, a class of **β-lactam antibiotics** that onsidered as **"the last line of antibiotic ce"** against MDR Gram-negative infections lso shown reports of resistance.

ded-spectrum β -lactamases (ESBLs) and o- β -lactamases (MBLs) are major nisms in bacteria conferring resistance t the majority of available antibiotics.

Hence, new strategies are in urgent need which can cross the line of resistance & are more efficient in combating resistant organisms.

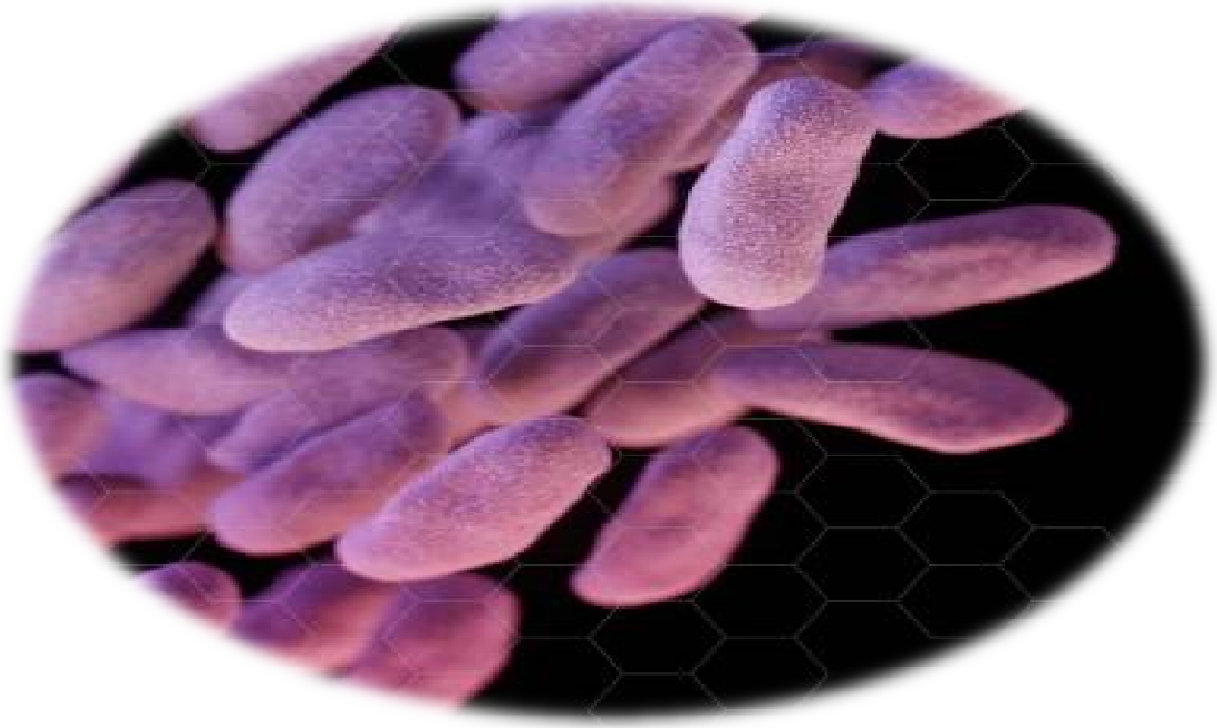
Monotherapy

It has long been implicated as an option to treat invasive infections

Combination therapy

An alternative to monotherapy for infections that do not respond to standard treatments

To explore novel combinations of antibiotics to inhibit extended-spectrum β-lactamases (ESBLs) and metallo- β-lactamases (MBLs) producers



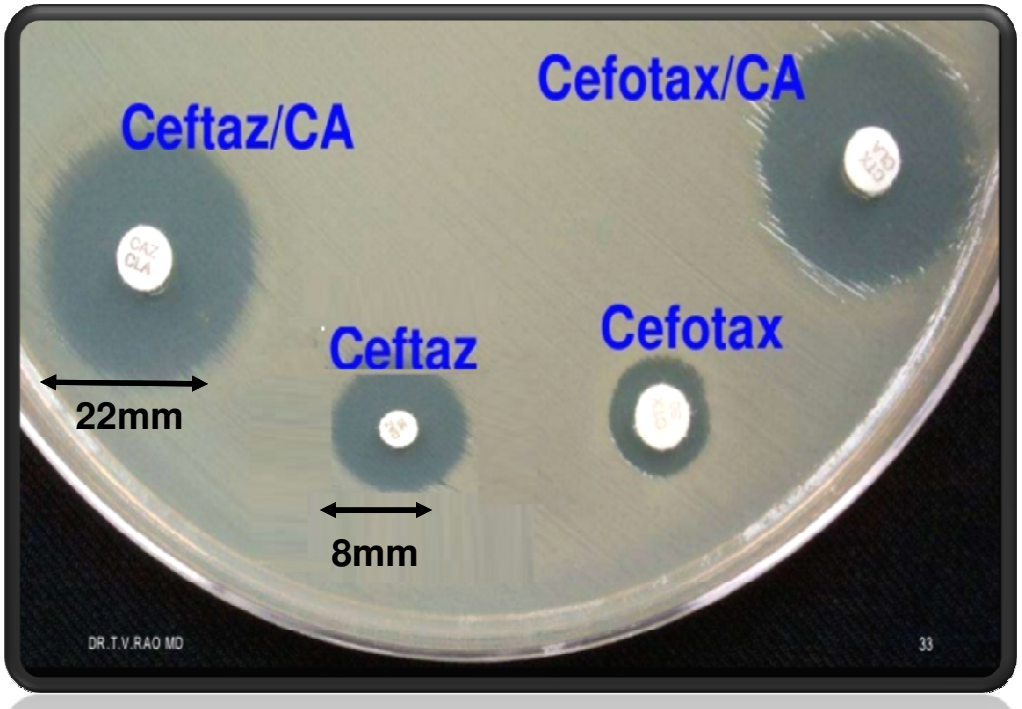
af **Hasan** and Asad U Khan (2013). Novel combinations of antibiotics to inhibit extended-spectrum β-lactam and metallo-β-lactamase producers in vitro: a synergistic approach. ***Future Microbiol***, 8: 939-944

- Samples were collected from nosocomial and community acquired infections However, this study includes 12 of those strains only.

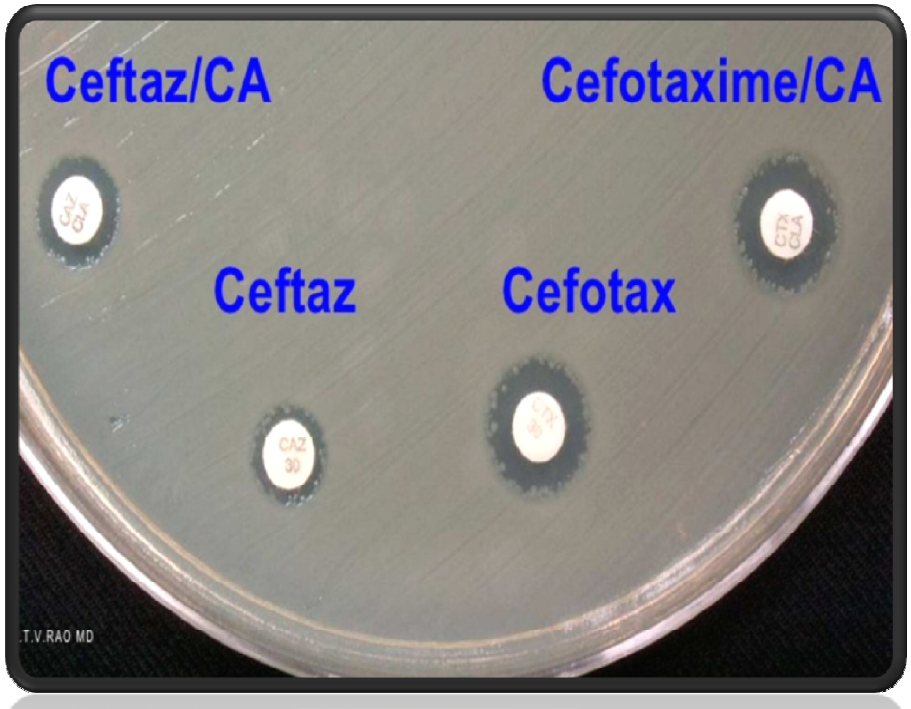
- These strains are well characterized by PCR amplification, Molecular typing and gene sequencing

- However, they were rechecked for ESBL and MBL production

ESBL Confirmatory Test
Positive

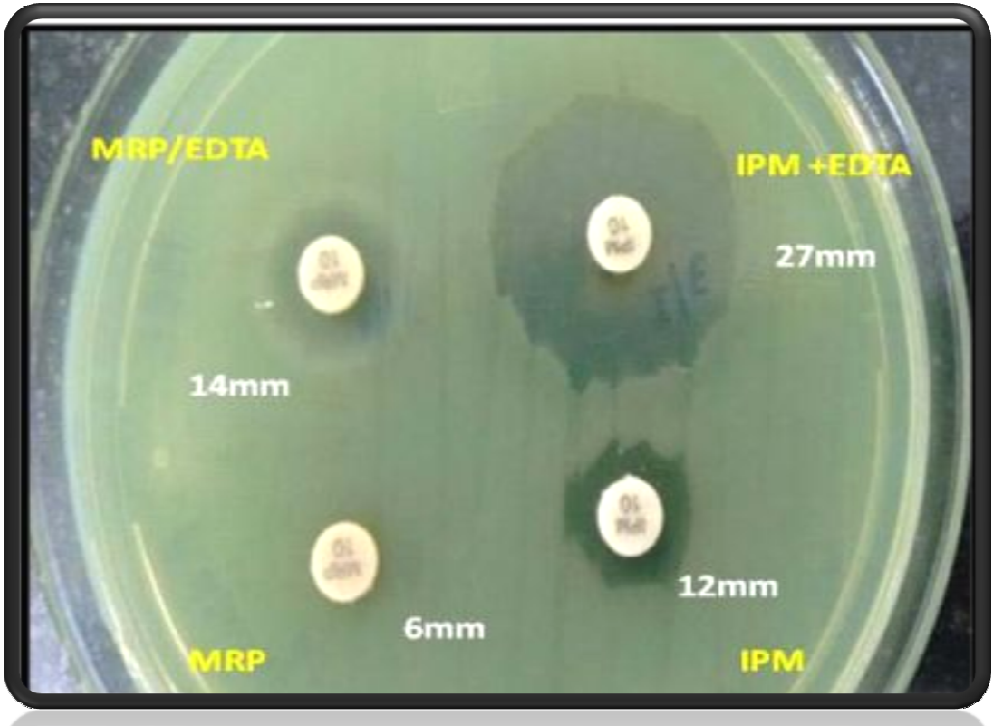


ESBL Confirmatory Test
Negative

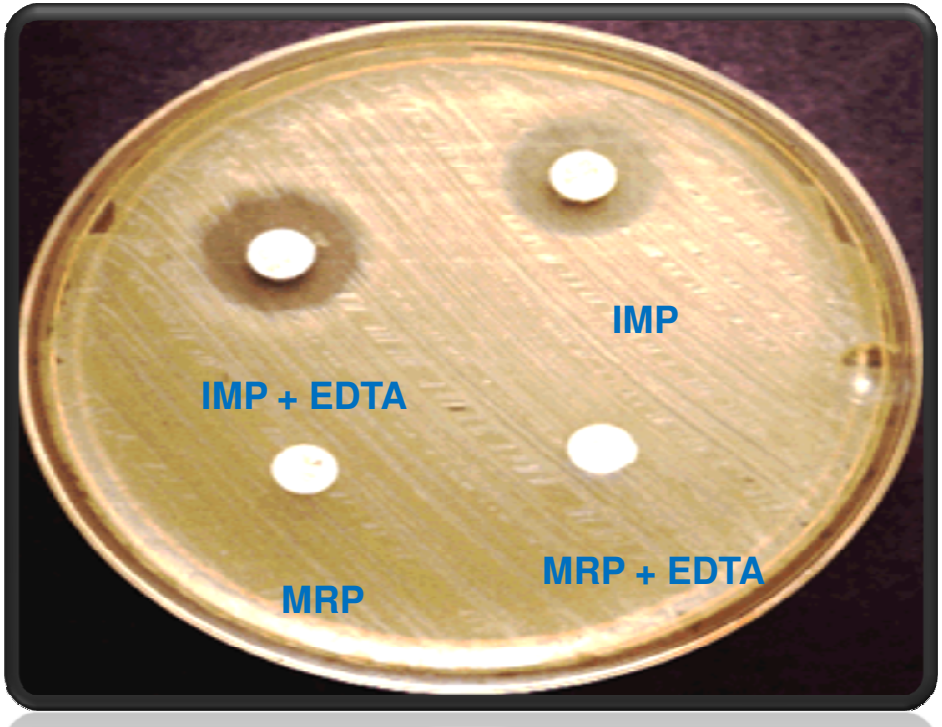


If there is a difference of ≥5mm in diameter of inhibition zone with a third generation cephalospor
combination with clavulanic acid (CA) compared with the antibiotic alone, confirms ESBL productio

MBL Confirmatory Test
Positive



MBL Confirmatory Test
Negative



e difference between zone of inhibition of IMP (or MRP) & IMP-EDTA (or MER-EDTA) is between 8-15m
nfirms MBL production.

ever, for MBL-negative isolates this difference will be between 1-5mm.

Characterized resistant markers in ESBL and MBL producing strains

Name of the organism	Strain no.	Resistance marker
E.coli	D8	bla_{CTX-15}
E.coli	D295	bla_{CTX-15}
E.coli	D253	bla_{CTX-15} and bla_{TEM-1}
K. pneumonia	KP113	bla_{CTX-3}, bla_{SHV-1} and bla_{TEM-1}
K. pneumonia	KP160	bla_{CTX-3}, bla_{SHV-1}, bla_{TEM-1}, bla_{OXA-1} and arm A
K. pneumonia	KP229	bla_{CTX-3}, bla_{TEM-1}
K. pneumonia	KP277	bla_{CTX-3}, bla_{TEM-1} and bla_{SHV-1}
K. pneumonia	**KP12**	bla_{CTX-15}, bla_{SHV-1}, bla_{TEM-1} and bla_{OXA-1}, **bla_{NDM-1}** and arm A
E. cloacae	**EC15**	bla_{CTX-15}, bla_{SHV-1}, bla_{TEM-1} and bla_{OXA-1}, **bla_{NDM-1}** and arm A

Plasmid encoded genes coding for β-lactamases

NDM-1: New Delhi Metallo Beta Lactamase
"The Superbug"

It was first detected in a *K.pneumonaie* isolate from a Swedish patient of Indian origin in 2009

DM-1(New Delhi metallo-ß-lactamase-1) is the gene that codes for metallo-beta-lactamase know
arbapenemase".

his drug inactivating enzyme (carbapenemase) cleaves the β lactam ring of carbepenem antibi
aking them ineffective. Hence, is virtually resistant to all antibiotics.

rbapenem antibiotics **(antibiotics of last resort).** These were considered as extremely powe
tibiotics and used to fight highly resistant bacteria (where other antibiotics have failed to work).

**bacterium with the NDM-1 gene has the potential to be resistant to nearly ALL CURRE
NTIBIOTICS that we have.**

RSA FALLS BUT BA

Help sto
sup

Superbug gene
Discovered in pneumonia and E.coli bugs resistant to last-line antibiotics

superbug in UK

hi metallo- -lactamase-1, or
or short, is an enzyme that can
e different bacteria. Any bacteria
it will be resistant to antibiotics

es where NDM-1 has spread

Islam
tan

Four more infected by new superbu

They are likely to have ... ter a two-month stay. The NDM-1 bacte- ... Cases have emerged in Britain, the ... other health problems that made them ... on top of the situation"

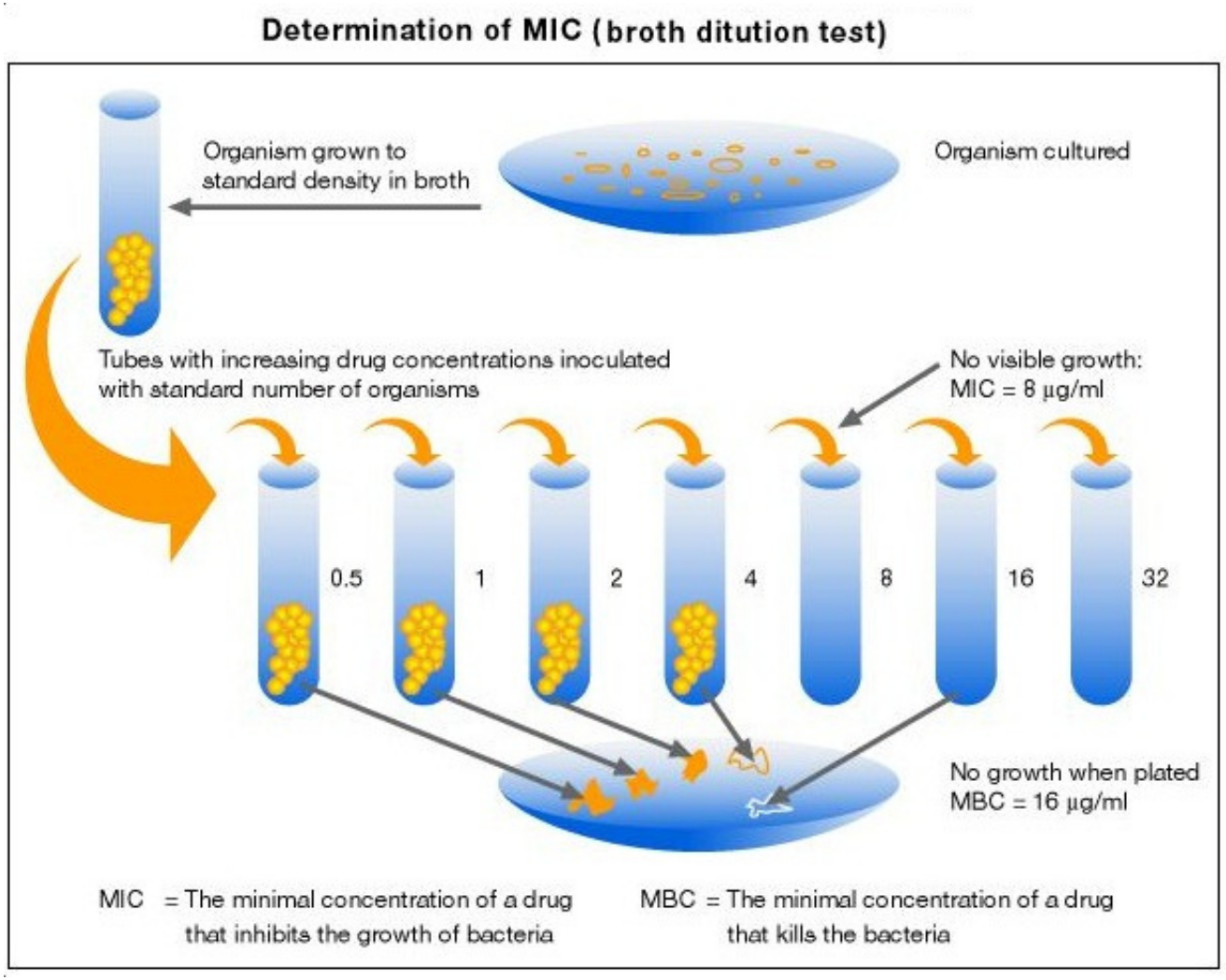
THE OSCILLATING BUG TRAIL

- **NDM-1 first identified in 2009 by Cardiff University researchers from bacteria taken from Swedish patient in Delhi**
- ***In August 2010, British researchers publish study which pointed to NDM-1 presence in patients from hospitals in Chennai, Haryana and other places***
- **Same group comes up with another study in April 2011, which showed the presence of the superbug in open water pools, sewage and drinking water samples collected from Delhi**
- ***British group concludes NDM-1 is not just a hospital-based infection but has spread to the community***
- **Indian scientists publish study showing no presence of NDM-1 in samples drawn from 1,000 healthy individuals**

Resistance mechanisms acquired by extended-spectrum β-lactamase (ESBLs) & metallo-β-lactamase (MBL) producing strains

Organism	Bacterial strain	Resistance markers	Resistance mechanisms
Escherichia coli	D8	bla_{CTX-15}	Hydrolysis of β-lactam antibiotics
	D253	bla_{CTX-15}	Hydrolysis of β-lactam antibiotics
	D295	bla_{CTX-15} and bla_{TEM-1}	Hydrolysis of β-lactam antibiotics
Klebsiella pneumoniae	KP113	bla_{CTX-3}, bla_{SHV-1} and bla_{TEM-1}	Hydrolysis of β-lactam antibiotics
	KP160	bla_{CTX-3}, bla_{SHV-1}, bla_{TEM-1}, bla_{OXA-1} and *armA*	Hydrolysis of β-lactam antibiotics Oxacillinase production to hydrolyze oxacillin Methylation of 16S rRNA to modify the target site
	KP229	bla_{CTX-3}, bla_{TEM-1}	Hydrolysis of β-lactam antibiotics
	KP277	bla_{CTX-3}, bla_{TEM-1} and bla_{SHV-1}	Hydrolysis of β-lactam antibiotics
	KP12	bla_{CTX-15}, bla_{SHV-1}, bla_{TEM-1}, bla_{OXA-1}, bla_{NDM-1} and *armA*	Hydrolysis of β-lactam antibiotics Hydrolysis of β-lactam antibiotics, oxacillinase and carbapenemase production Methylation of 16S rRNA to modify the target site
Enterobacter cloacae	EC15	bla_{CTX-15}, bla_{SHV-1}, bla_{TEM-1}, bla_{OXA-1}, bla_{NDM-1} and *armA*	Hydrolysis of β-lactam antibiotics Hydrolysis of β-lactam antibiotics, oxacillinase and carbapenemase production Methylation of 16S rRNA to modify the target site

In microbiology, **minimum inhibitory concentration** (**MIC**) is the **lowest concentration** of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation



C values of antibiotics tested against clinical MDR isolates by the broth microdilution method

anism	Bacterial strain	Resistance markers	Group of antibiotics													
			Aminoglycosides		*β-lactams*						*Broad spectrum (TET)*	*Cephalosporins*			*Carbapenems*	
			STR	*KAN*	*AMP*	*AMX*	*PIP*	*OXA*	*CLX*	*TIC*		*CTX*	*CRO*	*FOX*	*IPM*	*MER*	*ETP*
erichia coli	D8	bla_{CTX-15}	256	128	256	256	256	256	256	256	128	512	256	256	2	2	1
	D253	bla_{CTX-15}	64	64	256	256	256	256	256	256	256	256	256	256	2	2	2
	D295	bla_{CTX-15} and bla_{TEM-1}	128	64	128	128	256	128	256	128	64	256	256	128	2	4	2
siella umoniae	KP113	bla_{CTX-3}, bla_{SHV-1} and bla_{TEM-1}	64	128	256	128	64	64	64	128	32	256	256	256	8	8	8
	KP160	bla_{CTX-3}, bla_{SHV-1}, bla_{TEM-1}, bla_{OXA-1} and *armA*	128	256	512	256	256	512	256	256	128	256	256	256	16	8	8
	KP229	bla_{CTX-3}, bla_{TEM-1}	32	64	256	128	32	32	64	64	64	128	128	128	8	4	4
	KP277	bla_{CTX-3}, bla_{TEM-1} and bla_{SHV-1}	128	128	256	256	64	64	64	128	64	256	256	256	16	8	8
	KP12	bla_{CTX-15}, bla_{SHV-1}, bla_{TEM-1}, bla_{OXA-1}, bla_{NDM-1} and *armA*	512	256	512	512	512	512	512	512	256	512	512	256	8	8	16
robacter cae	EC15	bla_{CTX-15}, bla_{SHV-1}, bla_{TEM-1}, bla_{OXA-1}, bla_{NDM-1} and *armA*	256	256	256	512	256	512	512	256	128	256	512	256	8	16	8

lues are shown in µg/ml.
Ampicillin; AMX: Amoxicillin; CLX: Cloxacillin; CRO: Ceftriaxone; CTX: Cefotaxime; ETP: Ertapenem; FOX: Cefoxitin; IPM: Imipenem; KAN: Kanamycin; MER: Meropenem; OXA: Oxacillin; PIP: Piperacillin; Streptomycin; TET: Tetracycline; TIC: Ticarcillin.

Synergy Testing

Checkerboard assay

Fractional Inhibitory Concentration Index (FICI)

$$\text{FIC of drug A} = \frac{\text{(MIC of drug A in combination)}}{\text{(MIC of drug A alone)}}$$

$$\text{FIC of drug B} = \frac{\text{(MIC of drug B in combination)}}{\text{(MIC of drug B alone)}}$$

FICI = FIC of drug A + FIC of drug B

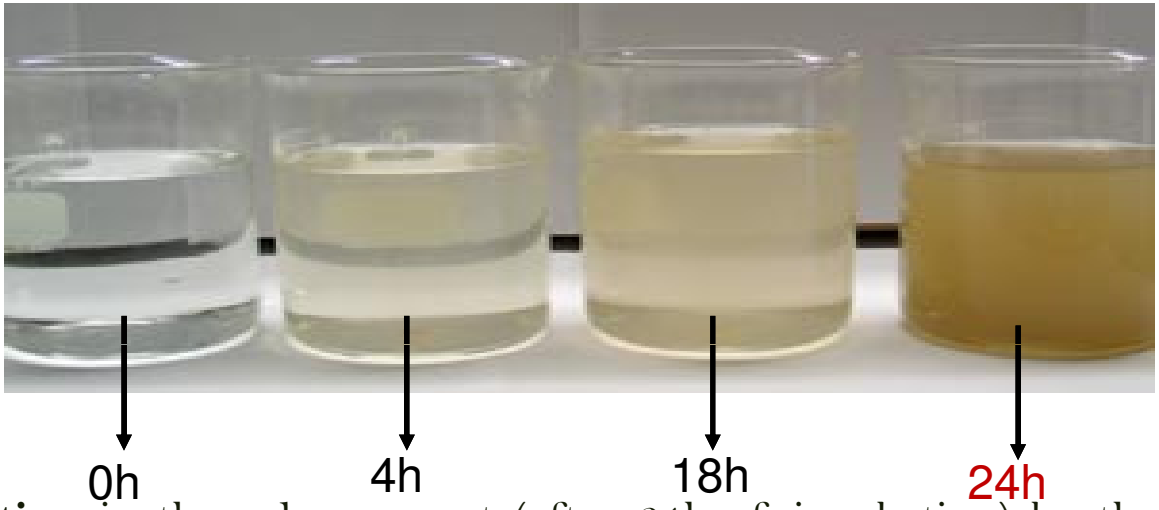
FICI<0.5 = synergy (our interest)

FICI > 0.5 ≤4 = no interaction

FICI >4 = antagonism

Time kill assay

Time-kill combinations			
2× MIC + 2× MIC†	2× MIC + 1/4× MIC‡	1/4× MIC + 2× MIC§	1/4× MIC + 1/4× MIC¶



gy: ≥ **100 fold reduction** in the colony count (after 24h of incubation) by the combination as compared **active agent** & ≥ **100- fold reduction** in the colony count (after 24h of incubation) as compared to the **m.**

rence: ≤10 fold or less reduction in the colony count (after 24h of incubation) by the combination as comp
le active agent.

nism: : ≥ **100 fold increment** in the colony count (after 24h of incubation) by the combination as compared **ctive agent.**

Potential synergistic combinations determined by checkerboard and time-kill assays showing cefoxitin as an active partner

Bacterial strain	Antibiotic combination	Checkerboard FICI (FICI ≤0.5)	Time-kill combinations			
			2× MIC + 2× MIC†	2× MIC + 1/4× MIC‡	1/4× MIC + 2× MIC§	1/4× MIC + 1/4× MIC¶
Escherichia coli (D8)	FOX# + STR	0.18 (S)	S	S	S	S
E. coli (D295)	FOX + STR	0.18 (S)	S	S	I	S
E. coli (D253)	FOX + STR	0.25 (S)	I	S	S	S
Klebsiella pneumoniae (KP113)	FOX + STR	0.31 (S)	S	S	S	I
K. pneumoniae (KP160)	FOX + STR	0.36 (S)	S	S	I	S
K. pneumoniae (KP229)	FOX + STR	0.28 (S)	I	S	S	S
K. pneumoniae (KP277)	FOX + STR	0.31 (S)	S	S	S	S
K. pneumoniae (KP12)	FOX + CTX	0.50 (S)	I	S	S	I
Enterobacter cloacae (EC15)	FOX + CTX	0.50 (S)	I	S	S	S

†Combination of 2× MIC of FOX and 2× MIC of STR or CTX.
‡Combination of 2× MIC of FOX and 1/4× MIC of STR or CTX.
§Combination of 1/4× MIC of FOX and 2× MIC of STR or CTX.
¶Combination of 1/4× MIC of FOX and 1/4× MIC of STR or CTX.
#FOX is present in every given combination as an active partner.
CTX: Cefotaxime; FICI: Fractional inhibitory concentration index; FOX: Cefoxitin; I: Indifference; S: Synergy; STR: Streptomycin.

Cefoxitin (FOX)

refractory against the hydrolytic activity of active site of β lactamases.

X is known to be a poor substrate to TEM-1, which doesn't allow the formation of an effective EN
STRATE complex.

X is known to induce conformational changes in the structure if enzyme, leading to its proteolytic digestion

catalytic efficiency of NDM is lowest for FOX as compared to CTX, MER or IPM.

Mechanism conjectured for the combination of FOX-CTX

OX

↓

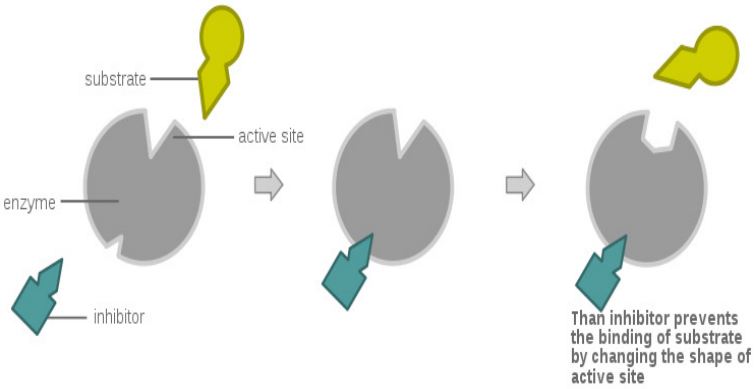
active site

↓

ional changes

↓

eactivation



CTX

↓

Will not be hydrolyzed

↓

Interfere with the peptidoglycan synthesis

↓

Destroy the bacteria

Mechanism for FOX-

FOX will disrupt peptidoglycan synt

↓

Allow rapid entry of

↓

STR will Inhibit pr synthesis

↓

Cell death

Summary

from monotherapy to combination therapy

ination therapy has proved to be a substitute for monotherapy for infections that fail to respond to standard ments. This approach involves a mechanism of synergy to combat these infections.

robable line of action in synergy is the combined action of different mechanisms of the antimicrobials, which uce an effect greater than the sum of their individual effects.

stic combinations

ombinations **cefoxitin-streptomycin** (ESBL) and **cefoxitin-cefotaxime** (MBL) were proven to be potential inations against multidrug-resistant strains.

ombination **cefoxitin-cefotaxime** was effective specifically against NDM-1-producing strains

Future perspective

gly propose these combinations for a possible empirical therapy against extended-spectrum β-lactamase and β-lactamase producers, where the use of single drug is ineffective.

hank you for your attentio