In vitro and *in vivo* evaluation of therapeutic efficacy of phages against multidrug resistant *Staphylococcus aureus* (MDRSA).

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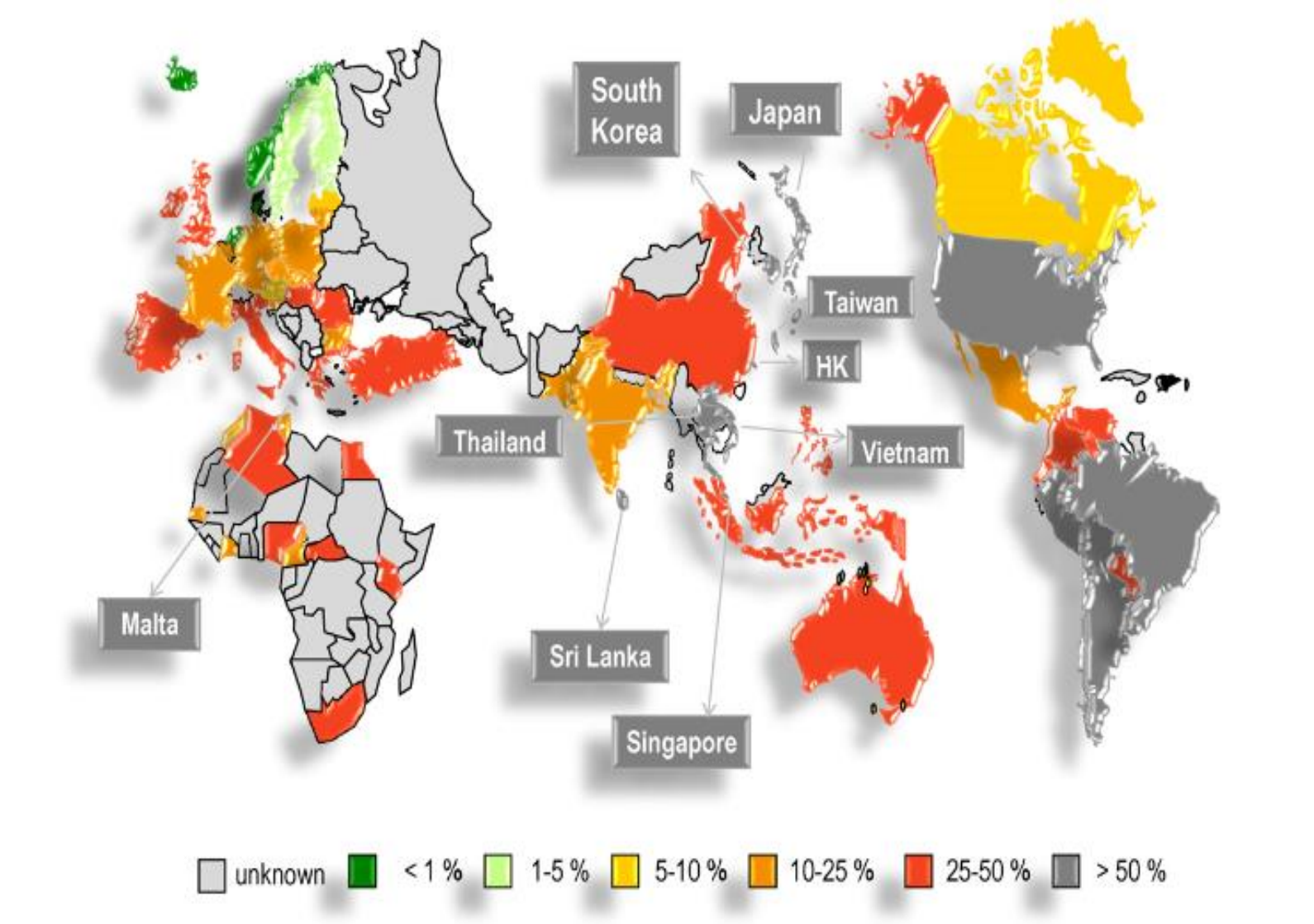
Introduction

- Multidrug resistant *S. aureus (*MDRSA) are emerging zoonotic pathogens.
- The infection is associated with high mortality rates but, there is shortage of novel antibiotics against the pathogen.
- *S. aureus* has reduced susceptibility to methicillin and a number of many other antibiotics currently available. Emergence of multi-drug resistant bacteria (MDR).
- Bacteriophages (phages): prokaryotic viruses that infect and devour bacteria (lytic phages).
- Are being used as therapeutic agent as Eastern Europe and renewed interest in USA.
- Phage therapy is considered as the option to antibiotics but, its efficacy and safety has been a subject of debate over the years .

(Sulakvelidze *et al*., 2001; Wittebole *et al.*, 2014)

Global prevalence of MDRSA



(Stefani *et al.*, 2012)

Is phage the best alternative to antibiotics?

Bacteriophages (phage therapy)	Antibiotics
All are bactericidal	Few are bacteriocidal
Fast and cheap to produce	Complex and expensive
“Intelligent drugs”	Non-localized
Auto dosing	Repeated administration
Highly specific.	Non-specific/broad spectrum.
Human microbiome	Pose adverse side effect
Used for a century	Used for seven decades

(Sulakvelidze *et al.*, 2001 & Chhibber *et al*., 2012)

General objective

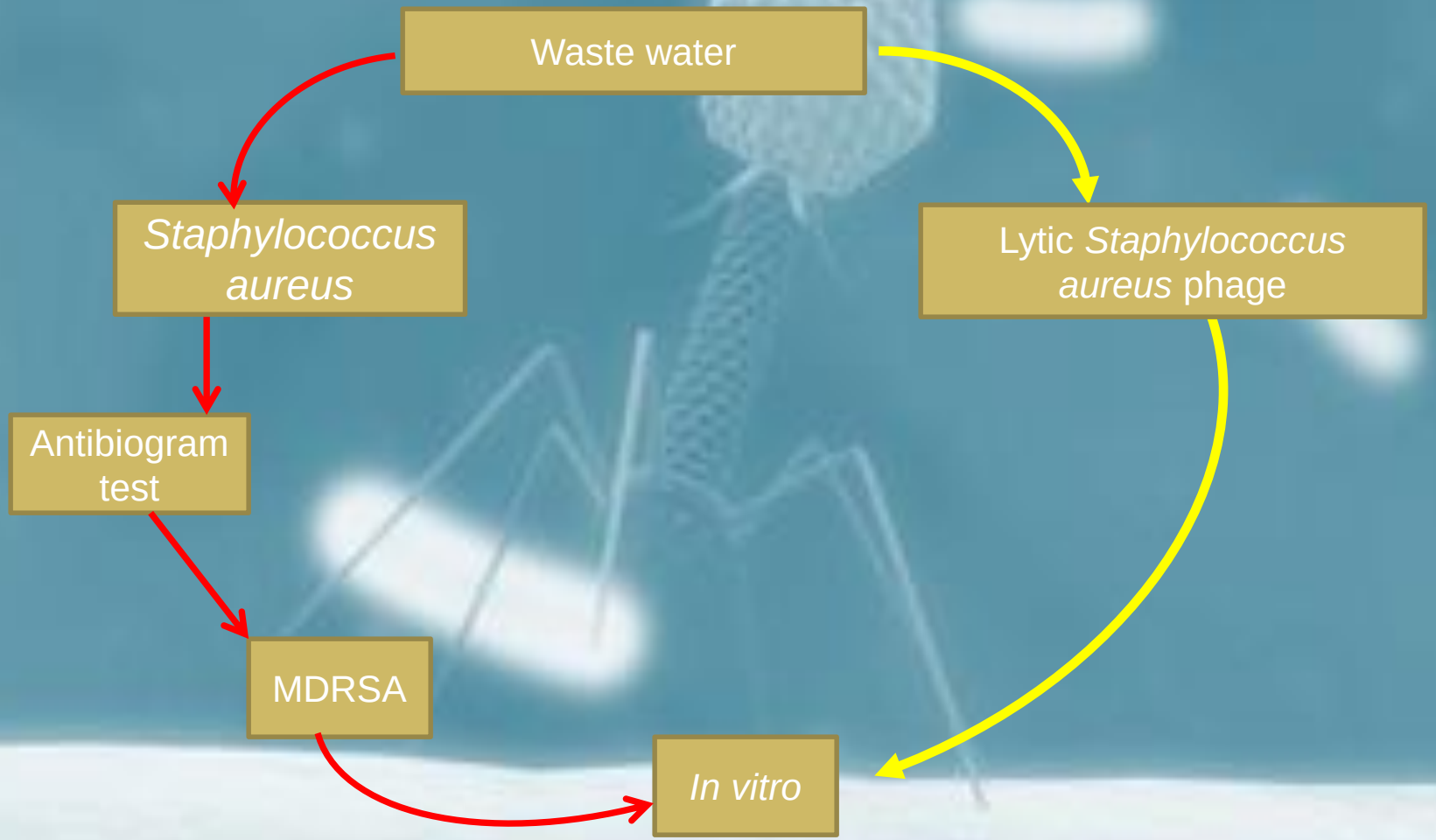
To evaluate the safety and efficacy of environmentally obtained lytic phages against MDRSA isolates.

Specific objective

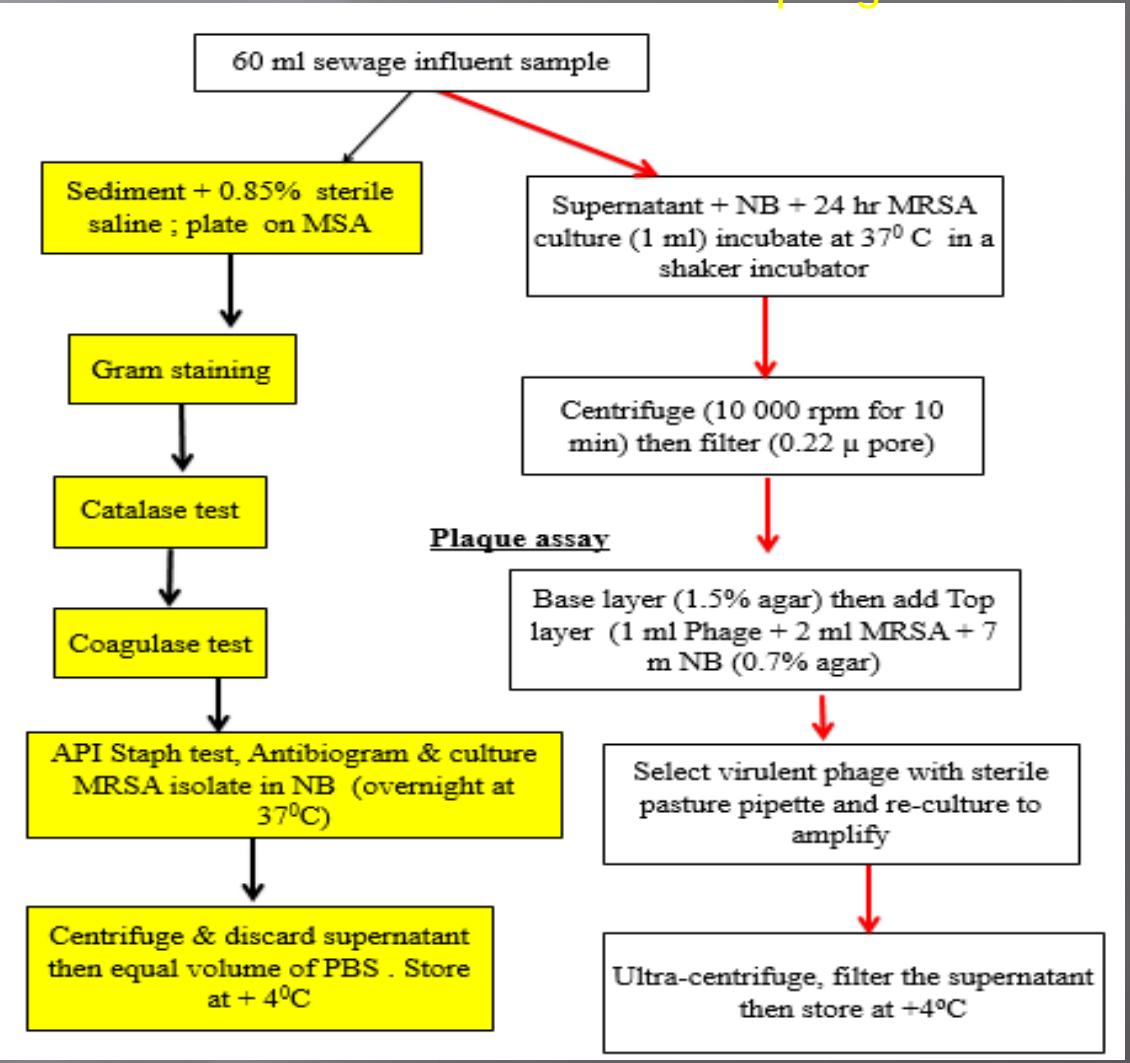
- To determine the presence of MDRSA isolate from environmental waste water and sewage drainage systems of Nairobi County.
- To determine the availability of lytic phage against environmental MDRSA isolate from Nairobi County.
- To evaluate the efficacy and safety of phage therapy against environmental MDRSA isolate from Nairobi County *in vivo* in BALB/c mice.

Methodology

Study I (*in vitro*)



Isolation of bacteria and phage



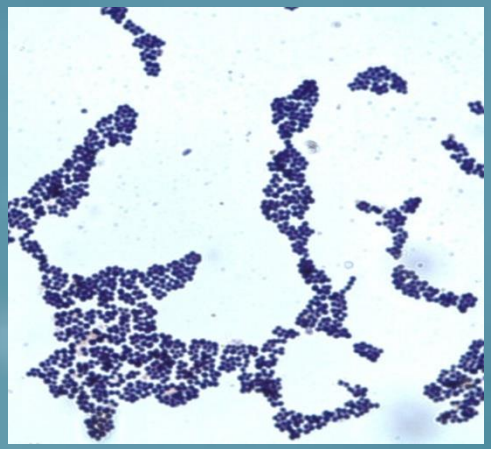
Spot assay of phages

Dispense 10 µl of different phage isolates to MRSA lawn on nutrient agar & incubate overnight at 37^{0}C .

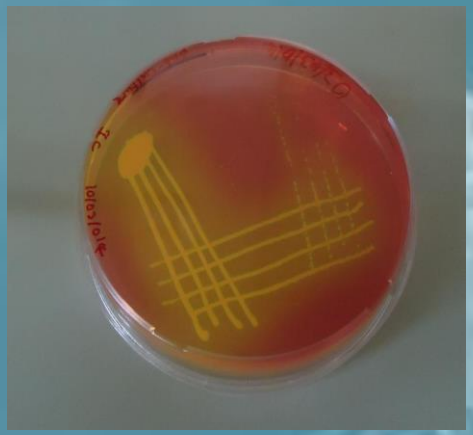
***In vitro* test**

Culture 1ml of MRSA (24 hrs old) with phage (100 µl) in NB of desired volume & incubate overnight at 37^{0}C.

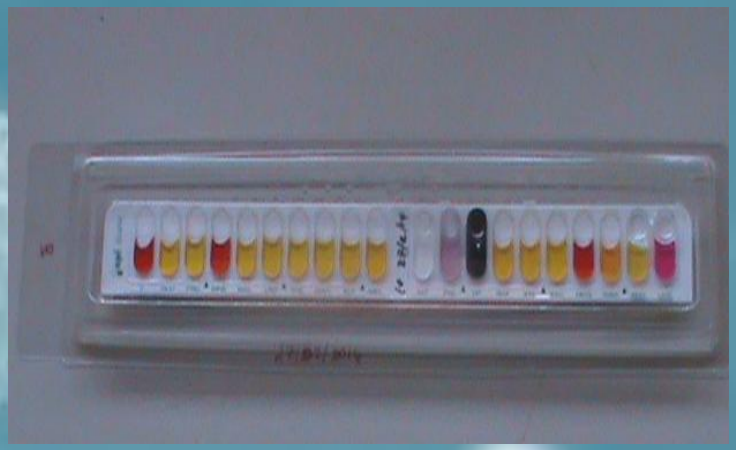
Isolation of MDRSA



A gram stain of isolated bacteria colonies

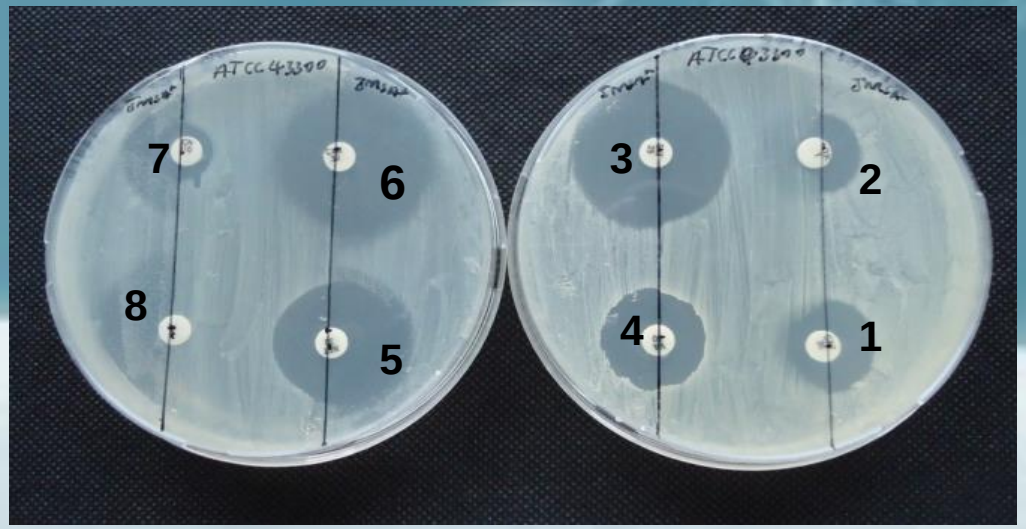


A culture of *S.aureus* in mannitol salt agar



Positive API confirmatory test for *S.aureus*

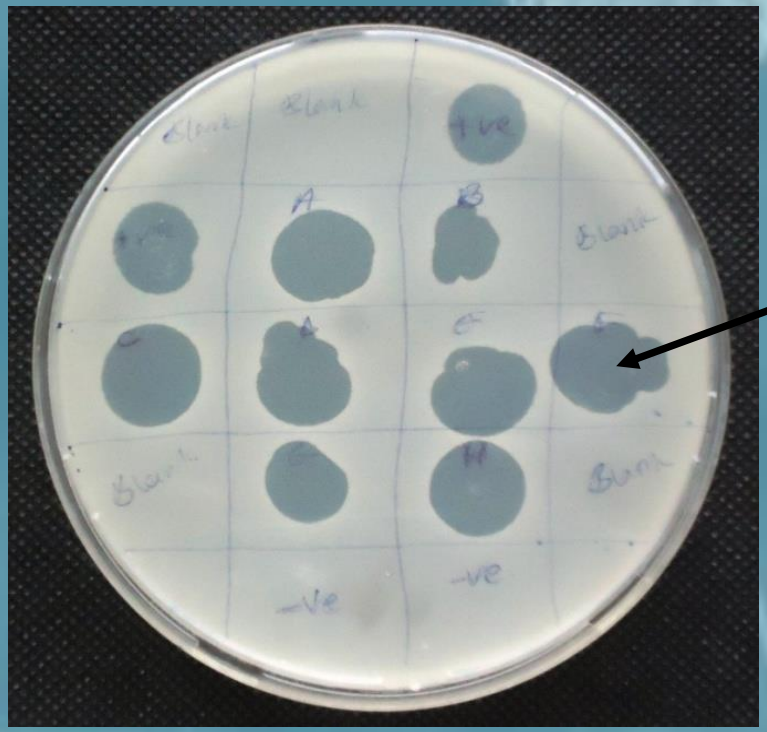
Antibiogram test



1. Ceftazidime (CAZ) 30µg (**R**).
2. Oxacillin (OX) 1 µg (**R**).
3. Cotrimoxazole (SXT) 25 µg (**S**).
4. Vancomycin (VAN) 30 µg (**R**).
5. Netilmicin (NET) 30 µg (**R**).
6. Cefuroxime (CXM) 30 µg (**S**).
7. Gentamicin (CN) 10 µg (**R**).
8. Erythromycin (E) 15 µg (**R**).

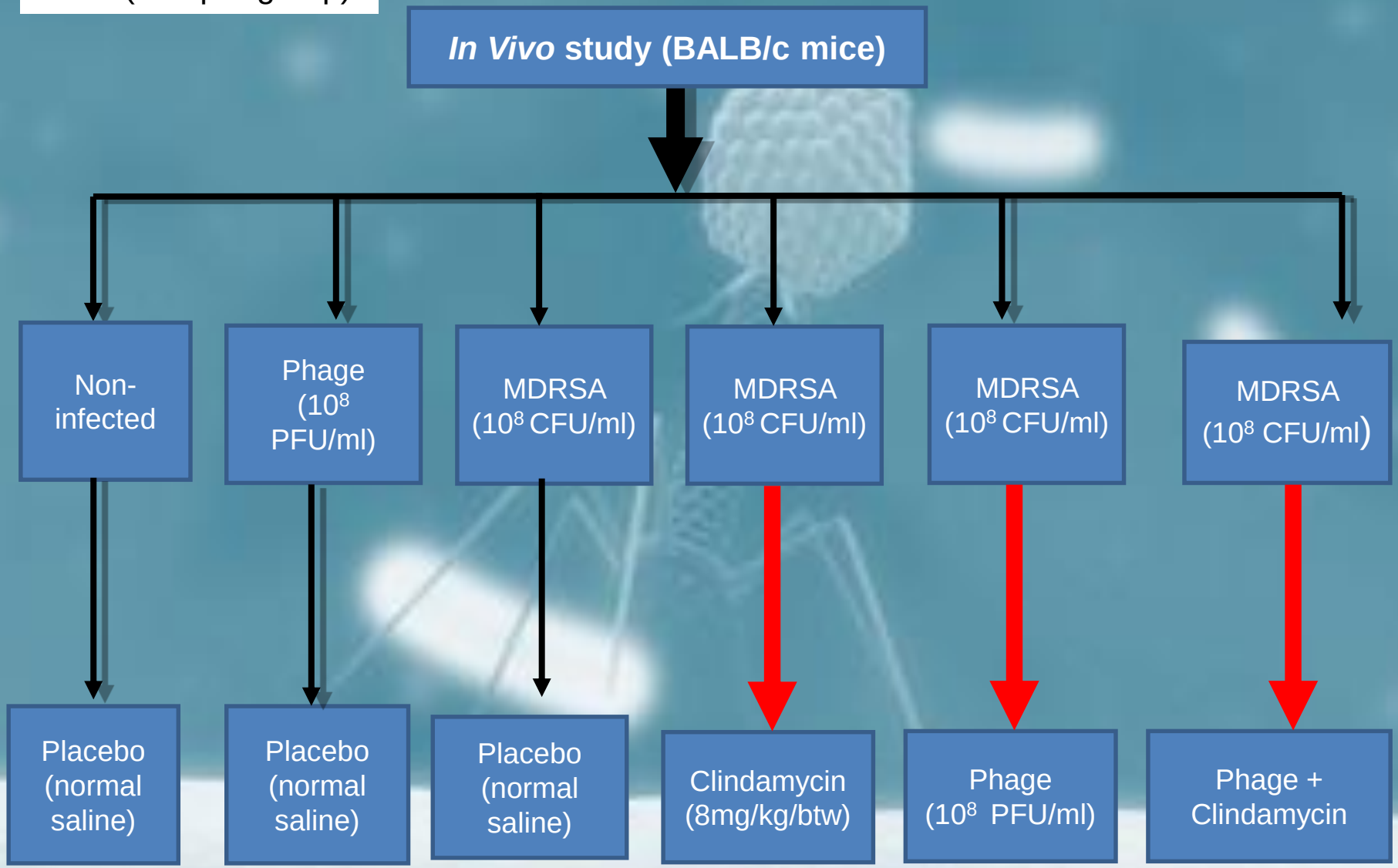
Isolation phages and their *in vitro* antibacterial activities

- Eight potent lytic phages were isolated i.e. A, B, C, D, E, F, G & H.
- One was most virulent (F ~ **x**).

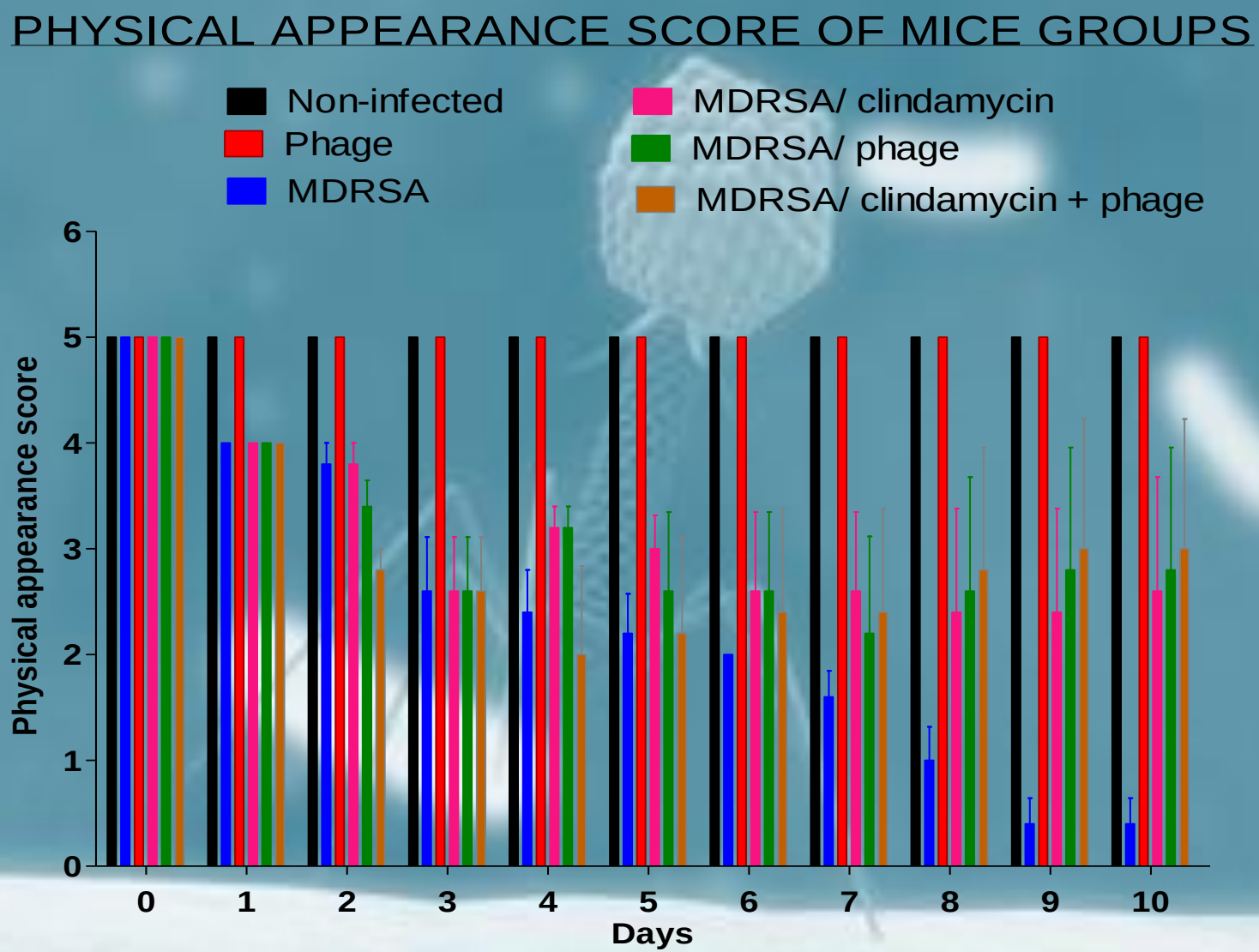


Study design II (*in vivo*)

N=30 (n=5 per group)



Results

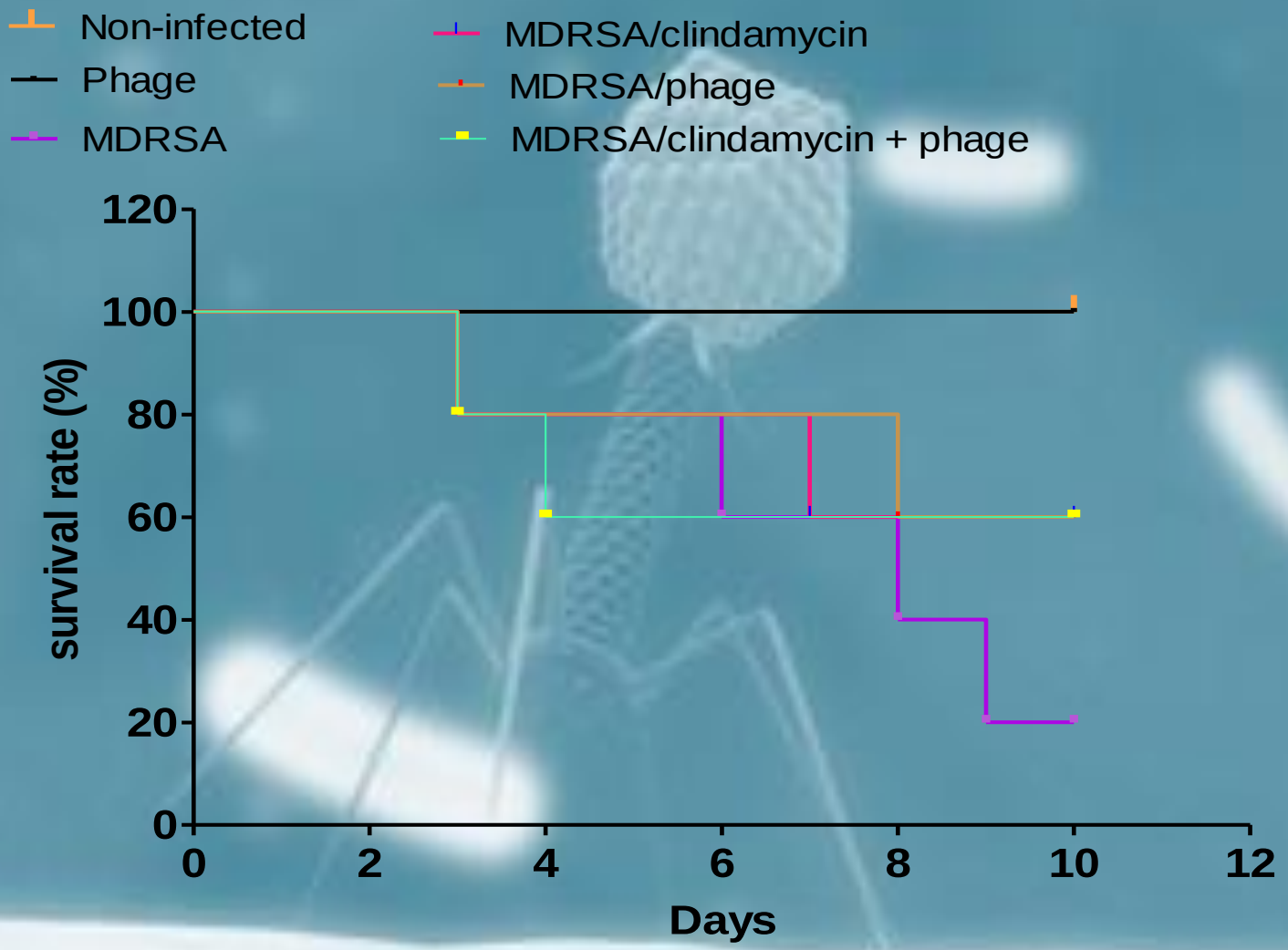

PHYSICAL APPEARANCE SCORE OF MICE GROUPS
Non-infected
Phage
MDRSA
MDRSA/ clindamycin
MDRSA/ phage
MDRSA/ clindamycin + phage
Physical appearance score
0
1
2
3
4
5
6
Days
0
1
2
3
4
5
6
7
8
9
10

Results

Groups	Initial number of mice	Number of mice 72 hours post-infection	Number of mice during treatment	Number of mice 7 days post-infection (end point)
A. All MDRSA infected mice	20	12	12	10
i. Non treated	5	3	3	1
ii. Clindamycin treatment	5	3	3	3
iii. Phage treatment	5	3	3	3
iv. Combination treatment	5	3	3	3
B. MDRSA non-infected group				
i. Phage infected mice	5	5	5	5
ii. Non-infected mice	5	5	5	5
Total	**30**	**22**	**22**	**20**

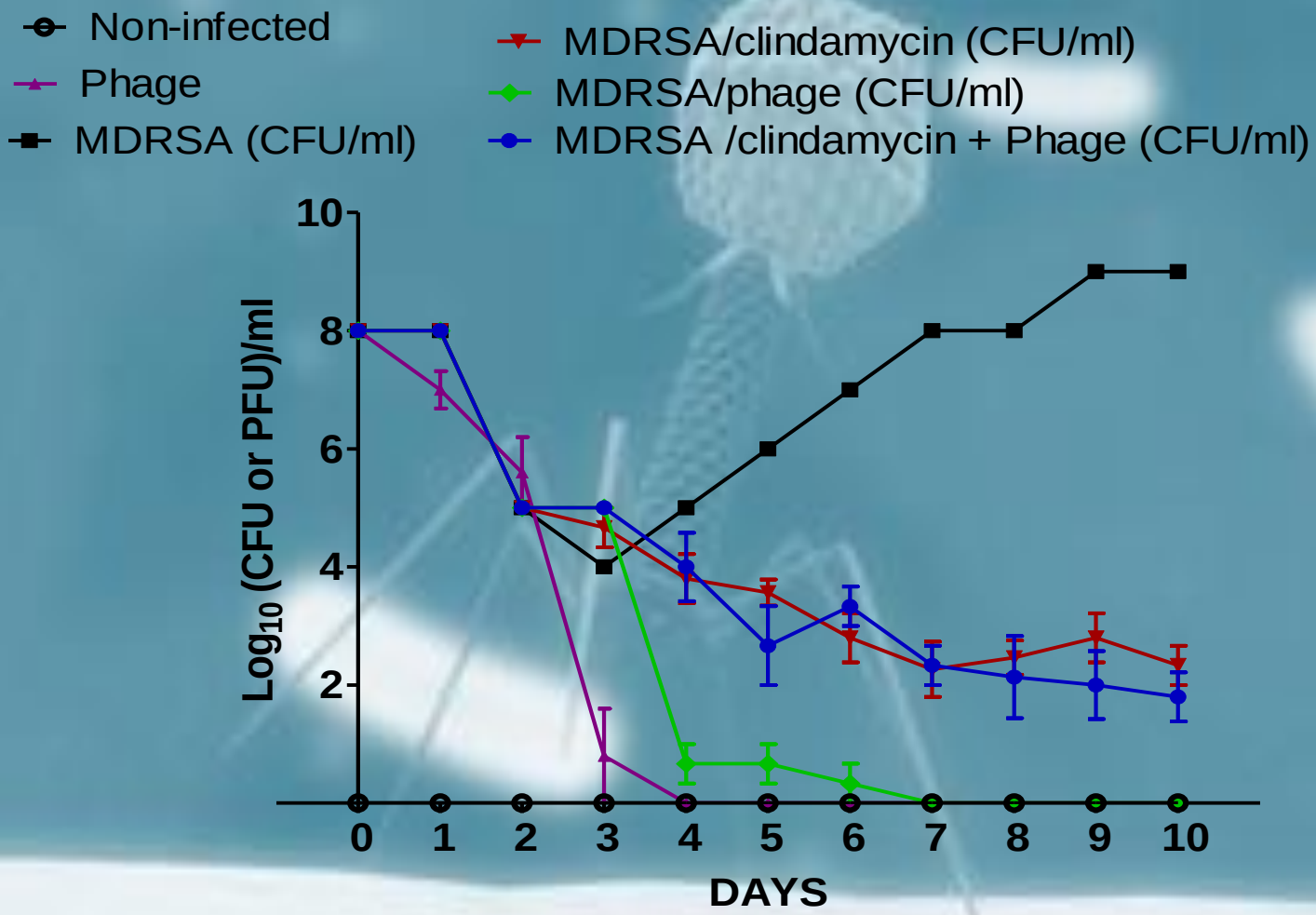
❖Number of surviving mice at 72 hours post-infection and 7 days post-treatment.

SURVIVAL RATE



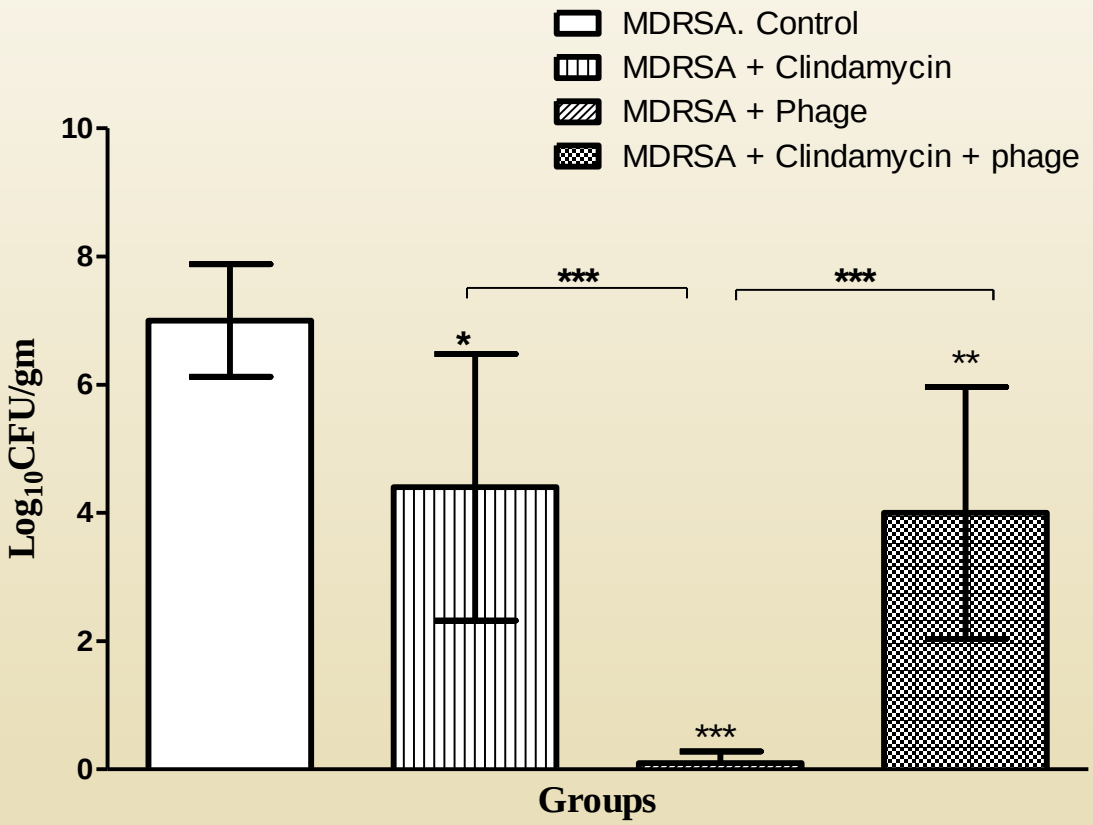
A dose of phage was as effective as a single dose of either clindamycin or combined antibiotic and phage.

BLOOD BACTEREMIA AND VIREMIA LEVEL OF THE MICE



Phage was more effective than clindamycin or clindamycin + phage

Bacterial Load

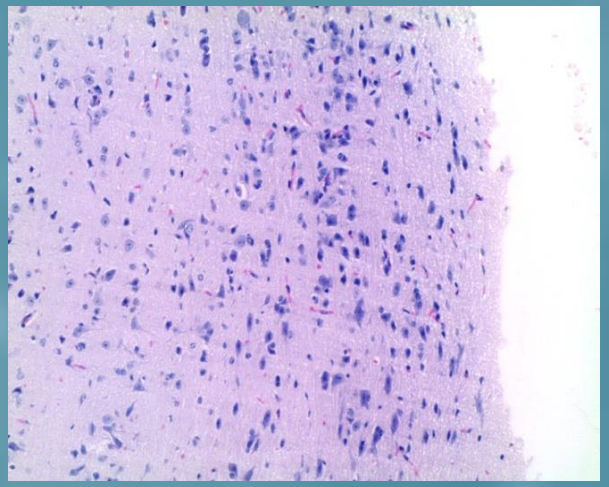


End point bacterial counts in blood.

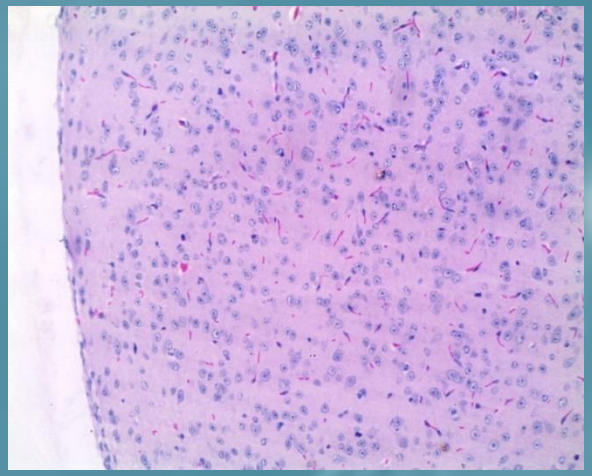
Groups	Treatment at 24 hrs post infection	% efficacy	Treatment at 72 hrs post infection	% efficacy
Non infected, non-treated	0.0	NIL	0.0	NIL
Phage + no treatment	0.0	NIL	0.0	NIL
MDRSA + no treatment	8.0± 0.2*	NIL	9.0± 0.2	NIL
MDRSA + clindamycin treatment	3.0 ± 0.2	62.25%	1.0 ± 0.2	87.5%
MDRSA + Phage treatment	0.0	**100%**	0.0	**100%**
MDRSA + (Phage-clindamycin treatment)	2.0 ± 0.2	75%	0.0	100%

*Mean log CFU/ml + SE

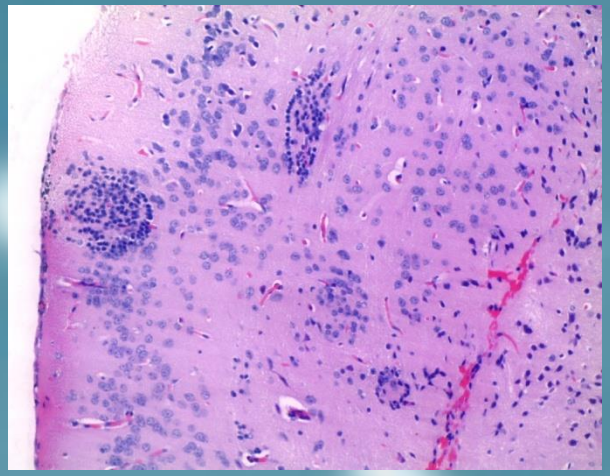
Brain tissues histopathological results



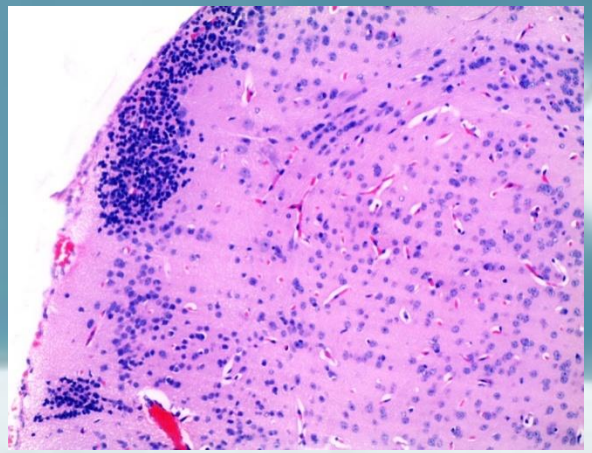
Non- infected, non-treated mouse



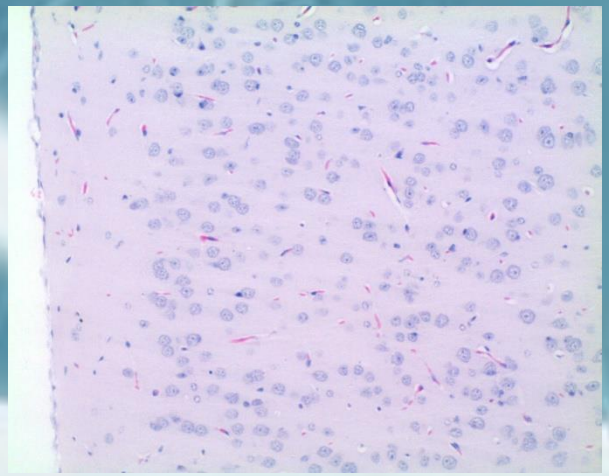
Phage infected, non-treated mouse



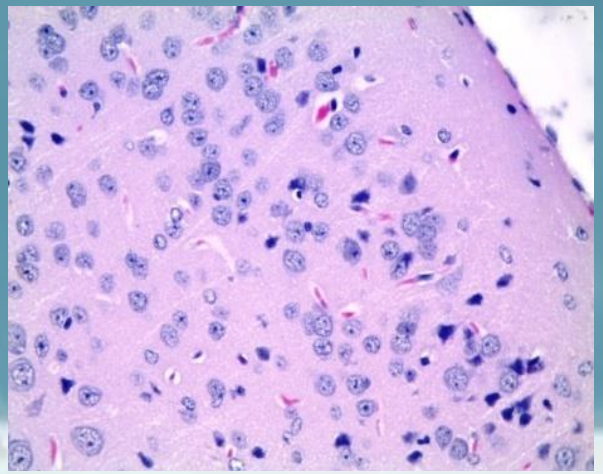
MDRSA ,non-treated mouse (inflammation)



MDRSA + clindamycin treated. (Inflammation) mouse

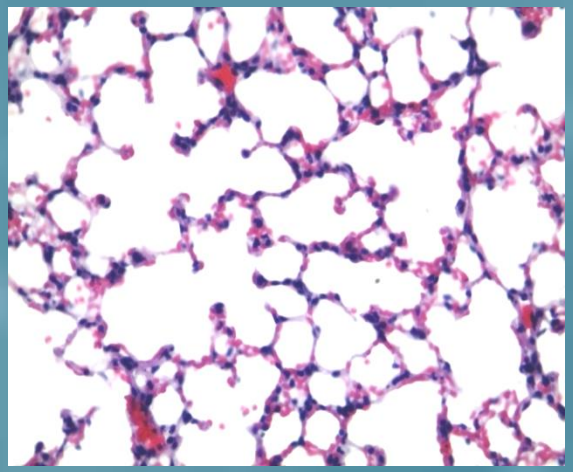


MDRSA + phage treated mouse

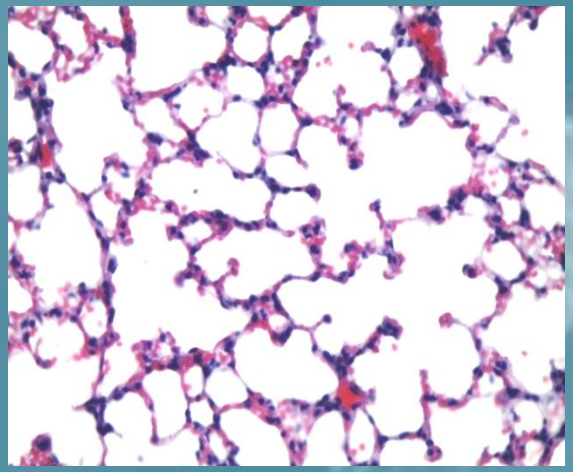


MDRSA + clindamycin - phage treated mouse

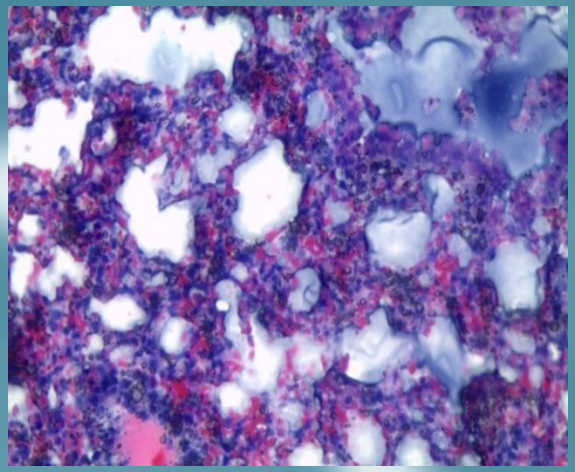
Lung tissues histopathological results



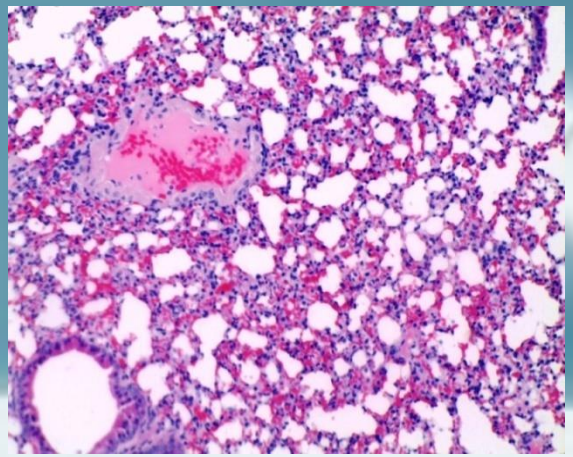
Non- infected, non-treated mouse



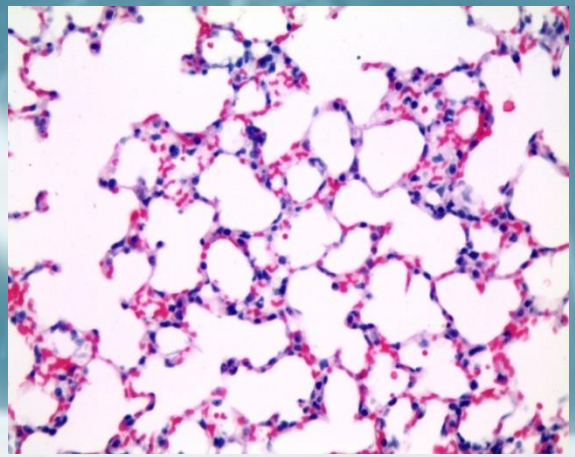
Phage infected, non-treated mouse



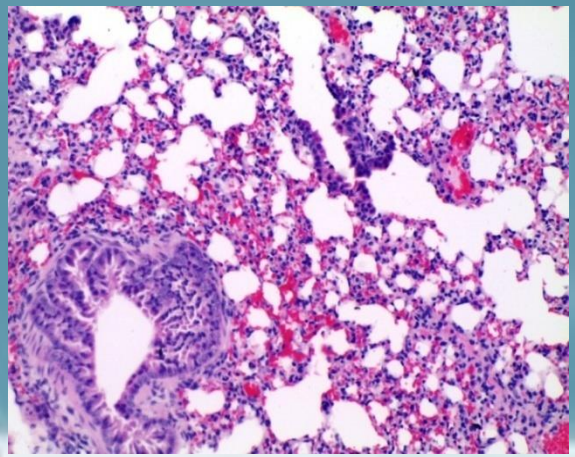
MDRSA infected, non-treated mouse – alveoli congestion



MDRSA , clindamycin treated mouse – mild alveoli congestion

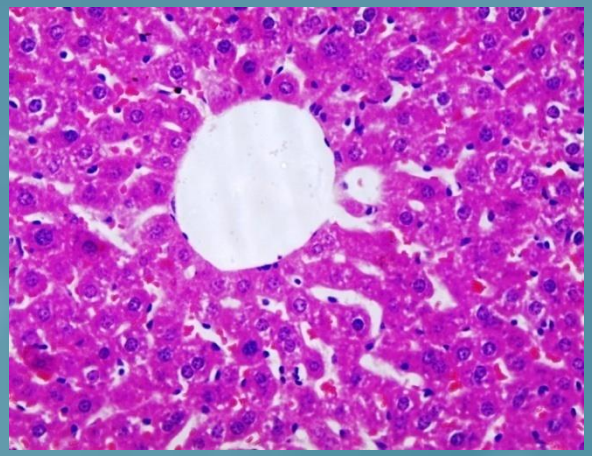


MDRSA , phage treated mouse

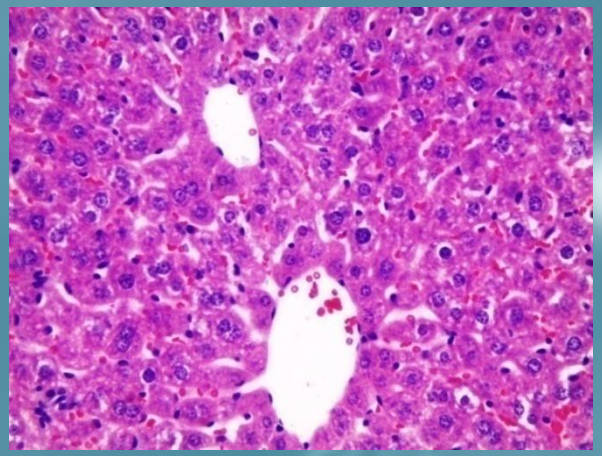


MDRSA, clindamycin-phage treated mouse – mild alveoli congestion

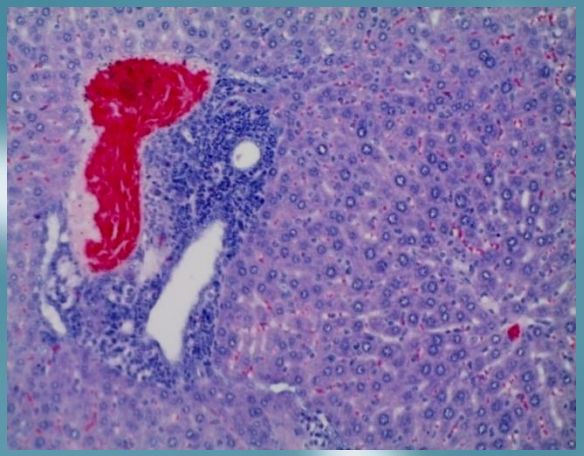
Liver tissue histopathological results



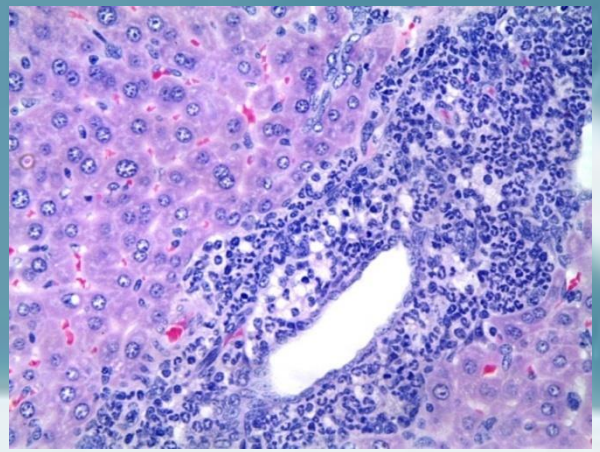
Non- infected, non-treated mouse



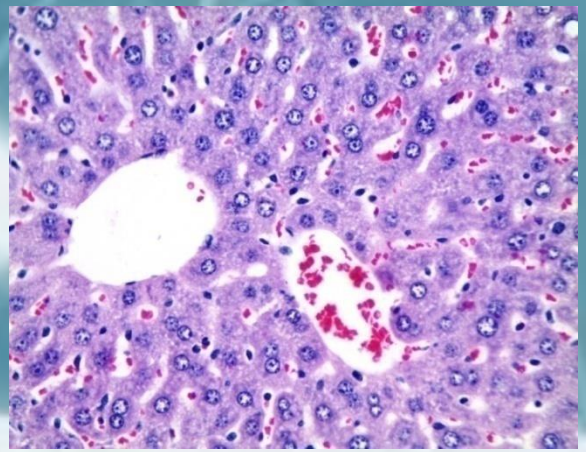
Phage, non-treated mouse



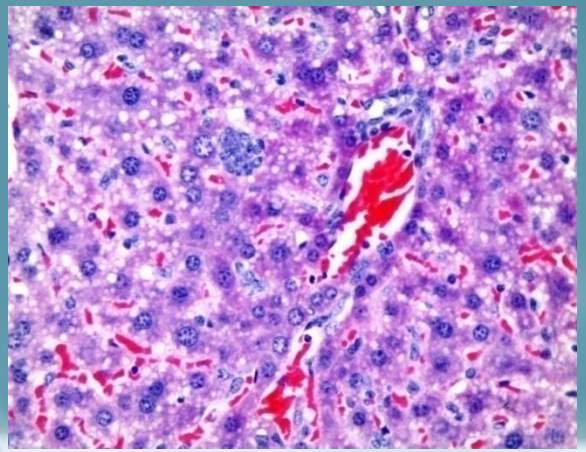
MDRSA , non-treated mouse (inflammation)



MDRSA , clindamycin treated mouse (inflammation)

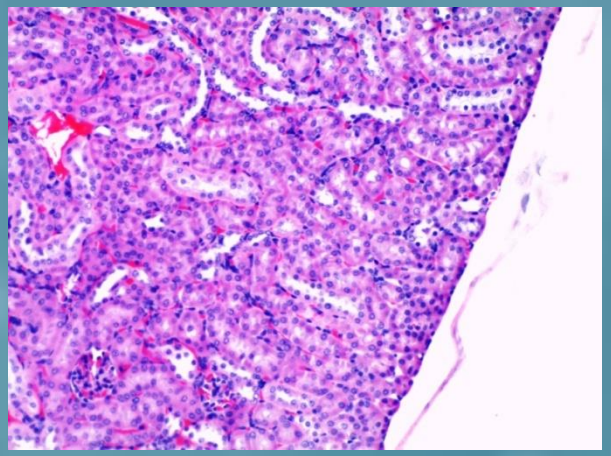


MDRSA , phage treated mouse

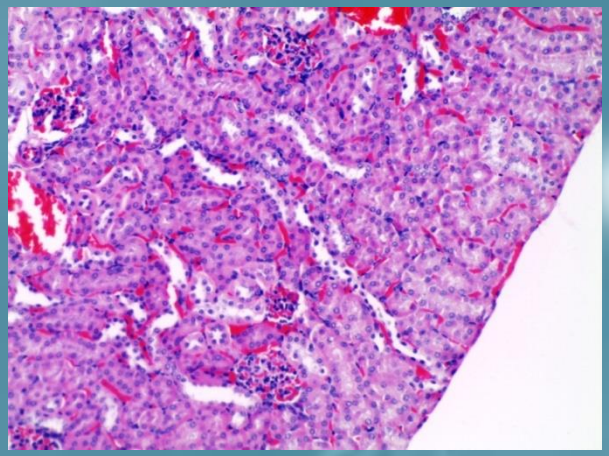


MDRSA , clindamycin - phage treated mouse

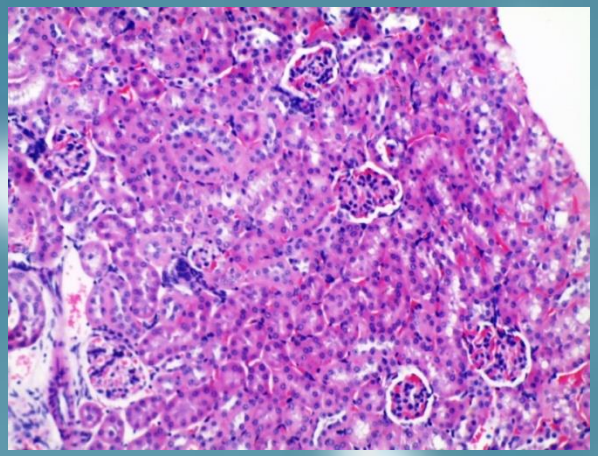
Kidney tissue histopathological results



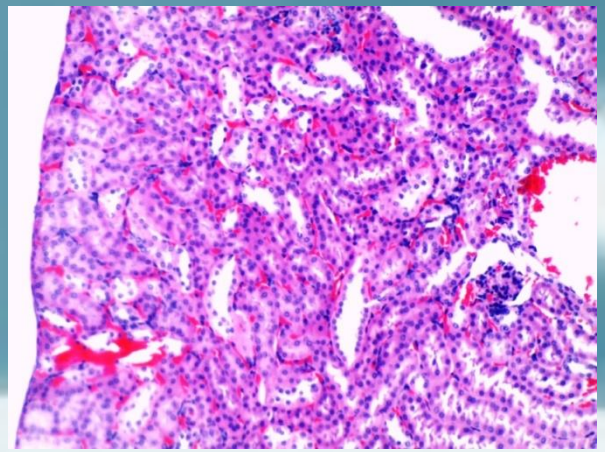
Non- infected, non-treated mouse



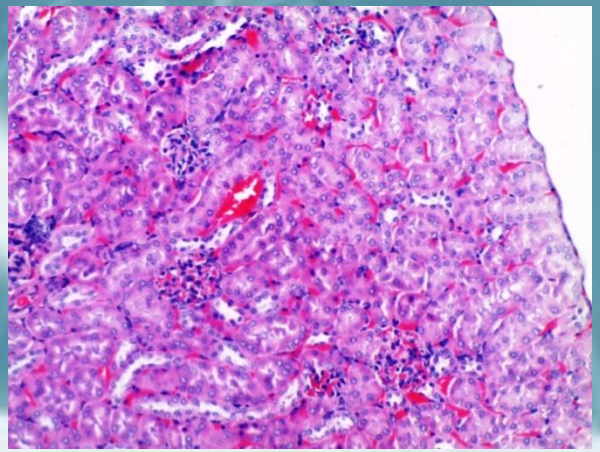
Phage, non-treated mouse



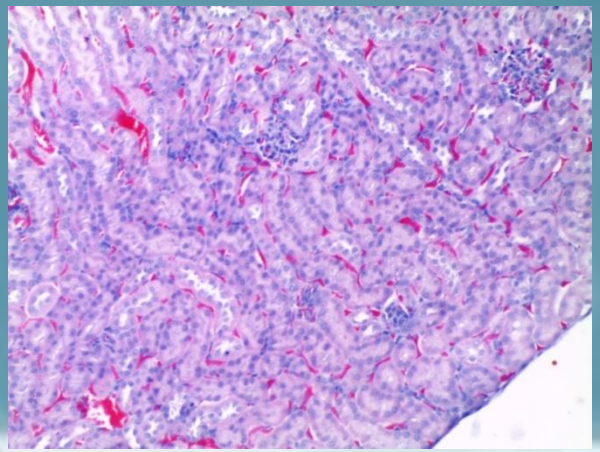
MDRSA , non-treated mouse (inflammation)



MDRSA , clindamycin treated mouse (inflammation)



MDRSA , phage treated mouse



MDRSA , clindamycin - phage treated mouse

Discussion and Conclusion

Discussion:

✓Phages are not pathogenic.

✓A dose of phage at 10^8 PFU/ml in MDRSA infected mice achieves 100% curative efficacy.

✓Cocktail treatment achieves range (80% 24hrs and 100% of 72hrs pi)

Conclusion:

➢The MDRSA are present within the environment.

➢Lytic phages from Nairobi County waste water have therapeutic potential.

➢Phage therapy is safe and effective against MDRSA bacterial infections.

Acknowledgement

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