LETTER TO THE EDITOR

Use of lytic bacteriophages in controlling multi drug resistant *staphylococcus aureus*

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Madam, due to increasing multidrug resistant (MDR) bacterial pathogens, clinicians are facing limitations in choice of antibiotics to treat infections. Global reports project mortality rates due to MDR infections to exceed those from cancer until 2050.[1,2] Use of lytic bacteriophages offers a promising alternative. Unlike antibiotics, they are very specific to their host which means their systemic use does not harm normal flora.

Staphylococcus aureus (*S. aureus*) is a deadly pathogen causing several different pathologies ranging from topical infections to life-threatening systemic ones such as meningitis.[3] There are worldwide efforts to develop credible phage therapies to control *S. aureus* infections.[4] We are isolating and characterizing bacteriophages specific to *S. aureus*.

Sewage samples were collected from different hospitals and enriched in *S. aureus* as follows: Samples were centrifuged at 4500 RPM for 10 minutes and filtered in 0.2μ filters. Then, 10 ml filtrate was added in 2x Lauria-Bertani (LB) broth and 0.5 ml log-phase *S. aureus* and cultured at 37°C for 10 hours. They were centrifuged at 180 RPM and filtered as mentioned above. Procedure was repeated thrice. After final enrichment, filtrate was serially diluted until 10^{-20}. Afterwards, 100μl of each dilution was mixed into 300μl of log-phase *S. aureus* and incubated at 37°C for 20 minutes. LB medium containing 0.8% agar was mixed in this culture and this mix was spread on LB agar plates for 24 hours. Appearance of visible plaques indicated presence of specific phage. Each plaque represented one type of phage. These plaques were collected using sterile microtips and inserted in 1.5ml tubes containing LB broth with 1% chloroform. Tubes were vortexed to dissolve phages in the medium. They were filtered and stored at -20°C until further use. Each isolated plaque was again enriched as mentioned above and tested in plaque forming assay against MDR *S. aureus*.

Two of our phage isolates, JPh-11 and MG-23, showed antibacterial activity against broad range of MDR *S.*

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Table: Effect of Phages on MDR *S. aureus* Isolates.

No.	Source	Resistance	Phages	
			Jph-11	MG-23
1*	Burn	Lev, Amo, Aug, Ery	1§	1
2	Burn	Lev, Amo, Chlo	1	0.4
3	Burn	Cip, Amo, Aug, Ery	0.7	0.2
4	Burn	Moxi, Amo, Ery	0.7	0.7
5	Burn	Lev, Aug, Ery, Lin	0.8	0.4
6	Burn	Lev, Cef, Aug, Gen	1	1.1
7	Burn	Cip, Aug, Ery	0.6	1.2
8	Burn	Cip, Aug, Chlo	0.8	0.2
9	Burn	Lev, Aug, Lin	1.1	0.8
10	Burn	Lev, Aug, Gen, Chlo	1.2	0.9
11	Burn	Lev, Aug, Gen	0.9	0.3
12	Burn	Cip, Aug, Gen	0.6	0.2
13	Burn	Cip, Aug, Gen	0.6	0.7
14	Burn	Lev, Amo, Ery	1.1	0.8
15	Accident	Lev, Cef, Gen	1	0.9
16	Accident	Lev, Aug, Lin	0.8	1.2
17	Accident	Lev, Aug, Ery, Chlo	0.6	0.6
18	Accident	Aug, Ery, Chlo	0.5	1.2
19	Accident	Lev, Amo, Gen	0.9	0.4
20	Accident	Lev, Amo,Aug,Ery,Gen	0.8	1.4
21	Accident	Lev, Cip,Aug,Chlo	0.6	1
22	Accident	Lev, Aug,Gen,Chlo	0.6	1
23	Accident	Moxi, Amo,Gen,Lin	0.9	0.4
24	Accident	Cip, Amo,Aug,Ery	1	0.5
25	Accident	Cip, Amo,Aug,Ery	0.9	1
26	Accident	Lev, Aug,Ery,Chlo	0.8	0.9
27	Accident	Lev, Cip,Amo,Aug,Ery	1.1	1.4
28	Accident	Lev, Amo, Aug, Ery	1.2	0.7
29	Accident	Lev, Amo, Aug, Ery	0.6	0.8
30	Accident	Lev, Cip, Amo, Ery	0.9	0.4
31	Obstetric	Lev, Amo, Chlo, Gen	1.1	1.2
32	Obstetric	Lev, Amo, Aug, Ery, Chlo	0.6	1.4
33	Obstetric	Cip, Amo, Ery, Gen	0.9	0.3
34	Obstetric	Cip, Amo, Ery, Gen	1.1	1.1
35	Obstetric	Cip, Amo, Aug, Chlo	0.9	1.3
36	Obstetric	Lev, Amo, Ery, Lin	0.7	1.3
37	Obstetric	Lev, Cip, Ery, Gen	0.9	1.4
38	Obstetric	Lev, Cip, Ery, Chlo	0.8	0.5
39	Obstetric	Moxi, Amo, Ery	0.8	0.2
40	Obstetric	Lev, Amo, Ery, Chlo	0.6	1.3
41	Obstetric	Cip, Amo, Aug, Chlo	0.9	0.6
42	Obstetric	Cip, Amo, Aug, Ery	0.6	0.9
43	Obstetric	Lev, Amo, Aug, Ery	1.2	1.3

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44	Obstetric	Lev, Amo, Ery, Chlo	0.6	1.1
45	Obstetric	Lev, Amo, Gen	0.6	0.6
46	Obstetric	Lev, Amo, Aug, Ery	0.7	0.1
47	Obstetric	Lev, Amo, Ery	1.2	0.3
48	Obstetric	Cip, Amo, Aug	0.6	1
49	Obstetric	Cip, Amo, Ery	1	0.1
50	Obstetric	Cip, Amo, Aug, Gen	0.7	1.3
51	Obstetric	Lev, Amo, Aug	0.8	0.3
52	Obstetric	Cip, Amo, Aug, Gen	0.8	0.2
53	Obstetric	Lev, Amo, Ery, Chlo	0.7	0.6
54	Obstetric	lev, Amo, Ery, Gen	0.7	0.1

* Phage samples were enriched in this isolate. § No. represents efficiency of plaque forming on different bacterial isolates relative to bacterial isolate 1.
Lev = Levofloxacin, Cip = Ciprofloxacin, Amo = Amoxycillin, Aug = Amoxycillin/Clavulanate, Ery = Erythrocin, Gen = Gentamicin, Chlo = Chloramphenicol, Lin = Linezolid.

aureus isolates. These bacterial isolates were isolated from burn, accidental and surgical wounds. Details of these isolates and phage activity of on them is given in the Table.

Our results indicate the potential of phage in treating skin infections caused by *S. aureus*. These phages should be tested in animal models of skin infections to document their therapeutic potential.

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Conflict of Interest: None.

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