

**In vitro antimicrobial and antibiofilm activity of phage cocktail against *Mammaliicoccus sciuri*, a causative agent of bovine mastitis**

Shirin Shahana PM, Archana Chandran, Surabhi PS, Rathish RL and Rahila MP

**SUPPLEMENTARY FILE**

Antibiotic /mcg	ADMS1	ADMS3	ADMS5
Amikacin (30)	13 mm	12 mm	16 mm
Amoxiclav (30)	21 mm	19 mm	14 mm
Ampicillin (10)	26 mm	22 mm	13 mm
Amoxicillin	19 mm	22 mm	19 mm
Azithromycin (15)	17 mm	16 mm	17 mm
Chloramphenicol	19 mm	22 mm	19 mm
Cefotaxime	-	16 mm	12 mm
Ciprofloxacin	24 mm	21 mm	-
Gentamicin	14 mm	14 mm	-
Ofloxacin	19 mm	19 mm	-
Tetracycline	14 mm	13 mm	-
Vancomycin	15 mm	16 mm	15 mm

**Table S 1:** Antibiogram showing the antimicrobial susceptibility of three *M.sciuri* isolates, (CLSI, 2015) Red -resistant, Yellow- Intermediate, Green- susceptible

ISOLATES	$\phi$ - <i>M.sciuri</i> A	$\phi$ - <i>M. sciuri</i> D
ADMS1( <i>Staphylococcus spp.</i> )	+	+
ADMS3 ( <i>Staphylococcus spp.</i> )	+	+
ADMS5 ( <i>Staphylococcus spp.</i> )	+	+
ADMS15( <i>Staphylococcus spp.</i> )	-	-
ADMS16( <i>Staphylococcus spp.</i> )	+	+
ADMS17( <i>Staphylococcus spp.</i> )	-	-
ADMS19( <i>Staphylococcus spp.</i> )	-	-
ADMS21( <i>Staphylococcus spp.</i> )	-	-
<i>E. coli</i> MTCC 1610	-	-
<i>Pseudomonas aeruginosa</i> (clinical isolate )	-	-
<i>S. typhi</i> MTCC 733	-	-
<i>S. aureus</i> MTCC 96	+	-

**Table S 2: host range of *M.sciuri* phages , (+)sensitive for phage; (-)not sensitive for phage**

## Figure legends

**Figure. 1 :PCR:** A) 16 S rRNA gene targeting products amplified by 27F/149R primers B) Amplification of *coa* gene of *Staphylococci* 1-positive control. S1-ADMS1, S3-ADMS3, S5-ADMS5 C) **Phylogenetic tree**, which depicts the diversity between the isolates.

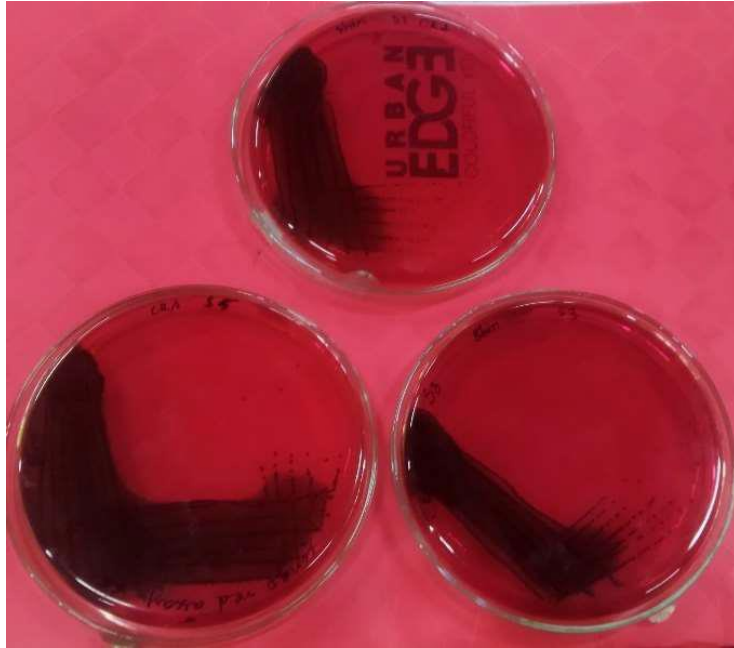
**Figure. S1: Congo red assay,** The ADMS1, ADMS3 and ADMS5 isolates form black colonies on CRA, indicating biofilm formation

**Figure. S2: Transmission electron micrograph** of  $\phi$ -*M. sciuri* A(A) and  $\phi$ -*M. sciuri* D(B), (200nm)

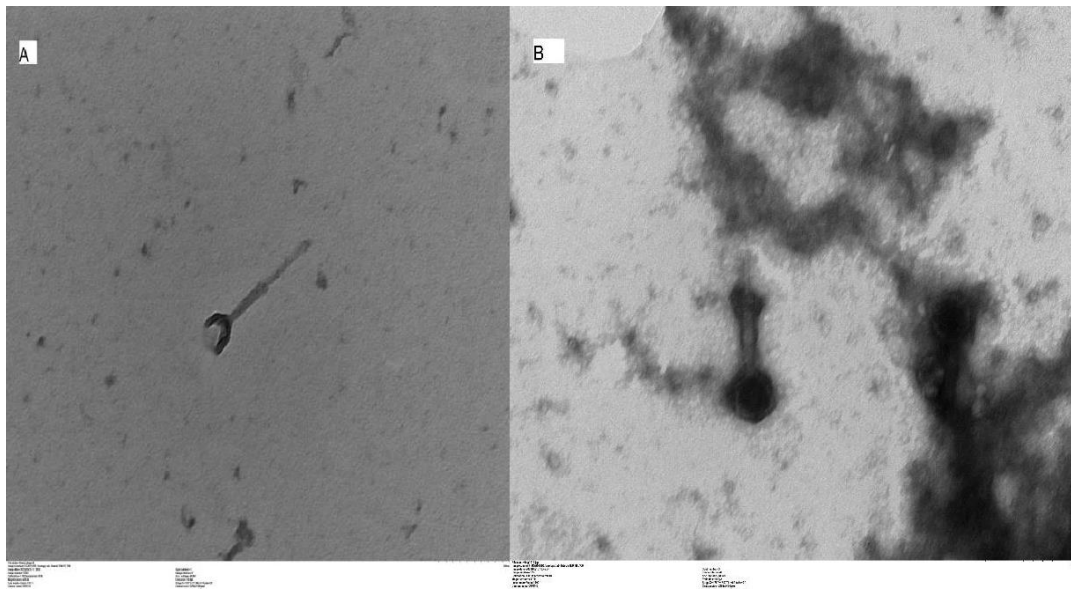
**Figure S3. Antibacterial activity of phages against coagulase-negative isolates.** Clear zone of lysis was observed for ADMS3 and ADMS5 isolates when infected with *M. sciuri* phages on spot assay

**Figure S4: Antibiofilm activity of phages.** *M. sciuri* isolates were allowed to form biofilm and treated with  $\phi$ -*M. sciuri* A,  $\phi$ -*M. sciuri* D and PM for 24 h followed by crystal violet assay to quantify biofilm . Significance was determined compared to untreated control. (If a p-value is less than 0.05, it is flagged with one star (\*). If a p-value is less than 0.01, it is flagged with 2 stars (\*\*). If a p-value is less than 0.001, it is flagged with three stars (\*\*\*))

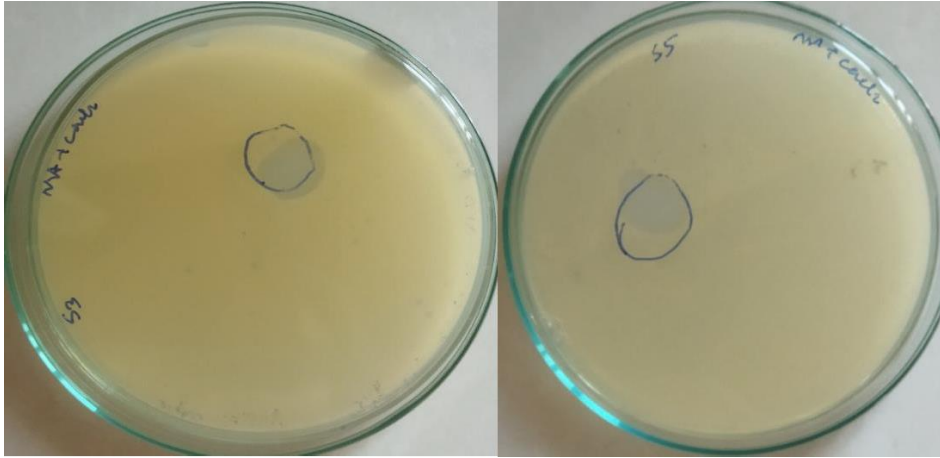
**Figure S5. In-vitro bacteriolytic activity of *M. sciuri* phages.** *M. sciuri*ADMS3 log-phase culture was infected with phages  $\phi$ -*M. sciuri* A(grey),  $\phi$ -*M. sciuri* D (orange), PM(yellow) at MOI of 10 and 100 respectively



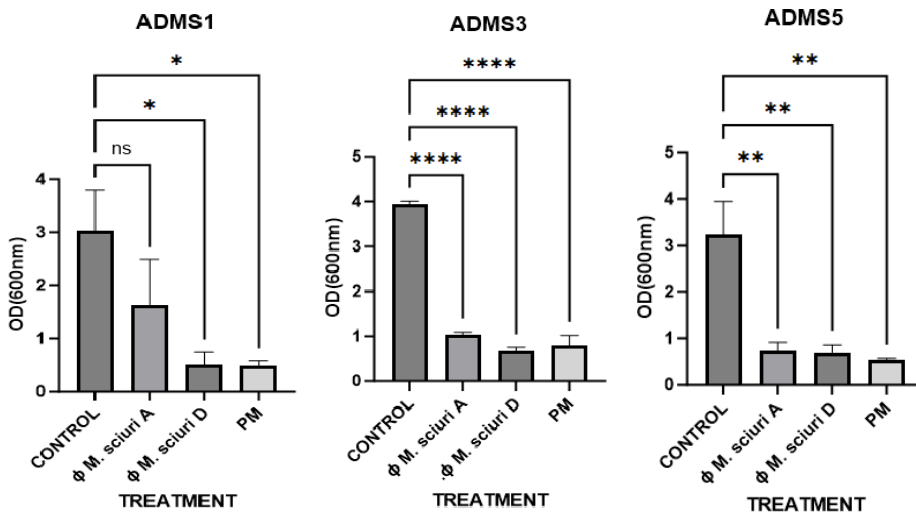
**Figure. S1 : Congo red assay**, The ADMS1, ADMS3 and ADMS5 isolates form black colonies on CRA, indicating biofilm formation



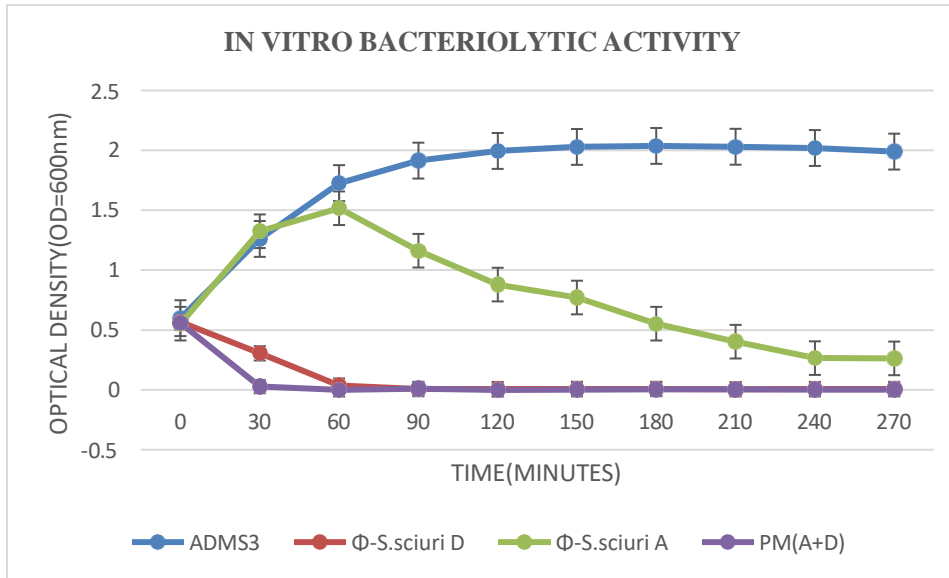
**Figure. S2: Transmission electron micrograph** of  $\phi$ -*M. sciuri* A(A) and  $\phi$ -*M. sciuri* D(B), (200nm)



**Figure S3. Antibacterial activity of phages against coagulase-negative isolates.** Clear zone of lysis was observed for ADMS3 and ADMS5 isolates when infected with *M. sciuri* phages on spot assay



**Figure S4: Antibiofilm activity of phages.** *M. sciuri* isolates were allowed to form biofilm and treated with  $\phi$ -*M. sciuri* A,  $\phi$ -*M. sciuri* D and PM for 24 h followed by crystal violet assay to quantify biofilm. Significance was determined compared to untreated control. (If a p-value is less than 0.05, it is flagged with one star (\*). If a p-value is less than 0.01, it is flagged with 2 stars (\*\*). If a p-value is less than 0.001, it is flagged with three stars (\*\*\*)



**Figure S5. In-vitro bacteriolytic activity of *M. sciuri* phages.** *M. sciuri*ADMS3 log-phase culture was infected with phages  $\phi$ -*M. sciuri* A (grey),  $\phi$ -*M. sciuri* D (orange), PM (yellow) at MOI of 10 and 100 respectively.