The Evaluation of Five Commercial Bacteriophage Cocktail Against Methicillin Resistant Staphylococcus Aureus Isolated From Nasal Swab Samples

Hilal Basak Erol

Ankara University Faculty of Pharmacy: Ankara Universitesi Eczacilik Fakultesi

Banu Kaskatepe (✉ bkaskatepe@ankara.edu.tr)

Ankara University: Ankara Universitesi <https://orcid.org/0000-0002-9722-4267>

Zekiye Bakkaloglu

Ministry of Health of Turkey: Turkiye Cumhuriyeti Saglik Bakanligi

Serap Suzuk Yildiz

Ministry of Health of Turkey: Turkiye Cumhuriyeti Saglik Bakanligi

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Abstract

Infections due to methicillin-resistant *Staphylococcus aureus* (MRSA) are a growing concern for public health resulting in increase in morbidity, length of hospital stay, and cost of treatment. MRSA nasal-swab screening may give clinicians additional information for decision of empiric antimicrobial agents. While increasing antibiotic resistance leads to new treatment approaches, bacteriophages are one of the most promising methods for these alternatives. It was aimed to determine the effectiveness of bacteriophages against MRSA isolates. Nasal swab samples were collected from outpatients without any evidence of infection who applied to Hatay, Mersin and Gaziantep family and immigration health centers. A series (35) were isolate from Turkish patients, G series (64) were isolated from Syrian immigrants. Methicillin resistance were determined by phenotypically and genotypically. Also, antibiotic susceptibilities of all isolates were determined against erythromycin, clindamycin, gentamicin, linezolid, rifampicin, and mupirocin. The total antimicrobial resistance rates of isolates were found 11%, 28%, 8%, 5%, 16%, 19%, and 29% respectively. The high susceptibility rate against ciprofloxacin (88.8%) was remarkable. The overall susceptibility of MRSA strains to ENKO, INTESI, PYO, SES, and STAPHYLOCOCCAL bacteriophages was 67.7%, 55.5%, 53.5%, 61.6% and 44.4%, respectively. The antibiotic susceptibility rates (except erythromycin), and efficacy of bacteriophages were higher in group A. Considering that high efficacy rates were not achieved in the study and the sensitivity rates of Turkish isolates to all phages were found higher than Syrian isolates, searching for phages in the geography where the pathogen is common, may be helpful to obtain suitable phages for treatment.

Introduction

Staphylococcus aureus (*S. aureus*) is the most important cause of community and hospital acquired infections all over the world and can cause different infection pictures including acute and pyogenic infections, skin and soft tissue infections, postoperative wound infections, and bacteremia. The limited treatment options due to the methicillin resistance makes the agent even more important.

Since the methicillin resistant *Staphylococcus aureus* (MRSA) are resistant to many antibiotics, they cause severe infections with high mortality in intensive care, surgical clinics, neonatal, hemodialysis and burn units, which require longer hospitalization and more antibiotics, and increase the rate of hospital acquired infections (Goetghebeur et al. 2007). For people who are seriously ill and need to be admitted to an intensive care unit or have had surgery, colonization with MRSA is a risk factor for a serious staphylococcal infection that can later lead to death. Over the past few decades, crucial requirements emerged to identify novel therapeutic strategies to control bacterial infections mainly due to multi-drug resistant bacteria. Bacteriophage therapy is among the prominent alternative treatment methods (Lyon 2017).

Bacteriophage therapy is a method of treating bacterial infections using unique properties of bacteriophages that only attack bacterial cells (Sulakvelidze et al. 2001). Lytic bacteriophages are similar to antibiotics in their pronounced antibacterial effects. However, bacteriophage preparations created for therapeutic purposes have some advantages and disadvantages compared to antibiotics. Bacteriophages have advantages such as protecting the natural microbiota, being non-toxic, cheap, and easily obtainable, migrating and reproducing wherever needed, regardless of the method of application, acting independently of antibiotic resistance, and being effective on biofilm, as well as some disadvantages. Hospital applications are still very limited due to the narrow spectrum of action, the fact that the agent- bacteriophage relationship has not been defined before the bacteriophage treatment and the inadequacy of clinical studies (Wittebole et al. 2014). Personalized bacteriophage therapy is seen as one of the most promising ways, especially in cases where the agent is identified and infections caused by multidrug resistant bacteria.

Bacteriophages have been used for the treatment of infectious diseases from the early 20th century, mainly in Eastern European countries whereas with the exploring of the antibiotics, lost their importance in Western countries. However, it has gained importance again with the increasing antibiotic resistance. The most essential feature, for the bacteriophage to be selected for phage therapy, is its strong lytic effect and host specificity. In some applications of bacteriophages, phages with a wide host range appear to be more useful. For this purpose, isolating and characterizing phages with a wide host range is a requirement for high therapeutic efficacy (Ross et al. 2016). Several studies have proved the efficacy of phages specific against this pathogen in different animal models and human clinical trials (Alvarez et al. 2019).

The aim of this study was to determine the efficacy of commercially available phage cocktails (PYO, INTESI, ENKO, SES, STAPHYLOCOCCAL (STP) Bacteriophages) against 99 *S. aureus* isolates characterized by phenotypic and genotypic methods.

Materials And Method

Bacteriophage Cocktails

Five commercially available bacteriophage cocktails produced by George Eliava institutions located in Tbilisi were tested. According to the manufacturers, they are all sterile-filtrate phage lysates of different bacterial species as listed below. These cocktails were purchased from the George Eliava Institute Pharmacy, Georgia. The preparation lot numbers applied during the current study are indicated. The following five commercial phage cocktails were included in this study.

PYO Bacteriophage

Pyo bacteriophage (lot # M1-1001; Eliava BioPreparations, Tbilisi, Georgia) is a mix of sterile filtrates of phage lysates active to: *Staphylococcus spp.*, *Streptococcus spp.*, Different types of *E. coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Proteus vulgaris*.

INTESI Bacteriophage

Intesti bacteriophage (lot # M2-1001; Eliava BioPreparations, Tbilisi, Georgia) is a mix of sterile filtrates of phage lysates active to: *Shigella* (*Shigella flexneri* serotype 1,2, 3, 4 and *Shigella sonnei*), *Salmonella* (*S. paratyphi* A, *S. paratyphi* B, *S. typhimurium*, *S. enteritidis*, *S. choleraesuis*, *S. oranienburg*), Different types of *E. coli*, *Proteus mirabilis*, *Proteus vulgaris*, *Staphylococcus* (*S. aureus*), *Pseudomonas aeruginosa* and *Enterococcus* spp.

ENKO Bacteriophage

Enko bacteriophage (lot # E-0119; Eliava BioPreparations, Tbilisi, Georgia) active against *Salmonella typhimurium*, *Salmonella enteritidis*, *Salmonella heidelberg*, *Salmonella newport*, *Salmonella cholerae*, *Salmonella oranienburg*, *Salmonella dublin* and *Salmonella anatum*; *Shigella flexneri* (serovars 1, 2, 3, 4) and *Shigella sonnei* (six different serovars), enteropathogenic *E. coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Staphylococcus saprophyticus*.

SES Bacteriophage

SES bacteriophage (lot # S-0119; Eliava BioPreparations, Tbilisi, Georgia) active against *Staphylococci* (*S. aureus*, *S. epidermidis* and *S. saprophyticus*); *Streptococci* (*Streptococcus pyogenes*, *Streptococcus sanguis*, *Streptococcus salivarius* and *Streptococcus agalactiae*) and different serotypes of enteropathogenic *E. coli* serovars.

STAPHYLOCOCCAL Bacteriophage

Staphylococcal bacteriophage (lot # P1-901; Eliava BioPreparations, Tbilisi, Georgia) is a mix of sterile filtrates of phage lysates active to: *Staphylococcus* spp.

Bacterial Collection and determination of methicillin resistant

The efficacy of commercial phage cocktails was evaluated to 99 MRSA isolates obtained from nasal swab samples. Nasal swab samples were collected from outpatients without any evidence of infection who applied to Hatay, Mersin and Gaziantep family health centers and immigration health centers. A series were isolate from Turkish patients, G series were isolated from Syrian immigrants.

Phenotypic identification

The nasal swab from each patient was inoculated on mannitol salt agar (HiMedia Labs, India) media and incubated aerobically overnight at 37°C. After 24 h of incubation, MSA media were observed for growth. All isolates were identified by MALDI TOF MS (Bruker MALDI Biotyper, Germany) also confirmed as *S. aureus* were further tested for detection of methicillin resistance by Kirby–Bauer disc diffusion method using cefoxitin 30 µg discs (HiMedia Labs, India). The standard bacterial suspension of *S. aureus* with turbidity equal to 0.5 McFarland was inoculated on Mueller Hinton agar (HiMedia Labs, India), using the following: gentamicin (10 µg), ciprofloxacin (5 µg), erythromycin (15 µg), clindamycin (2 µg), linezolid (10 µg), rifampisin (5 µg), and mupirocin (5 µg) (HiMedia Labs, India). The plates were incubated at 35°C for 24 hand the diameter of zone of growth inhibition were measured and as per European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (2021). The *S. aureus* ATCC 25923 was used for quality control strain.

Molecular characterization of MRSA strains

Molecular identification and characterization of isolated methicillin resistant *S. aureus* strains were performed by the detection of *S. aureus*-specific *nuc* [279 bp], *MRSA*-specific *mecA* 162 bp] and *mecC* [138 bp] genes using three different primer pairs that given in Table 1. In-house multiplex PCR was carried out on supernatants from crude DNA extracts; a bacterial suspension equal to McFarland 4 turbidity was prepared in sterile water and boiled for 10 min and subsequently centrifuged for 5 min at 10.000 g. 10 pmol of each primer was added to a DreamTaq PCR Master Mix kit (Thermo Scientific, CA, USA), and amplification was performed by using a GeneAmp PCR system 9700 thermal cycler (Applied Biosystems, CA, USA). The amplification conditions were 94°C for 5 min, followed by 35 cycles at 94°C for 30 s, 55°C for 30 s and 72°C for 45 s with a final 7 min elongation step at 72°C for. Amplification products were electrophoresed through a 3% agarose gel stained with 0.5 g of ethidium bromide/ml. The following controls were included in all amplification reactions: NCTC 12493 (*mecA*-positive *S. aureus*), NCTC 13552 (*mecC*-positive *S. aureus*), ATCC 25923 (*mecA*-negative *S. aureus*), and negative control (water).

Table 1
Primers used for PCR reactions and expected amplicon sizes

Genes	Sequences of Primers (5' 3')	Size (bp)	References
<i>mecA</i>	F-TCCAGATTACAACCTTCACCAGG R-CCACTTCATATCTTGTAACG'	162	(Oliveira and de Lencastre 2002)
<i>mecC</i>	F-GAAAAAAGGCTTAGAACGCCTC R- GAAGATCTTTCCGTTTTCAGC	138	(Stegger et al. 2012)
<i>nuc</i>	F- GCGATTGATGGTGATACGGTT R- AGCCAAGCCTTGACGAACATAAGC	279	(Zhang et al. 2004)

In vitro Susceptibilities of Bacteriophage Cocktails

Spot tests were performed to determine the effectivity of five commercially available bacteriophages on 99 MRSA isolates. For spot test, all MRSA isolates were incubated at 37°C for 18 h in Mueller Hinton Agar (MHA). Single colonies were incubated in LB broth for about 3 h. Then, bacterial culture

(A600 ca. 0.5) inoculated on the surface of the LB agar. 10 µL of phage suspension was applied on the bacterial layer after 10 min, and incubated at 37°C overnight. Bacterial sensitivity to phage preparations was evaluated according to the lysis of bacteria on the agar surface. Observing of opaque, semi-confluent and confluent lysis was accepted as the isolate susceptibility. When the lysis was not observed, the corresponding isolates were determined to be resistant. The spot test was repeated three times on different days.

Statistical analysis

The data were evaluated statistically with IBM SPSS 21 packet program. Firstly, it was examined whether the data showed a normal distribution. The difference between groups A and G in the efficacy of each commercial preparation was determined by the Mann-Whitney U test. The difference between commercial preparations was also investigated by the Kruskal Wallis test.

Results

Phage and antibiotic susceptibility of bacteria

The isolates grown in Mannitol salt agar and found to be resistant to methicillin were confirmed with molecular characterization of *mec A*, *mec C*, *nuc* genes. All isolates were identified as *S. aureus* with *nuc* gene and also all isolates were found to have the *mec A* gene but not the *mec C* gene. Multiplex amplification image is shown in Fig. 1. MRSA isolates were tested for antibiotic susceptibility. The total antimicrobial resistance rates of isolates were found as 11%, 28%, 8%, 5%, 16%, 19%, and 29% for ciprofloxacin, erythromycin, clindamycin, gentamicin, linezolid, rifampicin, and mupirocin respectively. It is also considerable that intermediate sensitivity rates are high for ciprofloxacin (88%) and mupirocin (67%). The antibiotic susceptibility results of the isolates are given in Table 2.

Table 2
The results of antibiotic susceptibility and effectivity of bacteriophage

No	Bacterial ID	Zone Diameter					Bacteriophage						
		CIP	ERY	DA	GEN	LNZ	RFM	MPR	ENKO	INTESTI	PYO	SES	STP
1	27A 347	27	6	25	27	26	30	26	+	-	-	+	-
2	27A 352	22	6	28	24	26	28	25	++	++	++	+	+
3	27A 354	23	6	29	21	23	30	16	-	-	-	-	-
4	27A 356	30	26	27	26	25	30	24	+++	+++	+++	+++	-
5	27A 360	30	25	28	22	30	31	30	+++	++	++	++	++
6	27A 387	28	29	30	27	27	30	26	++	++	++	++	++
7	27A 400	23	21	22	22	23	27	24	++	++	+++	+	+
8	27A 437	28	29	29	27	28	30	26	+++	+++	+++	+++	+++
9	27A 438	23	20	23	24	24	26	22	+++	+++	+++	+++	++
10	27A 441	23	6	29	24	26	29	20	+	-	-	-	-
11	27A 443	23	6	28	25	25	29	23	+	-	-	+	-
12	27A 446	26	26	29	27	29	33	26	-	-	-	-	+
13	27A 462	26	6	6	27	30	31	24	+	-	-	++	-
14	27A 467	27	25	28	24	25	28	22	+++	+++	+++	++	+
15	27A 471	6	22	25	11	25	30	20	++	+++	+++	+++	+++
16	27A 485	30	35	35	28	26	30	21	+	+	+	+	+
17	27A 489	25	23	25	23	23	24	22	++	++	+++	++	+
18	27A 497	27	25	27	23	25	30	21	+	+	-	+	+
19	27A 510	25	19	23	20	23	24	22	+++	+++	+++	+	+
20	27A 513	24	21	24	21	23	28	21	+	-	-	+	-
21	27A 523	27	6	26	26	27	30	21	-	-	-	-	-
22	27A 531	29	27	28	25	25	29	25	-	+++	+++	-	-
23	27A 534	21	8	28	18	21	17	6	+	-	-	+	-
24	27A 537	18	24	28	23	22	26	20	-	-	-	-	-
25	27A 538	17	8	6	23	6	6	6	-	-	-	-	-
26	27A 80	27	26	30	27	28	28	27	++	++	++	+	+
27	27A 81	30	29	32	27	29	34	24	+++	+++	+++	+++	+++
28	27G 177	26	25	25	24	26	27	20	+++	+++	+++	++	++
29	27G 190	23	6	29	19	20	23	16	+	+	+	+	+
30	27G 200	13	8	28	17	18	22	15	++	-	-	++	-
31	27G 217	24	6	30	26	29	30	25	-	-	-	-	-
32	27G 241	21	16	19	18	17	21	16	-	-	-	+	-
33	27G 266	21	21	18	18	18	23	16	-	-	-	-	-
34	27G 270	30	25	28	21	27	27	22	+	++	+	+	++
35	27G 280	18	22	22	20	24	23	20	+	-	-	++	-
36	27G 283	22	18	17	20	20	23	18	+	+++	+++	+	+++
37	27G 284	25	17	25	25	26	13	6	+	+	+	-	-
38	27G 31	23	18	19	11	22	25	20	-	-	-	-	-
+++ : confluent lysis (i.e. complete lysis), ++ : semiconfluent lysis (i.e. Sporadic bacteria in the lysis zone), + : opaque lysis (i.e. many bacteria in the lysis zone), -: No lysis. CIP: ciprofloxacin, ERY: erythromycin DA: clindamycin, GEN: gentamicin, LNZ: linezolid, RFM: rifampicin, MPR: mupirosin													

No	Bacterial ID	Zone Diameter					Bacteriophage						
		CIP	ERY	DA	GEN	LNZ	RFM	MPR	ENKO	INTESTI	PYO	SES	STP
39	27G 310	22	21	20	21	23	25	19	+++	+++	+++	++	++
40	27G 338	25	11	28	21	28	26	18	++	++	+++	++	++
41	27G 341	30	25	28	28	30	31	28	++	+	+	++	+
42	27G 343	30	6	20	27	27	21	26	++	+	+	++	+
43	27G 349	22	21	22	21	22	24	18	-	-	-	-	-
44	27G 356	25	22	27	21	23	25	17	-	-	-	-	-
45	27G 363	23	20	19	19	17	25	16	+++	+++	+++	++	+
46	27G 368	21	20	22	21	21	25	17	+	-	-	-	-
47	27G 398	23	19	19	18	18	20	17	+++	+	++	+++	-
48	27G 405	24	21	18	21	22	23	17	++	++	++	++	++
49	27G 49	23	17	21	22	24	11	6	+++	+++	+++	++	+
50	31A 172	27	17	24	23	27	17	6	-	-	-	-	-
51	31A 271	33	22	30	24	30	18	6	-	-	-	-	-
52	31A 350	30	28	28	26	30	25	30	-	-	-	-	-
53	31A 55	25	20	25	22	22	28	20	-	-	-	-	-
54	31G 248	25	25	26	24	28	30	26	-	-	-	-	-
55	31G 29	24	10	30	26	28	29	24	+++	+++	+++	+++	+++
56	31G 369	27	25	28	17	30	30	27	-	-	-	-	-
57	31G 411	24	28	30	25	26	28	22	+++	+++	+++	+	+
58	31G 542	25	25	29	26	27	30	23	-	-	-	-	-
59	31G 545	16	19	20	21	20	23	15	-	-	-	-	-
60	31G 549	24	27	30	26	30	27	24	+	+	+	-	-
61	31G 556	24	26	29	24	27	28	23	+	-	-	+	-
62	31G 568	18	22	22	20	22	23	17	+	-	-	+	-
63	31G 577	26	24	26	20	26	26	24	+	+	+	-	-
64	33A 140	25	20	21	20	23	20	18	-	+	+	-	-
65	33A 212	21	20	22	21	21	25	17	++	++	++	+++	-
66	33A 230	26	25	27	21	27	26	22	+++	+++	+++	+++	-
67	33A 248	27	23	27	22	27	28	23	+++	+++	+++	+++	+++
68	33G 136	24	20	22	21	20	25	17	++	-	-	++	-
69	33G 138	23	17	18	19	18	22	17	+++	+++	+++	++	++
70	33G 142	23	23	26	23	25	26	23	+++	+++	+++	++	+
71	33G 147	25	6	26	19	24	30	20	+++	+++	+++	+++	+++
72	33G 15	6	6	32	33	32	31	27	+	+	+	+	+
73	33G 162	15	25	30	21	26	30	26	-	-	-	-	-
74	33G 170	21	22	24	22	21	25	20	-	-	-	-	-
75	33G 184	30	30	35	30	35	38	29	+++	+++	+++	+++	+++
76	33G 192	30	8	6	22	6	6	6	-	-	-	-	-
77	33G 199	20	19	23	20	19	19	18	+	+	-	-	-

+++ : confluent lysis (i.e. complete lysis), ++: semiconfluent lysis (i.e. Sporadic bacteria in the lysis zone), +: opaque lysis (i.e. many bacteria in the lysis zone), -: No lysis. CIP: ciprofloxacin, ERY: erythromycin DA: clindamycin, GEN: gentamicin, LNZ: linezolid, RFM: rifampicin, MPR: mupirocin

No	Bacterial ID	Zone Diameter					Bacteriophage						
		CIP	ERY	DA	GEN	LNZ	RFM	MPR	ENKO	INTESTI	PYO	SES	STP
78	33G 249	24	18	22	19	21	23	16	-	-	-	-	-
79	33G 25	23	20	23	23	20	26	16	++	++	++	++	+
80	33G 26	27	25	27	25	26	28	23	+++	++	+++	++	+
81	33G 287	27	28	30	26	26	30	26	+	+	+	+	+
82	33G 289	22	30	32	26	26	32	23	+++	+++	+++	++	-
83	33G 290	25	19	26	26	28	14	6	+	-	-	-	-
84	33G 301	28	25	27	22	26	27	26	-	-	-	-	-
85	33G 303	6	22	21	15	20	23	17	+++	++	++	+	++
86	33G 321	26	6	6	20	26	26	22	++	++	++	++	+
87	33G 327	30	18	30	27	32	19	22	-	-	-	-	-
88	33G 336	30	6	30	28	30	30	26	-	-	-	-	-
89	33G 34	28	10	28	28	20	30	23	+	-	-	+	+
90	33G 375	27	30	30	27	28	33	27	-	-	-	-	-
91	33G 408	27	18	23	26	28	14	6	++	++	++	+	++
92	33G 421	30	28	29	26	30	18	6	-	-	-	-	-
93	33G 423	23	22	23	23	22	27	22	-	-	-	-	-
94	33G 434	28	23	19	24	27	30	25	+	++	+++	++	-
95	33G 500	27	28	30	25	28	33	26	+++	+++	+++	++	+
96	33G 512	25	23	27	23	23	28	23	+++	+++	+++	+++	+++
97	33G 65	23	6	20	27	28	21	6	+++	+++	+++	++	++
98	33G 67	25	6	28	23	23	28	22	-	-	-	-	-
99	33G 80	27	6	25	27	26	30	26	+	+	+	++	-
Total R (n)		11	28	8	5	16	19	29	-	-	-	-	-
		A: 3	A:11	A:2	A:1	A:1	A:6	A:7					
		G:11	G:17	G:6	G:4	G:15	G:13	G:22					
Total I (n)		88	18	12	-	-	21	67	-	-	-	-	-
Total S (n)		-	53	79	94	83	59	3	-	-		-	-
% Confluent lysis									26.3	25.2	30.3	13.1	9.1
% Semi confluent lysis									16.2	16.2	11.1	26.3	12.1
% Opaque lysis									25.2	14.1	12.1	22.2	23.2
% Total Lytic Effect of phage		-	-	-	-	-	-	-	67.7	55.5	53.5	61.6	44.4
+++ : confluent lysis (i.e. complete lysis), ++ : semiconfluent lysis (i.e. Sporadic bacteria in the lysis zone), + : opaque lysis (i.e. many bacteria in the lysis zone), - : No lysis. CIP: ciprofloxacin, ERY: erythromycin DA: clindamycin, GEN: gentamicin, LNZ: linezolid, RFM: rifampicin, MPR: mupirocin													

As shown in Table 2, the overall susceptibility of MRSA strains to ENKO, INTESTI, PYO, SES, and STP bacteriophages was 67.7% (including 26/99 with '+++ and 16/99 with '++'), 55.5% (including 25/99 with '+++ and 16/99 with '++'), 53.5% (including 30/99 with '+++ and 11/99 with '++'), 61.6% (including 13/99 with '+++ and 26/99 with '++') and 44.4% (including 9/99 with '+++ and 12/99 with '++'), respectively.

According to the results, the most effective bacteriophage preparation was ENKO-bacteriophage (67.7%) and the least effective one was STP bacteriophage (44.4%). 72 (72.7%) of 99 MRSA strains included in the study were found to be susceptible to bacteriophage cocktails. While 72 strains were found to be susceptible to at least one phage tested, 38 *S. aureus* strains were found resistant to all bacteriophage tested. Although the efficacy percentages were high in group A, no statistical difference was found between the group A and G ($p > 0.05$). There was a statistically significant difference between the activity of STP phage and the activity of Enko ($p = 0.003$), Pyo (0.038) and Intesti ($p = 0.050$) phages when all commercial preparations were compared according to the lysis type. The comparison of lytic activity results in A and G groups is given in Table 3.

Table 3
The comparison of lytic activity results in A and G groups.

			ENKO	INTESTI	PYO	SES	STAP
99 MRSA	A series (35)	Lytic activity (+)	24	20	19	23	16
		Lytic activity (-)	11	15	16	12	19
		% Lytic activity	68,6	57,1	55,3	65,7	45,7
	G series (64)	Lytic activity (+)	33	25	34	38	28
		Lytic activity (-)	31	39	30	26	36
		% Lytic activity	51,6	39	53	59,4	43,8

Discussion

The development of resistance to most antibiotics in many clinically important bacteria has led to increased interest in bacteriophages. They have also been used therapeutically for ~ 100 years, with a good safety record and there are studies in the literature showing the safety of phage applications (Abedon et al. 2017). Even though in some countries bacteriophage application has not approved yet, several examples of successful applications of personalized phage therapy have been reported (Jennes et al. 2017; Nir-Paz et al. 2019; Tkhilaishvili et al. 2020). Lytic bacteriophages that used therapy are similar to antibiotics in terms of their pronounced antibacterial effects. However, there are some advantages and disadvantages of bacteriophage preparations created for therapeutic purposes compared to antibiotics. Regardless of the way bacteriophage is applied, the factor specifically has disadvantages such as migration and reproduction there where it is needed, to act independently from antibiotic resistance, to be effective on biofilm. Hospital applications are still very limited due to the disadvantages of the narrow spectrum of action and the fact that the agent / bacteriophage relationship has not been identified before bacteriophage therapy, the potential of lysogenic bacteria to produce resistant bacteria causing changes in bacterial DNA and inadequate clinical trials. However, in today's emphasis on the need to apply personalized antibiotic therapy, personalized bacteriophage therapy is one of the most promising ways, especially in cases where the agent is identified, and when there are no other options for infections with multiple resistance microorganisms.

In our study, 99 MRSA isolates were taken from nasal swab samples of Turkish patients and Syrian immigrants. Antibiotic susceptibilities of the isolates were examined and their susceptibility to commercial phage preparations was evaluated. 35 of 99 MRSA isolates originate from Turkey patients (A group) and 64 of them originate from Syrian patients (G group). Antibiotic resistance rates of isolates in group A against CIP, ERY, DA, GEN, LNZ, RFM, MPR were detected as 8.5%, 31.4 %, 5.7%, 2.85%, 2.85%, 17.1%, and 20% respectively. In group G the these resistance rates were found as 12.5%, 26.5 %, 9.3%, 6.25%, 23.4%, 20.8%, and 34%. Although resistance rates were higher in the G group, except for erythromycin, this difference was statistically significant only for linezolid ($p = 0.008$).

Sensitivity percentages of the isolates to commercial phage preparations were found as ENKO 67.7%, INTESTI 55.5%, PYO 53.5%, SES 61.6%, and STP bacteriophages 44.4%. Sensitivity percentages of ENKO, INTESTI, PYO, SES, STP commercial bacteriophages were found as 68.6%, 57.1%, 55.3%, 65.7%, 45.7% for Turkish patient isolates, and 51.6%, 39%, 53%, 59%, 43.8% for Syrian immigrants respectively. Although the rates were not statistically significant between A and G groups, the sensitivity rates of Turkish isolates to all commercial phages were higher. Searching for phages to be used in treatment in the geography where the pathogen is common may be helpful in terms of reaching suitable phages. Commercial preparations are likely to be found more effective due to Turkey's border with Georgia and its close commercial and social relations.

In a small-scale study in Turkey by Ozkan et al.(2016) was found that PYO-bacteriophage cocktail was effective on 80% (8/10) of *S. aureus* isolates and 90% (9/10) of *P. aeruginosa* isolates, FERSISI-bacteriophage cocktail was effective on 100% (10/10) of *P. aeruginosa* and *S. aureus* (10/10) isolates and INTESTI-bacteriophage cocktail was effective on 90% (9/10) of *S. aureus* isolates and 80% (8/10) of *P. aeruginosa* isolates. A small number of isolates taken from a single center in this study is thought to be due to high phage sensitivity. At the same time, it is highly probable that the pathogens in our study have not been encountered in Georgia before, and therefore the relevant phage is not included in the regularly updated phage cocktails. Fortunately, these resistant strains were sensitive to at least one bacteriophages. It should be noted that the majority of *S. aureus* strains included in the collection (72 of 99; 72.7%) were sensitive to at least one of the tested phage products. There was no significant difference in the efficacy of lysis between antibiotic resistance. We found that phage products cause the lysis of antibiotic sensitive and resistant bacteria. This is a very important factor in deciding whether phages can be used as a clinical alternative to antibiotics. We could not find a relationship between the susceptibility of the bacterial species to the phages tested and the sensitivity to antibacterial agents.

Kuptsov et al. (2020) was found that monovalent bacteriophage products STAPHYLOCOCCAL bacteriophages demonstrated 86% efficacy against *S. aureus*. Another study Dvorackova et al. (2019) found that the commercial polyvalent bacteriophage preparation 'Stafal phage' (Bohemia Pharmaceuticals, Czech Republic) effectively killed 83% of MRSA and 99% of MSSA (methicillin-sensitive *Staphylococcus aureus*). In our study, STP phage had statistically significant lower activity to MRSA isolates compared to ENKO, INTESTI, and PYO. It is thought that the use of phages as monophages is effective in seeing low efficiency. The use of phages as cocktails is thought to increase lysis efficiency.

Conclusion

As a result, infections caused by MDR pathogens pose challenges in treatment around the world. To overcome these difficulties, phage therapy as a forgotten method has regained the attention of the rest of the world except Eastern European countries. For this reason, besides in vitro phage susceptibility studies on in vivo clinical phage applications have also gained momentum. However, different activity rates of phages to different isolates due to their host specificity are one of the most important factors limiting their widespread use. It would be meaningful to expand phage libraries with effective phages to multi-drug resistant strains that cause problems in treatment, or to expand phage libraries in accordance with countries' own isolates. Well characterization of the phages obtained for this purpose and determination of their activities by in-vivo studies are also important parameters.

Declarations

• Funding

Not applicable

• Conflicts of interest/Competing interests

The authors declared that they have no conflict of interest

• Ethics approval

This study was approved by Ankara City Hospital Ethics Committee, under the approval number E19-007, 2019

• Consent to participate (include appropriate consent statements)

This manuscript is an original work and has been prepared by the authors and They aware of its content and approves its submission.

• Consent for publication (consent statement regarding publishing an individual's data or image)

The manuscript has not been published elsewhere in part or in entirety and is not under consideration by another journal. The author give their consent for publication in the Archives of Microbiology.

• Availability of data and material (data transparency)

Not applicable

• Code availability (software application or custom code)

Not applicable

• Authors' contributions

The authors have contributed to designing this study, performed research, analyzed data, and wrote the article.

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Figures

Figure 1

Multiplex amplification for the detection of *S. aureus*-specific (*nuc*), MRSA-specific (both *mecA* and *mecC*) genes. M: 50 bp size DNA Ladder (Thermo Scientific, CA, USA); Lane 1: *nuc* gene positive sample (35GSM25); Lane 2 and 3: *nuc* and *mecA* gene positive sample (27ASM400 and 35GSM22, respectively); Lane 4: ATCC 25923 (*mecA*-negative *S. aureus*); Lane 5: NCTC 12493 (*mecA*-positive *S. aureus*); NCTC 13552 (*mecC*-positive *S. aureus*); Lane 7: negative control (water)