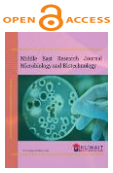

Middle East Research Journal of Microbiology and Biotechnology
ISSN: **2789-8644 (Print & Open Access)**
Frequency: Bi-Monthly
DOI: https://doi.org/10.36348/merjmb.2026.v06i02.008

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Phage Typing of *Staphylococcus aureus* Isolated from Cancer and Noncancer Patients in Iraq

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Abstract: ***Background*:** The bacteriophages have been considerably taken as an important aspect of infectious diseases attacking cancer patients particularly the normal flora causing opportunistic diseases among immunocompromised individuals with tumors. ***Materials and Methods*:** 400 patients were included and they were 200 patients with cancer diseases and 200 patients complaining of other illnesses. Swabs were taken from nares, sores and wounds and Staphylococcus aureus was isolated and identified. The bacteriophage typing was carried out in the in Unit of Phages/ Division of Bacteriology/ Central Public Health Laboratory/ Baghdad. ***Results*:** The present study revealed that 60% (105/175) were typable using the 23 types of bacteriophages which are internationally recognized for lysis of *S. aureus*. The other 40%(70/175) isolates were un-typable. It was demonstrated that the most common bacteriophages attacking staphylococci were 42E, 88A, 3C and 71 and the total numbers of S. aureus isolates susceptible to these phages were 34, 28, 24 and 22 respectively. The analysis demonstrates marked variability in lysis interaction frequencies among phage types. Phages 42E, 54, and 83A exhibited the strongest lytic interaction frequencies, suggesting high phage compatibility and dominant lysis behavior. Phages 53, 47, and 96 showed weak interaction frequencies, indicating limited lytic association. ***Conclusions*:** It was found that most of the isolates of *S. aureus* were lysed by the phage group III. A low number of bacterial isolates were lysed by phage group M. It was concluded that 18.7% of the bacterial isolates lysed by phage 29 were susceptible to phage 54 at the same time. It was demonstrated that the most common bacteriophages attacking staphylococci were 42E, 88A, 3C and 71 and the total numbers of S. aureus isolates susceptible to these phages were 34, 28, 24 and 22 respectively. The Chi-square test demonstrated a statistically significant difference in the distribution of *Staphylococcus aureus* phage types between cancer and noncancer patients ($p < 0.05$). This indicates that some phage types were more frequently associated with either cancer or noncancer isolates.

Keywords: Cancer Patients, Wounds, *S. Aureus*, Phage Typing.

Research Paper

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How to cite this paper:
Mohemid Maddallah Al-Jebouri & Ibraheem Abdul-kareem Al-Ani; "Phage Typing of *Staphylococcus Aureus* Isolated from Cancer and Noncancer Patients in Iraq" Middle East Res J. Microbiol Biotechnol., 2026 May-Jun 6(2): 118-127.

Article History:
| Submit: 23.05.2026 |
| Accepted: 25.06.2026 |
| Published: 30.06.2026 |

INTRODUCTION

The bacteriophages have been considerably taken as an important aspect of infectious diseases attacking cancer patients particularly the normal flora causing opportunistic diseases among immunocompromised individuals with tumors. In a study carried out by Ketchel and Rodriguez [1-4], they concluded that infectious diseases caused almost 20% of deaths among solid tumor patients. Most of the dangerous diseases were caused by Gram-negative bacteria like *Escherichia coli* [5], *Pseudomonas aeruginosa* [6], and *Klebsiella* [7]. Other infectious diseases of tumor patients were caused by Gram-positive bacteria like *Staphylococcus aureus* and *Staphylococcus epidermis* [7, 8]. These infectious diseases were usually endogenous, community – acquired or hospital-acquired infections [9-11]. The changing of anatomical barriers of cancer patients leads to immunological deficiencies particularly skin and mucous membranes causing invading of the opportunistic normal flora the human body and causing infections for those immunodeficient patients with tumor [12, 13]. Radiation can cause cancer. Highly energetic ionizing radiation (capable of knocking electrons out of atoms and molecules) is the most

hazardous. But non-ionizing radiation such as ultra-violet light, plays an important role in the genesis of human skin cancer. Survivors from Hiroshima and Nagasaki showed a marked elevation in the incidence of leukemia five years after the explosions in 1945. After a later period, the incidence of breast, thyroid, lung, and bowel cancers were found to be modestly elevated [14, 15]. The effect of radiation on man can be observed as weakness, age shortening, redness of the skin, and the elevation of incidence with leukemia [16, 17]. The effect of radiation on human cells appears within 10^{-16} second, usually called the direct physical effect of radiation. The biological effect of radiation not only depends on the dose, but also depends on the relative biological effect (R B E) which is consequently dependent on the type of radiation used. This is defined as the quality factor [16-19]. The effect of the American and affiliated forces attacks using the depleted uranium munitions and the rate of incidence of cancer diseases among the Iraqi peoples by comparing the incidence of cancers before and after the war in 1991[12-21].The present study concerned with the phage typing of most common infectious pathogen disseminated among cancer patients which was *Staphylococcus aureus*, The predominant phage group is belonging to the mixed group. Phage group (II) considered as an epidemiological marker correlated to b-lactamase hyper producer isolates. Staphylococcal isolates particularly MRSA types indicated high prevalence of phage group (II) with highly increase for phage types (Ø3A), related to the skin. Phage types (Ø80/Ø81) played an important roll in Community Acquired Methicillin Resistant *S. aureus* (CAMRSA). Phage groups (M) and (II) were found almost to be integrated for Gentamycin (GN) resistance especially phage type (Ø95) which relatively increased up to 20% in MRSA. Tetracycline (TE) resistant isolates typed by groups (II) and (III). It could be concluded that *S. aureus* isolates from the wound that originated and colonized, and started to build up multi-resistance against the topical treatment [22]. Typing was performed with the 23 phages of the international system: group I, phages 29, 52, 52A, 79, and 80; group II, phages 3A, 3C, 55, and 71; group III, phages 6, 42E, 47, 53, 54, 75, 77, 83A, 84, and 85; and group M (miscellaneous), phages 81, 94, 95, and 96. The methodology described by Blair and Williams was used in accordance with the recommendations of the International Subcommittee for the Phage Typing of *S. aureus* [23, 24]. *S. aureus* strains which were susceptible to phages from two or more of the abovementioned lytic groups were assigned to the mixed-group phage patterns. Strains that could not be typed at 100 times the routine test dilution (100 RTD) were considered non-typeable [25-29]. Most of the studies locally carried out on cancer patients were mostly for survey purposes neglected the epidemiological tools like serotyping, biotyping, resistotyping and phage typing [30, 31].

MATERIALS AND METHODS

Patients

This study was carried out in hospital of atomic medicine, Al-Khansaa Hospital and general hospital of Mosul city, Iraq. 400 patients were included and they were 200 patients with cancer diseases and 200 patients complaining of other illnesses. The acceptance for participation in the present study was taken from all the participants whose native language is Arabic. They were not mentally retarded and they were completely healthy considering hearing and speaking.

Sampling

Swabs were taken from nares, sores and wounds. Swabs were rinsed with sterile nutrient broth as a transport media. 200 swabs of cancer patients and 200 swabs were from noncancer patients [32].

Isolation

All swabs were inoculated on blood and mannitol-salt agars (Oxoid). Inoculated plates were incubated at 37 ^{0}C for 24 hours [33].

Identification of Staphylococcus aureus

The purified isolates of suspected S. aureus were conventionally identified following methods of workers [34, 35].

Bacteriophage Typing

The bacteriophage typing was carried out as described before [21]. This experiment was carried out in the in Unit of Phages/ Division of Bacteriology/ Central Public Health Laboratory/ Baghdad. The set of international phage types used in the present study were:
Group I: 29, 52, 52A, 79, 80.
Group II; 3A, 3C, 55, 71.
Group III: 6, 42E, 47, 53, 54.
Group M (miscellaneous): 81, 94, 95, 96.

Statistical Analyses

All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics such as means, standard deviations, and frequency distributions were computed to summarize the data [36-39].

RESULTS

Phage Typing of Staphylococcus Aureus

The present study revealed that 60% (105/175) were typable using the 23 types of bacteriophages which are internationally recognized for lysis of *S. aureus*. The other 40% (70/175) isolates were un-typable. The phage typing used showed that all isolates were human in origin (Table 1). It was found that most of the isolates of *S. aureus* were lysed by the phage group III. A low number of bacterial isolates were lysed by phage group M (Table 2). It was revealed that 61 isolates of S. aureus were susceptable to more than one group of phages used. 18.1% of the isolates were lysed by group III and M but

only 7 isolated (6.6%) were lysed by group I and group M (Table 3).

Table 1: Distribution of *Staphylococcus aureus* phage types isolated from cancer and other diseases patients

Source	Phage typed isolates: No.(%)	Non-phage typed isolates: No.(%)	Total isolates
Cancer patients	54 (30.9)	44(25.1)	98(56)
Non-cancer patients	51 (29.1)	26(14.9)	77(44)
Total	105(60)	70(40)	175(100)

Table 2: Distribution of *Staphylococcus aureus* isolates according to their phage typing

Phage group	Phage typed cancer isolates: No.(%)	Phage typed non-cancer patients: No.(%)	Total No.(%)
Group I	12(7.7)	17(11.0)	29(18.7)
Group II	22(14.2)	10(6.5)	32(20.6)
Group III	29(18,8)	38(24.5)	67(43.2)
group M	9(5,8)	18(11.6)	27(18.1)
Total	72(46.5)	83(53.5)	155(100)

Table 3: Distribution of *Staphylococcus aureus* isolates according to their lysis by more than one phage type

Sharing phages	Isolates No. (%)
I+II	9(8.6)
I+III	17(16.2)
I+M	7(6.6)
II+III	9(8.6)
III+M	19(18.1)
Total	61(100)

Frequency of Bacteriophage Types among Staphylococcus Aureus Isolates from Cancer and Noncancer Patients

It was demonstrated that the most common bacteriophages attacking staphylococci were 42E, 88A, 3C and 71 and the total numbers of S. aureus isolates susceptible to these phages were 34, 28, 24 and 22 respectively (Table 4). Phage type 53 was only able to lyse one isolate of cancer patient. The Chi-square test demonstrated a statistically significant difference in the distribution of *Staphylococcus aureus* phage types between cancer and noncancer patients ($p < 0.05$). This indicates that some phage types were more frequently associated with either cancer or noncancer isolates (Figure 1).

Table 4: Frequency of *Staphylococcus aureus* phages among isolates from cancer and noncancer patients

Phage type	Cancer patients		Noncancer patients		Total	
	No.	%	No.	%	No.	%
42E	11	20.3	23	45.1	34	32.3
B3A	15	27.7	13	25.5	28	26.6
3C	16	29.6	8	15.6	24	22.8
71	16	29.6	6	11.7	22	20.9
95	5	9.2	14	27.4	19	18.1
29	6	11.1	10	19.6	16	15.2
52	7	12.9	9	17.6	16	15.2
85	7	12.9	8	15.6	15	14.3
54	5	9.2	8	15.6	13	12.3
77	4	7.4	7	13.7	11	10.5
94	3	5.5	7	13.7	10	9.5
81	2	3.7	6	11.7	8	7.6
80	2	3.7	4	7.8	6	15.7
55	4	7.4	2	3.9	6	5.7
3A	5	9.2	1	1.9	6	5.7
B4	3	5.5	1	1.9	4	3.8
96	3	5.5	1	1.9	4	3.8
79	0	0	3	5.8	3	2.8
75	2	3.7	1	1.9	3	2.8
47	1	1.8	2	3.9	3	2.8
53	1	1.8	0	0	1	1.0
Total	**118**	**46.8**	**134**	**53.2**	**252**	**100**

*= Chi-square (χ^2) = 32.06; Degrees of freedom (df) = 20; p-value = 0.0427.

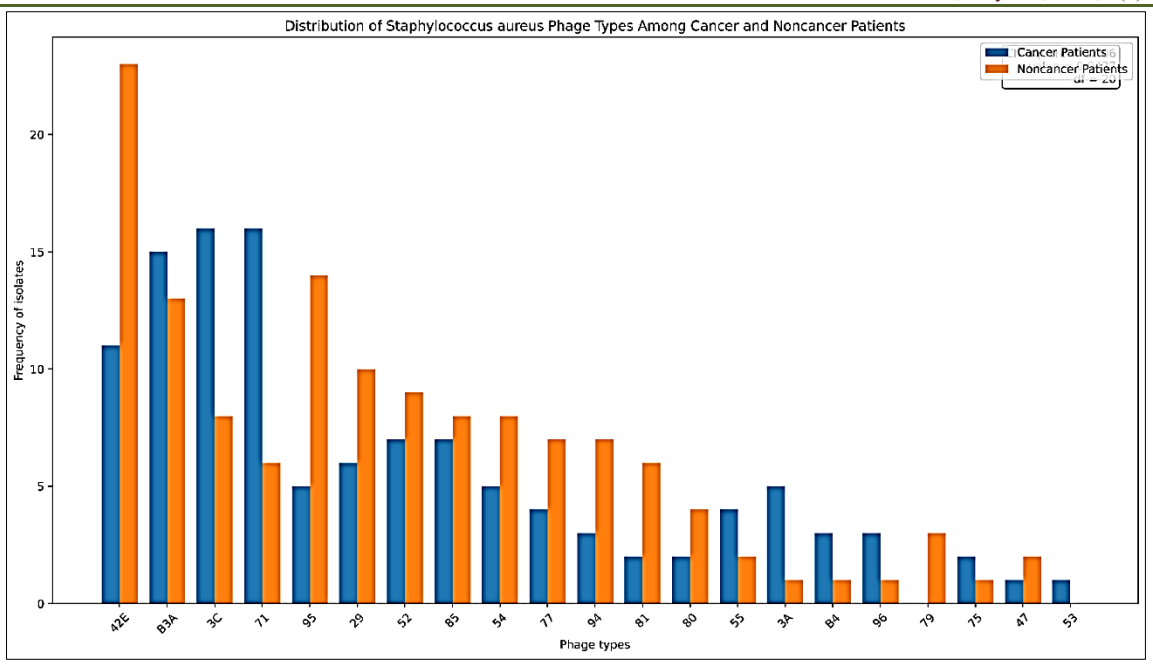

Figure 1: Distribution of *Staphylococcus aureus* Phage Types among Cancer and Noncancer Patients

2x2 Matrix of Phages Causing Lysis to Staphylococcus Aureus

The present study revealed that the 2x2 matrix analysis which showed the number of the susceptible isolated of S, aureus to 2 phages at the same time (Table 5. The data presented in Table 5 shows that the most common pair of phages was 71/3C that abled to lyse 14 isolates at the same time whereas the two pairs 83A/42E and 83A/77 were able to lyse 9 isolates at the time each. Statistical analyses showed that the mean interaction frequency was 26.35 with standard deviation equal to 16.22. The maximum value was 55 and the minimum was 1. The analysis demonstrates marked variability in lysis interaction frequencies among phage types. Phages 42E, 54, and 83A exhibited the strongest lytic interaction frequencies, suggesting high phage compatibility and dominant lysis behavior. Phages 53, 47, and 96 showed weak interaction frequencies, indicating limited lytic association. The dashed horizontal line represents the overall mean interaction frequency across all phage types (Figure 2).

Table 5: Matrix shows number of *Staphylococcus aureus* lysis at 2x2 pattern of the same time

29	1																			
52	0	7																		
79	0	1	1																	
80	0	3	3	1																
3A	0	1	4	0	0															
3C	0	2	6	0	0	5														
55	0	2	4	0	0	2	5													
71	0	1	6	0	0	5	14	5												
54	0	3	3	3	1	3	3	1	2											
53	0	0	0	0	0	0	0	0	0	0										
47	0	0	0	0	0	0	0	0	0	0	1									
42E	0	3	6	3	3	2	3	2	3	7	0	1								
85	1	3	0	1	1	1	2	1	0	4	0	1	3							
84	0	0	0	0	0	0	0	0	0	0	0	0	0	1						
83A	2	3	3	2	1	1	1	1	1	4	0	0	9	8	3					
77	0	2	2	1	1	1	1	0	0	3	0	0	6	4	1	9				
75	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	3	1			
81	1	2	2	2	2	0	0	0	0	3	0	0	6	1	0	6	3	1		
94	2	2	2	2	1	1	0	0	0	3	0	0	5	1	0	3	3	0	5	
95	1	3	1	3	2	0	1	1	0	6	0	0	4	2	0	6	2	0	3	6
	96	29	52	79	80	3A	3C	55	71	54	53	47	42E	85	84	83A	77	75	81	94

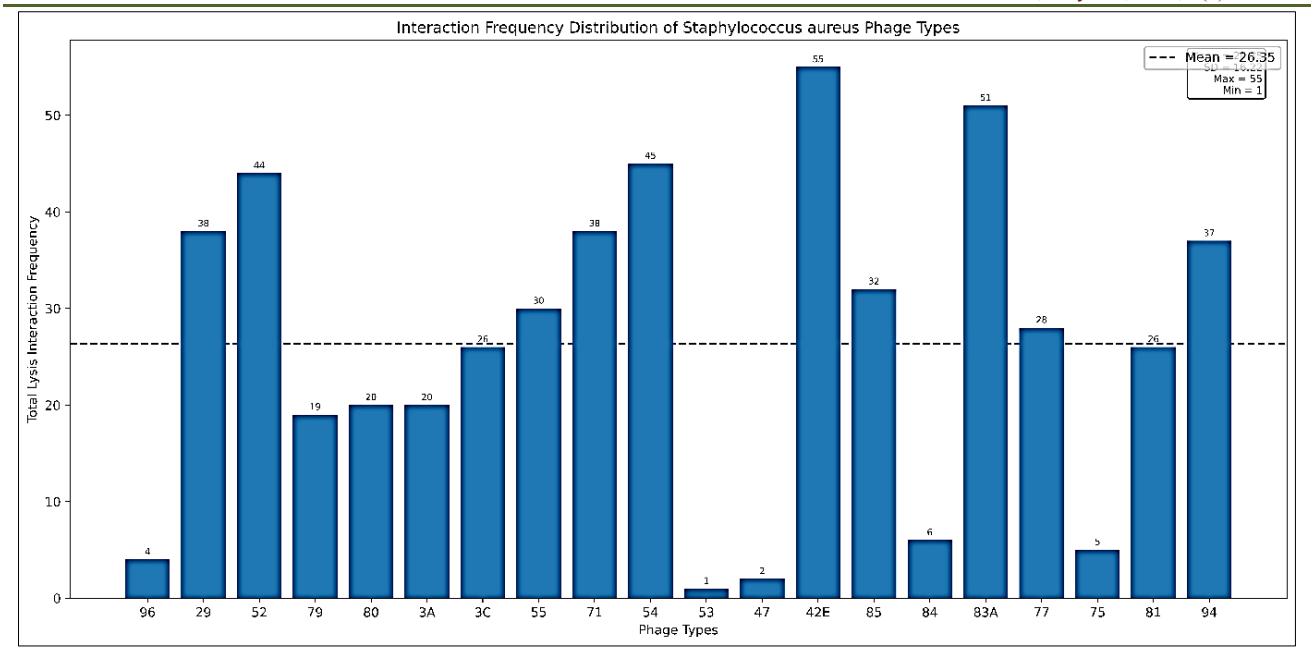

Figure 2: Interaction Frequency Distribution of *Staphylococcus aureus* Phage Types

2x2 Matrix of Percentages of Staphylococcus Aureus Cultures Lysed

It was concluded that 18.7% of the bacterial isolates lysed by phage 29 were susceptible to phage 54 at the same time (Table 6). It was noticed also that 23.1% of the isolates that susceptible to phage 54 were lysed by phage 29 at the same Time. It was also concluded that the similarity in frequency of lysis was limited but still existed, e.g. the phage pair 29/52 Frequency was 43.7% in both upper and lower triangles of the matrix. This character is so important for epidemiological Study as a tool for following the distribution of the most common phages that able to lyse the bacterial isolates. The statistical analyses revealed that mean lysis frequency was 275.71 with standard deviation equal to 107.20. The linear regression coefficient of determinant value was (R^2) value equal to 0.006 with regression P value = 0.7448. The data showed substantial variability in lysis frequencies among different phage types. Phages 54, 83A, 94, and 80 demonstrated the highest lysis activity. The regression analysis indicated a very weak overall linear trend, as reflected by the low R^2 value, suggesting that lysis frequencies vary independently across phage categories rather than following a consistent linear pattern (Figure 3).

Table 6: Frequency matrix of total number of *Staphylococcus aureus* lysis by each phage

	96	29	52	79	80	3A	3C	55	71	54	53	47	42E	85	84	83A	77	75	81	94	95
96	-	25	0	0	0	0	0	0	0	0	0	0	0	25	0	50	0	0	25	50	25
29	6.2	-	43.7	6.2	18.7	12.5	12.5	12.5	2.6	18.8	0	0	18.7	18.7	0	18.7	12.5	0	12.5	12.5	18.7
52	0	43.7	-	6.2	18.7	37.5	25	25	37.5	18.7	0	0	37.5	0	0	18.7	12.5	0	12.5	12.5	6.2
79	0	33.2	33.2	-	33.2	0	0	0	0	100	0	0	100	33.3	0	66.6	33.3	0	66.6	66.6	100
80	0	50	50	16.6	-	0	0	0	0	25	0	0	75	25	0	25	25	0	50	25	50
3A	0	16.6	66.6	0	0	-	83.3	33.3	83.3	50	0	0	33.3	16.6	0	16.6	16.6	0	0	16.6	0
3C	0	8.3	25	0	0	20.8	-	20.8	58.3	12.5	0	0	12.5	8.3	0	4.2	4.2	0	0	0	4.2
55	0	33.3	66.6	0	0	37.3	83.3	-	83.3	83.3	66.6	0	0	33.3	16.6	0	16.6	0	0	0	16.6
71	0	4.5	27.2	0	0	22.7	63.6	22.7	-	9.1	0	0	13.6	0	0	4.5	0	0	0	0	0
54	0	23.1	23.8	23.1	7.7	23.1	23.1	7.7	15.4	-	0	0	53.8	30.8	0	30.7	23.1	0	23.1	23.1	46.2
53	0	0	0	0	0	0	0	0	0	0	-	100	0	0	0	0	0	0	0	0	0
47	0	0	0	0	0	0	0	0	0	0	33.3	-	33.3	33.3	0	0	0	0	0	0	0
42E	0	8.8	17.6	8.8	8.8	5.8	8.8	5.8	8.8	20.6	0	2.9	-	8.8	0	26.4	17.6	0	17.6	14.7	11.7
85	1	20	0	6.6	6.6	6.6	17.3	6.6	0	26.6	0	6.6	20	-	6.6	53.3	26.6	6.6	6.6	6.6	13.3
84	0	0	0	0	0	0	0	0	0	0	0	0	0	25	-	75	25	25	0	0	0
83A	7.1	10.7	10.7	7.1	3.5	3.5	3.5	3.5	3.5	14.3	0	0	32.1	28.5	10.7	-	32.1	10.7	21.4	10.7	21.4
77	0	2	18.2	18.2	9.1	9.1	9.1	9.1	0	0	17.3	0	0	54.5	26.3	9.1	-	9.1	27.2	27.2	18.2
75	0	0	0	0	0	0	0	0	0	0	0	0	0	0	33.3	33.3	3.5	-	12.5	0	0
81	12.5	25	25	25	25	0	0	0	0	37.5	0	0	7.5	12.5	0	75	37.5	12.5	-	50	30
94	20	20	20	20	10	10	0	0	0	30	0	0	50	10	0	30	30	0	50	-	31.5
95	5.3	15.7	5.3	15.7	10.5	0	5.3	5.3	0	30.5	0	0	21.1	10.5	0	31.5	10.5	0	15.8	31.5	-

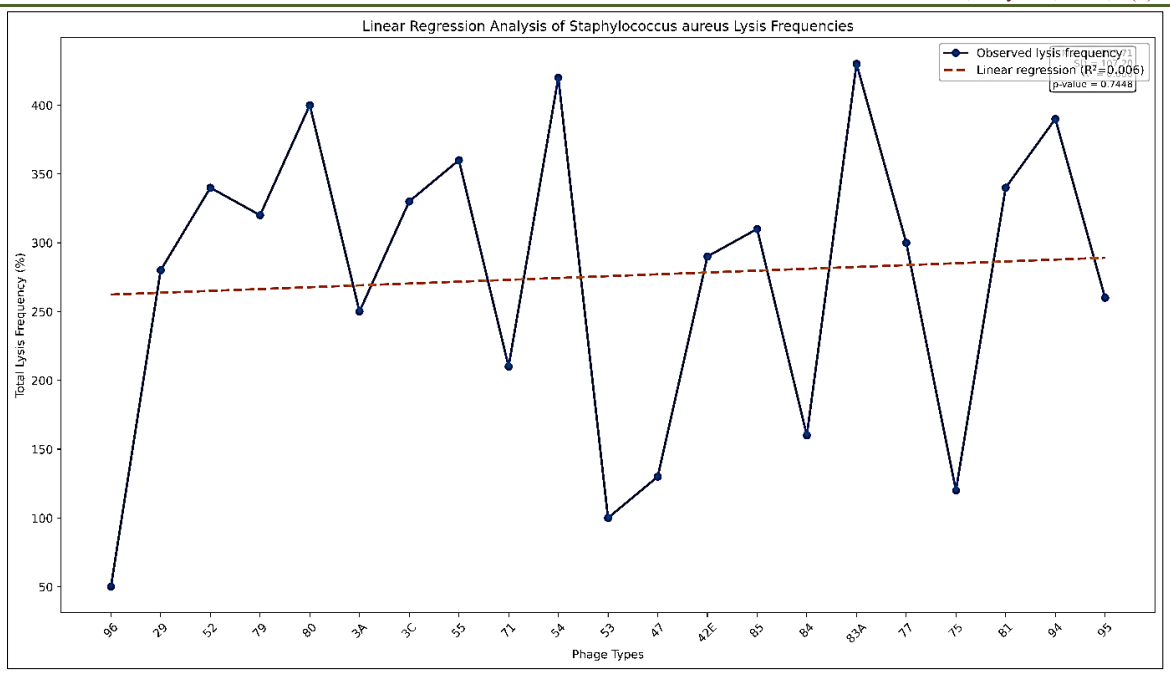

Figure 3: Linear Regression Analysis of *Staphylococcus aureus* Lysis Frequencies

DISCUSSION

The present study revealed that 60% of the *Staphylococcus aureus* isolates were susceptible to phage types used in this investigation [Table 1]. The other 40% isolates were un-typable. At the same time, it was found that phage typing showed that all bacterial isolates were human in origin that is why there was no need to use phage types of animal origin. It was revealed that 61 isolates of *S. aureus* were susceptible to more than one group of phages used. 18.1% of the isolates were lysed by group III and M but only 7 isolated (6.6%) were lysed by group I and group M. The present results were almost similar to those concluded elsewhere [29], who found that 50-80 % of the coagulase – positive staphylococci were abled to be typed by one or more of international phages. Khalaf and his co-workers [40], found at Basra district that 63.8% of S. aureus isolates were susceptible to the same international phages. On the other hand, Ma *et al*., [31-41], demonstrated that most of staphylococcal isolates were not susceptible to set of international phage types that used in the present study. MacFarlane and his colleagues [42-44], demonstrated that 19% of the S. aureus isolates were resistant to phages and this might be another indication that the source and geographical site of isolates were limitations of bacterial susceptibility to phages. Moreover, it was found that the un-typable isolates of bacteria were under steadily increase in number particularly inside the hospitals, and suggested that this phenomenon might be due to genetical changes of phages to become unable to lyse the bacteria [45-48]. It was found in the present study that most of the isolates were susceptible to group III of the phages used. However, It was found elsewhere that most of the S. aureus isolates from Baghdad were highly susceptible phage group I [49], and the results were different compared to the findings concluded in the present study but almost similar to the patterns demonstrated by MacFarlane and his co-workers [42-51]. It was found also that most of the isolates studied here were susceptible to one or more of the phages belonging to groups I and III. These results were almost similar to those of Khalaf *et al*., [40]. It was also demonstrated by Williams and Rippon [53], that isolates typeable by more than one group of phages made patterns of epidemiological concern [54-57]. The present study showed that most of the bacterial isolates were susceptible to phages type 42E, 83A, 3C and 71 and these phages were belonging to groups II and III. It was concluded the susceptible isolates to these group are usually associated with wound pus, bacteremia and food-poisoning caused by *S. aureus* [Parker 158]. The frequency of 42E, 83A, 71 and 3C phages lysed isolates of cancer patient. The Chi-square test demonstrated a statistically significant difference in the distribution of *Staphylococcus aureus* phage types between cancer and noncancer patients ($p < 0.05$). This indicates that some phage types were more frequently associated with either cancer or noncancer isolates were mostly different from those of noncancer patients isolates like 42E, 95, 83A and 52. It was found that the phage type 3C was more prevalent in Mosul area compared to other phage types specific for staphylococcal isolates in Basra, Baghdad and Tikrit areas of Iraq [59].

The 2x2 matrix analyses of the phages pair showed that 71/3C pair was the most prevalent causing *S. aureus* isolates lysis at the same time which able to attack 14 isolates. The second most common pairs were 42E/83A and 77/83A which abled to lyse 9 isolates by each of them at the same time. Phage type 53 was only able to lyse one isolate of cancer patient. The Chi-square test demonstrated a statistically significant difference in

the distribution of *Staphylococcus aureus* phage types between cancer and noncancer patients ($p < 0.05$). This indicates that some phage types were more frequently associated with either cancer or noncancer isolates. It was tabulated a matrix of percentages of bacterial isolated lysed (Table 6) by dividing the results in Table 5 over data of Table 4. It was noticed that the results of this numerical analyses (Table 6) were not commonly symmetrical, e.g. if you noticed the second line of the matrix you will see that 18.7% of the isolates lysed by phage 29 was lysed by phage 54 at the same time but one can found in line 10 that 23.1% of bacterial isolates which were lysed by phage 54 were lysed by phage 29. Generally, Table 6 shows that the symmetrical pattern was rarely seen, e.g. phage 52/29 showed a symmetrical manner between upper and lower triangles of the matrix when the percentage was 43.7%. This case had a characteristic feature in epidemiological studies for following up these patterns of bacteriophages which are more common with respect to lysis of bacterial isolates. Furthermore, it was found that the most common phage patterns were 52/80, 52A/52, 29/52 and 29/79 with percentages were 78, 87, 68 and 48% respectively [60-63]. The present study showed that the most common phages were 42E, 83A and 3C with frequencies were 32.3, 26.6 and 22.3% respectively. Typing was performed with the 23 phages of the international system: group I, phages 29, 52, 52A, 79, and 80; group II, phages 3A, 3C, 55, and 71; group III, phages 6, 42E, 47, 53, 54, 75, 77, 83A, 84, and 85; and group M (miscellaneous), phages 81, 94, 95, and 96. The methodology described by Blair and Williams [21], was used in accordance with the recommendations of the International Subcommittee for the Phage Typing of S. aureus [64, 65]. S. aureus strains which were susceptible to phages from two or more of the abovementioned lytic groups were assigned to the "mixed-group" phage patterns. Strains that could not be typed at 100 times the routine test dilution (100 RTD) were considered non-typeable [67]. The resurgence of interest in staphylococcal phages is no longer a peripheral scientific curiosity – it has become a central theme in the broader effort to confront the antimicrobial resistance crisis. From foundational discoveries to current preclinical and clinical developments [68-73], the growing body of evidence supports bacteriophages not only as therapeutic agents but also as powerful tools to understand bacterial evolution, defense systems, and gene transfer. Their versatility extends across medical, veterinary, agricultural, and industrial applications [74-78].

CONCLUSIONS

It was found that most of the isolates of *S. aureus* were lysed by the phage group III. A low number of bacterial isolates were lysed by phage group M. It was concluded that 18.7% of the bacterial isolates lysed by phage 29 were susceptible to phage 54 at the same time. It was demonstrated that the most common bacteriophages attacking staphylococci were 42E, 88A, 3C and 71 and the total numbers of S. aureus isolates susceptible to these phages were 34, 28, 24 and 22 respectively. The Chi-square test demonstrated a statistically significant difference in the distribution of *Staphylococcus aureus* phage types between cancer and noncancer patients ($p < 0.05$). This indicates that some phage types were more frequently associated with either cancer or noncancer isolates.

Acknowledgements

The authors extend their appreciation to the Department of Scientific Research at University of Tikrit for funding this work.

Statement of Ethics

All the procedures involving human participation were conducted in strict accordance with ethical standards of Institutional Research Committee, Department of Scientific Research, Tikrit University as well as the 1964 Helsinki Declaration and its subsequent amendments or equivalent ethical norms.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of Interest Statement: The author declares that he has no conflicts of interest, financial or otherwise.

Funding Sources: The author extends his appreciation to the Department of Scientific Research of University of Tikrit.

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