

PHAGE TYPING OF STAPHYLOCOCCUS AUREUS ISOLATED FROM DAIRY COWS IN EGYPT

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SUMMARY

Phage typing occupies an interesting and important capital in this work. This review records observations of phage typing of *S. aureus* strains obtained during this investigation from mastitis and normal milk of dairy cows. (using the basic international set of 21 phages, which also includes 8 phages of Davidson recommended bovine set (1972).

A total of 103 *S. aureus* cultures (60 mastitis and 43 normal milk) were subjected to phage typing using phages at routine test dilution (RTD). In the current study 58.3% of cultures were typable.

It is interesting to mention that phage 42D of GIV was dominant and capable of lysing the largest number of cultures.

There were 16 recognizable phage patterns in mastitis and 6 in normal milk *S. aureus* strains. The phage patterns were made up of phages form groups. 1 III, Iv and none from group. II.

INTRODUCTION

A primary goal of this study is the phage typing of

S. aureus strains obtained during investigation of dairy cattle in Egyptian farms.

Phage typing is an established technique in epidemiological studies of *S. aureus* infection in man (Blair & Williams, 1961 and Frost, 1967). The system is well developed for typing human strains and systems for typing various animal strains are in different stages of development (Wilson and Miles, 1975). However, phage typing has become widely used as an internationally accepted technique for the study of *S. aureus* (animal origin), particularly those associated with mastitis in dairy cattle (Davidson, 1961; Frost, 1967 and Davidson, 1972).

Some workers have used the international basic human set to type bovine strains (Mac-Donald, 1946; Davidson, 1961, and Frost, 1962), other have introduced new phages where the human phages were unsatisfactory (Smith, 1948 a & b, Seto and Witson, 1958, Nakagawa, 1960, Davidson, 1961 and Frost, 1967).

The opportunity was taken here to record observations of phage typing of *S. aureus* strains obtained during this investigation from dairy cattle (Normal and mastitis milk) using the basic international set of 21 phages which also includes

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8 phages of Davidson recommended bovine set (1972). It is believed that no such work has been performed before in Egypt.

MATERIAL AND METHODS

Strains:

One hundred and three *S. aureus* strains originated from bovine milk specimens (60 mastitis & 43 normal) were used in testing the strain range specificity of phages. All strains were catalase positive, fermented glucose anaerobically, coagulase positive, acetoin produced form glucose, and fermented maltose rapidly (Holt et al., 1994 and Quinn et al., 1994).

Phage set:

The international basic set of 21 phages (human and bovine phages) for typing staphylococci were obtained from American Type Culture Collection (ATCC), 12301 Parkloun Dr, Rochville, MD 20852 USA (Table 1).

Phage typing:

This was carried out using phages listed in table (1). Phages were all used at routine test dilution (RTD). The basic technique of phage typing followed that published by Blair and Williams (1961) and described by Cruichshank et al., (1975).

Interpretation of Results:

Strains showing lysis (ranging from more than 50 plaques to confluent lysis) were recorded as typable.

RESULTS

PHAGE TYPING OF BOVINE *S. AUREUS* STRAINS

A total of 103 strains of *S. aureus* from cows milk were subjected to the set of 21 typing available phages. The *S. aureus* isolates consisted of 60 from mastitis milk and 43 from normal milk. Of

Table (1) The composition of the International basic phage set for typing of *S. aureus*.

Group	Bacteriophages
I	②9, 52, ⑤2A, 79, 80
II	③A, 3C, 55
III	⑥, 42E, 47, ⑤3, 54, ⑦5, 77, 83A, ⑧4, 85
IV	④2D
Misc	81, 187

○ Also in the international basic set for typing bovine *staphylococci* Davidson, (1972) and Carroll & Francis (1985).

Table (2): Percentage of *Staphylococcus aureus* isolates lysed by individual phages

Group	Phage	No. of isolates tested	isolates lysed*	
			No.	%
I	29	103	0	0
	52	103	23	22.3
	52A	103	2	1.94
	79	103	11	10.7
	80	103	0	0
II	3A	103	0	0
	3C	103	0	0
	55	103	0	0
	71	103	0	0
III	6	103	0	0
	42E	103	2	1.94
	47	103	0	0
	53	103	0	0
	54	103	0	0
	75	103	24	23.3
	77	103	2	1.94
	83A	103	6	5.8
	84	103	0	0
	85	103	0	0
IV	42D	103	35	34
Misc.	81	103	9	8.7
	187	103	0	0

* Strong reaction at RTD

Misc. = Miscellaneous group.

Table (3): Percentage of *S. aureus* isolates from normal and mastitis milk lysed by individual phages.

Group	Phage	Mastitis			Normal		
		Isolates tested	Isolates lysed		Isolates tested	Isolates lysed	
			No.	%		No.	%
I	52	60	21	35	43	2	4.7
	52A	60	1	1.7	43	1	2.3
III	79	60	11	18.3	43	0	0
	42E	60	2	3.3	43	0	0
	75	60	22	36.7	43	2	4.7
	77	60	2	3.3	43	0	0
	83A	60	2	3.3	43	4	9.3
IV	42D	60	34	58.6	43	1	2.3
Misc.	81	60	8	13.3	43	1	2.3

Table (4): Percentage frequency of phage patterns of 53 typable strains of *S. aureus* from mastitis cow milk

Phage patterns lysis	Group	No. of strains lysed	Frequency%
75, 42D	III, IV	3	5.7
52, 79, 42D,	I, IV	1	1.9
75, 42D, 81	III, IV, Misc	2	3.8
52, 79, 75, 42D	I, III, IV	2	3.8
52, 75, 42D	I, III, IV	7	13.2
42D	IV	13	24.5
52	I	3	5.7
52, 81	I, Misc.	1	1.9
79	I	7	13.2
52, 75, 42D, 81	I, III, IV, Misc.	5	9.4
52A, 42D	I, IV	1	1.9
42E, 77	III	2	3.8
75	III	2	3.8
83A	III	2	3.8
52, 79	I	1	1.9
52, 75	I, III	1	1.9
Total		53	88.3

It is shown from table (4) that among the fifty three typable strains of *S. aureus* recovered from mastitis milk, there were 16 recognizable phage patterns.

Table (5): Percentage frequency of phage patterns of 7 typable strains of *S. aureus* from normal cow milk.

Phage patterns lysis group	Group	No. of strain lysed	Frequency %
52, 75, 83A, 81	III, Mise.	1	14.3
52A, 75, 83A	I, III	1	14.3
83A	III	1	14.3
75, 83D	III	1	14.3
52	I	2	28.6
42D	IV	1	14.3
Total		7	16.3

these isolates, 60 (58.3%) were typable at the RTD of available phages. The remaining cultures were considered untypable.

The percentage frequency with which the isolates were lysed by individual phages can be seen in tables (2 and 3).

DISCUSSION

In this study, the bacteriophage typing of *S. aureus* isolates from mastitis and normal milk of cows was performed.

Bovine mastitis caused by *S. aureus* is serious

problem in all the major dairy countries (Frost, 1967 and Wilson and Richards, 1980). There are many different phage types of *S. aureus* and epidemiologically it is of significance to be able to tell the distribution of these types involved in udder infection and their lytic pattern. Phage typing is an established technique in epidemiological studies of *S. aureus* infection in man. Davidson (1972) established an internationally recommended set of bacteriophage for typing *S. aureus* mastitis in dairy cattle.

One hundred and three *S. aureus* cultures isolated from cow milk (60 mastitis and 43 normal) were subjected to phage typing using international basic set of 21 phages (including 8 phages of

* Unfortunately, the complete Davidson's recommended set could not be obtained.

Davidson's international basic set for typing, bovine staphylococci) at Routine Test Dilution (RTD).

In the current study, 58.3% of cultures were typable. The typable strains consisted of 53 (88.8%) from mastitis milk and 7 (16.3%) from normal milk (Tables 2 and 3). This finding indicates that the set of phages used is capable of typing the majority of mastitis strains but not normal strains reflecting the difference in distribution of phage types involved in udder infection and their suggested role in mastitis. Further experiments must be carried out to determine this point.

It would appear therefore, that *S. aureus* population has become less susceptible to lysis of this set of 21 phages used in this study.

The number of typable mastitis cultures at RTD (88.8%) is somewhat different with the results (73.6%) of Carrol and Francis (1985) and also with the range of results (69-73%) obtained by Davidson (1972) when typing bovine *S. aureus* cultures.

Holmberg (1975), employing at RTD 16 phages, was able to type 75.4% of *S. aureus* strains isolated from bovine milk in Sweden. However, Davidson (1972) found that 84% of U. K. strains were typable using a set of 9 phages and up to 89% when using a wider range of 30 phages. It would seem therefore that these variable range of typable strains could be attributed to the difference in phages set and dilution used as well as phage types exists within a geographically distinct area.

Some phages particularly 42 D from Group IV and 75 from group III and 52 from group I were particularly appropriate than others since they lysed 34% and 23.3% and 22.3% of isolates respectively. Nevertheless, some phages

particularly from G II would seem to be totally inappropriate for future typing of bovine *S. aureus* strains since none of phages belonged to GII could lyse any of isolates. This result would agree with that of other workers (Frost, 1967, Olson et al. 1969, Jones et al. 1972, Rahman and Baxi, 1983).

It is interesting to mention that phage 42 D of group IV was dominant and capable of lysing the largest numbers of cultures, a result which reflects its particular significance in typing of bovine population of *S. aureus*. Some authors have found a predominance of phage group IV in lysis of bovine *S. aureus* (Bonin and Blobel, 1967; Meyer, 1967; and Wilson and Miles, 1975; Mackie et al., 1986; Nag and Ghosh-Ray, 1990 and Shimizu et al., 1990).

Amongst phages of group I, group III and miscellaneous group, 10.7% of strains were susceptible to phage (79), 5.8% to phage (83 A) and 8.7% to phage (81) respectively. Susceptibility to other phages of these groups was of lower order (less than 2%). Different results were noted by Nag and Ghosh-Ray (1990) on the susceptibility of bovine staphylococci to individual phages within the groups who mentioned that the majority of strains were susceptible to phages 6 and 42E of group II while susceptibility to different phages of group I was of lower order followed by group II and miscellaneous group. This finding in turn confirms what is previously suggested that differences in phage susceptibility exist within geographical origin of strains (Holmberg, 1975 and Overturf et al 1991).

Among the staphylococcus strains examined there were 16 recognizable phage patterns in mastitis strains and 6 in normal milk strains.

The most common phage pattern was 42 (24.5%) which was made up of single phage from group IV. All the remaining phage patterns w

made up of phage from group I, III, IV and none from group II. In this concern, Surjala and Saloniemi (1987) recorded that the most common phage patterns was made up of phages of group IV (62.8%) I+M (16.1%), III + IV (6.6%) and III (3.9%).

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