

Phage Typing of *Staphylococcus aureus* Isolates from Poultry Meat in Spain

Rosa Capita*, Maite Álvarez-Astorga, Carlos Alonso-Calleja, Benito Moreno and María del Camino García-Fernández

Department of Food Hygiene and Food Technology, Veterinary Faculty, University of León, Campus Universitario de Vegazana, s/n. 24071. León, Spain

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Phage typing is currently used for typing of *Staphylococcus aureus* strains beyond the species level in epidemiological studies. A total of 168 *Staphylococcus aureus* isolates from chicken meat and chicken by-products were phage-typed using the international bacteriophage set for typing *Staphylococcus aureus* of human origin. One hundred and forty-eight (88.09%) strains were phage-typeable (at least one phage produced 20 or more plaques of lysis). Lysis by phages of group III was the most frequent with 99 (58.93%) sensitive strains. This fact coincides with results of other authors. Twenty-nine different phage patterns were observed and three (95, 75/84 and 6/1030/W57) were most common. One hundred and thirty-two (89.19% of typeable strains) showed these or indistinguishable (only one phage reaction difference) patterns. Twenty-six out of seventy chicken samples (37.14%) harboured more than one phage type of *Staphylococcus aureus*. This fact emphasizes the convenience of subtyping several *Staphylococcus aureus* isolates from the same sample in epidemiological studies. 80% of sausages and hamburgers contained the same *Staphylococcus aureus* phage types, which were not found in any of the other food types. This fact suggests a cross contamination during the processing of these foods. Phages 6, 75, 84, 1030 and W57 showed the greatest activity. None of the *Staphylococcus aureus* strains were sensitive to phages 47, 81 and 94.

Key words: *Staphylococcus aureus*, typing, phage typing, poultry, Spain

Staphylococcus aureus enterotoxigenic strains have been demonstrated as one of the major causes of foodborne illness throughout the world (9, 26, 33, 41). As a consequence, there is a widespread requirement for reliable methods to detect and type *Staphylococcus aureus* either for food quality assurance programmes or for epidemiological studies. Their presence in poultry meat and poultry by-products (44) emphasizes the need for laboratory surveillance for this bacterial pathogen. Various methods are used in epidemiological research. Coagulase typing (43, 45) and enterotoxin typing (24) are of limited value because they do not allow for detailed classification. Molecular based typing methods such as plasmid profile analysis and chromosomal probe fingerprinting (ribotyping) (20) or restriction fragment length polymorphism (RFLP) of genomic DNA determined by means of pulsed-field gel electrophoretic (PFGE) analysis (20, 36, 40, 42) seem to be very promising. However, they are generally too expensive, time consuming, and are not yet suitable for application in routine typing, especially in smaller laboratories. Phage typ-

ing is currently used for typing of *Staphylococcus aureus* strains in epidemiological studies. Since 1952 this technique has found widespread use, and an international system has been established for typing human strains (10).

The aim of this study was to characterize the *Staphylococcus aureus* strains isolated from chicken meat and chicken by-products by phage typing in order to investigate the possible source and the relationship of the strains, to determine their diversity within and between samples, to obtain data for epidemiological studies, and to evaluate the usefulness of this typing method used for epidemiological investigation of *Staphylococcus aureus* originating from poultry.

Materials and Methods

Strains

Forty chicken carcasses, fifteen chicken parts (5 legs, 5 wings and 5 gIBLETS-livers and hearts) and fifteen processed chicken products (5 red sausages, 5 white sausages and 5 hamburgers) purchased in retail outlets in León (Spain) were tested for *Staphylococcus aureus* by surface

* To whom correspondence should be addressed.
(Tel) 34-87-243123, 34-87-238162; (Fax) 34-87-243123
(E-mail) dhtrcg@unileon.es

plating using Baird-Parker agar with egg yolk/tellurite emulsion (Oxoid Ltd., Hampshire, England). Typical *Staphylococcus aureus* colonies were randomly taken off the Baird-Parker plates and identified on the basis of Gram-stain reaction (22), catalase test (11), modified oxydase test (16), anaerobic growth in a glucose-containing soft agar (15), lysostaphin sensitivity (39), coagulase test (29), thermo-stable nuclease (34), carbohydrate (maltose and mannitol) dissimilation, and acetoin production (7). The identification schemes proposed by Evans and Kloos (15), Schleifer and Kloos (38) and Schleifer (37) were used. One hundred and sixty-eight isolates were identified as *Staphylococcus aureus*.

Phage typing

Phage typing of all strains was carried out according to standard methods (10) with the 23 phages of the international bacteriophage set for typing *Staphylococcus aureus* of human origin (6): 29, 52, 52A, 79, 80 (Group I); 3A, 3C, 55, 71 (Group II); 6, 42E, 47, 53, 54, 75, 77, 83A, 84, 85 (Group III); 81, 94, 95, 96 (Miscellaneous). For reversed phage typing, additional phages 1030, W57, 2009 and 18042 were also used. The phages were purified and propagated on susceptible strains in soft agar (Oxoid Nutrient Agar CM3 overlaid with: Oxoid Nutrient Broth +0.5% Oxoid Agar L11+400 µg/ml CaCl₂). For the cultivation of *Staphylococcus aureus*, Oxoid Nutrient Agar CM3 with 400 µg/ml CaCl₂ plates were used.

Initially, strains were typed in routine test dilution (RTD, the highest dilution producing confluent lysis). In the cases of negative reactions, RTD×100 concentration (100 RTD) was also used. No phage typeable strains were tested by reversed phage typing. The lytic reactions were read as follows: ++ = more than 50 plaques (strong lysis); + = 20-50 plaques (moderate lysis); ± = less than 20 plaques (weak lysis); - = no plaques (no lysis). A strain was considered phage-typeable when it was lysed strongly (++) or moderately (+) by at least one phage.

Two *Staphylococcus aureus* strains are considered to be different when one is lysed strongly by at least two phages which produce no lysis of the other to any degree (two phage reaction differences).

Strains were phage typed in the Centro Nacional de Microbiología, Virología e Inmunología Sanitarias del Instituto de Salud Carlos III (Majadahonda, Madrid, Spain).

Results and Discussion

Staphylococcus aureus was detected in 100% of carcasses, sausages and hamburgers, 60% of wings and giblets and 40% of legs. Percentages of phage typeable strains were 93.75%, 60%, 33.33%, 75%, 80%, 88.46% and 92.86% for carcasses, wings, legs, giblets, red sausages, white sausages and hamburgers, respectively. The

Table 1. Results of phage typing of 168 *Staphylococcus aureus* isolates from poultry

Group	RTD	100 RTD	Reversed phage typing	Total
I	1 (0.59) ^a	-	1 (0.59) ^c	2 (1.19)
II	1 (0.59)	-	-	1 (0.59)
III	23 (13.69)	43 (25.59)	33 (19.64) ^c	99 (58.93)
M ^b	4 (2.38)	13 (7.74)	-	17 (10.12)
Mixed ^c	3 (1.79)	1 (0.59)	2 (1.19) ^c	6 (3.57)
AP ^d : 1030, W57, 2009, 18042	-	-	23 (13.69)	23 (13.69)
Total	32 (19.05)	57 (33.93)	59 (35.12)	148 (88.09)

^anumber (percentage) of typeable isolates

^bmiscellaneous

^cphages from different groups

^dadditional phages

^eall strains were also lysed by additional phages

overall proportion of strains typed is shown in Table 1. Results indicate that of the 168 *Staphylococcus aureus* strains isolated from chicken meat and chicken by-products, 148 (88.09%) were phage typeable either in routine test dilution (RTD), at hundred-fold RTD (100 RTD) or by reversed phage typing. This percentage of phage typeable strains is higher than those reported by other authors where percentage of phage typeable strains with the international bacteriophage set for typing *Staphylococcus aureus* of 0% (*Staphylococcus aureus* strains of poultry origin; 27), 4.04% (isolates from milk in Yugoslavia; 32), 12.12% (isolates from milk in India; 30), 26% (strains for restaurant workers in Kuwait; 5), 50.2% (strains from milk samples in Trinidad; 3), 54.4% to 54.8% (strains of poultry origin; 28), 59.6%, 66.4% and 72.5% (isolates from human handlers, bulk milk and composite milk, respectively, in Trinidad; 4), 63.79% (strains from food samples, including poultry meat, in India; 19), 67.7% or 77.5% (strains from foods or raw meat, respectively, in Trinidad; 2), 68% (strains of various procedences in Atlanta; 8), 69.23% (isolates from meat and fish in India; 12), 76.2% (strains isolated from raw milk in India; 35), 79.9% (isolates from milk samples in Denmark; 1) have been reported. This high percentage of phage-typeable strains found in our study corroborates the general idea that phage-typing is an important tool in epidemiological studies.

Lysis by phages of group III was the most frequent with 99 (58.93%) sensitive strains. Twenty-three, seventeen, six, two and one strains were lysed by additional phages, group miscellaneous, phages in the different groups (mixed), group I and group II, respectively. According to Hájek and Horák (21) the majority of the strains of poultry origin are sensitive to phages of group III. Other authors have also found that the phages of group III (2, 13, 14, 17, 18, 23, 25, 31, 35), or the phages of groups I and III (5, 8) show the highest activity against the *Sta-*

Table 2. Grouping of the phage patterns and strains

	Total	Common phage lysis patterns	One phage reaction difference with the common phage lysis pattern	≥ Two phage reaction differences with the common phage lysis pattern
Phage patterns	29 (100%)	3 (10.34%)	15 (51.72%)	11 (37.93%)
Strains	148 (100%)	38 (25.68%)	94 (63.51%)	16 (10.81%)

phylococcus aureus strains isolated from foods (including poultry).

Only 32 (19.05%) strains could be phage typed at RTD. Using phages at 100 RTD, the number of typeable strains increased to 89 (52.98%). Other authors were also able to typify a larger number of strains at 100 RTD than at RTD using the human bacteriophage set: 37.4% and 30.3% (2) or 39.5% and 15.3% (28), at 100 RTD or at RTD, respectively. By using reversed phage typing we managed to increase the number of typeable strains by 59 (35.12%).

Twenty-nine different patterns were observed in the 148 typeable strains. Three were most common, and were exhibited by a total of 38 strains (Table 2). A further ninety-four strains (fifteen phage patterns) gave one phage strong reaction different from those three common patterns and would thus be considered indistinguishable from at least one of these patterns. The remaining 16 strains, which showed two or more than two phage strong reactions different from at least one of the three common patterns, formed a further eleven patterns. Table 3 shows the number of strains included in each pattern and the number of indistinguishable strains (one major difference) of each pattern.

More than one phage type of *Staphylococcus aureus*

Table 3. Occurrence of the three most common phage lytic patterns in the 148 typeable *Staphylococcus aureus* strains

Most common lytic pattern	Number of strains showing common phage lysis pattern	Number of strains showing only one phage reaction difference (i.e. indistinguishable) from common phage pattern
95	17	1
75/84	4	54
6/1030/W57	17	39

was detected in 26 (37.14%) samples (14 chicken carcasses, and one, four, four, and three samples of giblets, red sausage, white sausage and hamburger, respectively). This fact emphasizes the convenience of subtyping several *Staphylococcus aureus* isolates from the same food sample in epidemiological studies. Relatedness of *Staphylococcus aureus* strains isolated from sausages and hamburgers were demonstrated using the phages patterns detected. This fact, together with the elevated contamination rates in processed chicken products suggests a common origin of contamination during the processing. The production of sausages and hamburgers requires a significant amount of handling of meat and thus increases

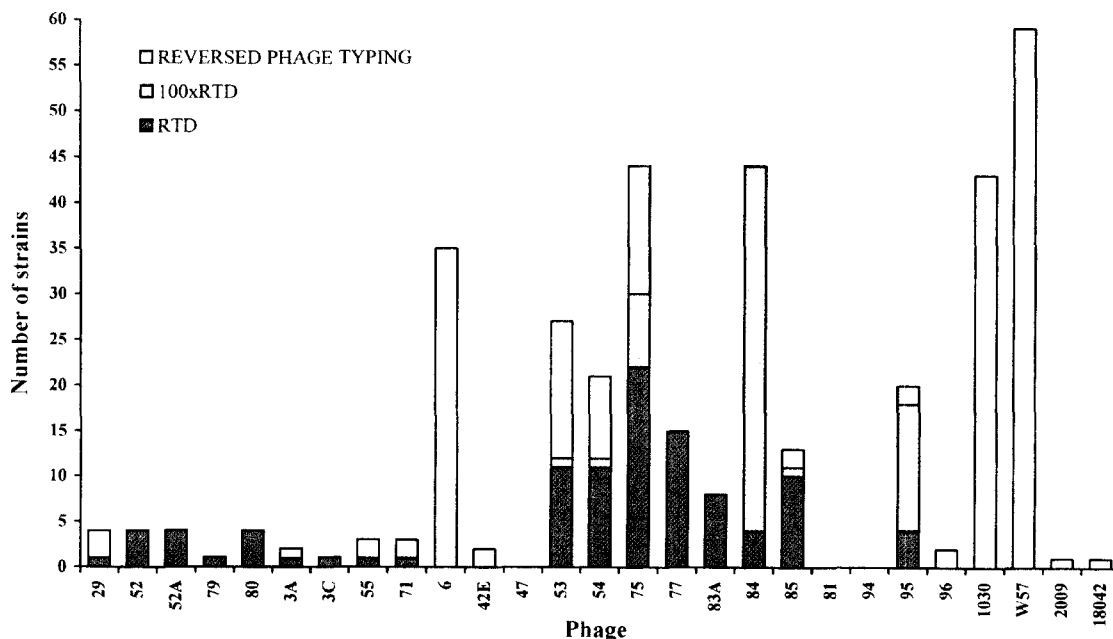


Fig. 1. Number of *Staphylococcus aureus* strains lysed by phages of the human bacteriophage set.

Table 4. Activity, frequency of lysis, specificity index, percentage of strong reactions of the phages used at RTD and relation of each one to the rest of the phages

Lytic action of the 23 phages on the 32 typed strains							
Phage number	With the indicated phage			With the other 22 phages		Specificity index (5)	Percentage of strong reactions (++) (6)
	Number of sensitive strains	FREQUENCY OF LYSIS ON:		Total number of reactions (3)	Phage with higher number of reactions (4)		
		Total reactions (1)	Total strains (2)				
29	1	0.97	3.12	4	52, 52A, 80, 79 (1)	20	100
52	4	3.88	12.50	16	52A, 80 (4)	20	100
52A	4	3.88	12.50	16	52, 80 (4)	20	100
79	1	0.97	3.12	4	29, 52, 52A, 80 (1)	20	0
80	4	3.88	12.50	16	52, 52A (4)	20	50
3A	1	0.97	3.12	3	3C, 55, 71 (1)	25	0
3C	1	0.97	3.12	3	3A, 55, 71 (1)	25	0
55	1	0.97	3.12	3	3A, 3C, 71 (1)	25	0
71	1	0.97	3.12	3	3A, 3C, 55 (1)	25	0
6							
42E							
47							
53	11	10.68	34.37	39	75, 77 (10)	22	36.36
54	11	10.68	34.37	35	75 (11)	23.91	36.36
75	22	21.36	68.75	62	54 (11)	26.19	90.91
77	15	14.56	46.87	49	75 (11)	23.44	40
83A	8	7.77	25	32	54, 75, 77 (8)	20	75
84	4	3.88	12.50	8	54, 75, 77, 83A (2)	33.33	0
85	10	9.71	31.25	29	75 (8)	25.64	70
81							
94							
95	4	3.88	12.50	0	-	100	0
96							

(1), (number of reactions produced by this phage/number total of reactions at RTD)×100

(2), (number of strains lysed by this phage/number of total strains studied typed at RTD)×100

(3), number of reactions produced by the rest of the phages on the strains lysed by the phage in study

(4), phage which most showed simultaneous reaction with the phage in study, in brackets, number of times

(5), (number of strains sensitive to this phage/number of total reactions on these strains)×100

(6), (number of strong reactions produced by this phage/number of total reactions produced by this phage)×100

Table 5. Activity, frequency of lysis, specificity index, percentage of strong reactions of the phages used at 100 RTD and relation of each one to the rest of the phages

Lytic action of the 23 phages on the 57 typed strains							
Phage number	With the indicated phage			With the other 22 phages		Specificity index (5)	Percentage of strong reactions (++) (6)
	Number of sensitive strains	FREQUENCY OF LYSIS ON:		Total number of reactions (3)	Phage with higher number of reactions (4)		
		Total reactions (1)	Total strains (2)				
47							
53	1	1.54	1.75	3	54, 75, 85 (1)	25	0
54	1	1.54	1.75	3	53, 75, 85 (1)	25	0
75	8	12.31	14.03	7	84 (4)	53.33	87.5
77							
83A							
84	40	61.54	70.17	5	75 (4)	88.89	15
85	1	1.54	1.75	3	53, 54, 75 (1)	25	0
81							
94							
95	14	21.54	24.56	1	84 (1)	93.33	0
96							

Note. For interpretation see Table 4.

Table 6. Activity, frequency of lysis, specificity index, percentage of strong reactions of the phages used by reversed phage typing and relation of each one to the rest of the phages

Lytic action of the 27 phages on the 59 typed strains							
Phage number	With the indicated phage			With the other 26 phages		Specificity index (5)	Percentage of strong reactions (++) (6)
	Number of sensitive strains	FREQUENCY OF LYSIS ON:		Total number of reactions (3)	Phage with higher number of reactions (4)		
		Total reactions (1)	Total strains (2)				
29	3	1.55	5.08	24	1030, W57 (3)	11.11	0
52							
52A							
79							
80							
3A	1	1.04	1.69	7	W57, 6, 75, 1030, 2009, 18042, 54, 18042 (1)	12.5	0
3C							
55	2	1.04	3.39	22	29, 71, 95, 6, 75, 85, 96, 42E, 53 (2)	8.33	0
71	2	1.04	3.39	22	29, 95, 6, 75, 85, 96, 55, 42E, 53 (2)	8.33	0
6	35	18.13	59.32	113	W57 (35)	23.65	80
42E	2	1.04	3.39	22	29, 71, 95, 6, 75, 85, 96, 55, 53 (2)	8.33	0
47							
53	15	7.77	25.42	80	W57, 1030, 6 (15)	15.79	13.33
54	9	4.66	15.25	47	W57, 6, 75, 1030 (9)	16.07	22.22
75	14	7.25	23.73	81	6, 1030, W57 (14)	14.74	14.29
77							
83A							
84							
85	2	1.04	3.39	22	29, 71, 95, 6, 75, 96, 55, 42E, 53 (2)	8.33	0
81							
94							
95	2	1.04	3.39	22	29, 71, 6, 75, 85, 96, 55, 42E, 53 (2)	8.33	0
96	2	1.04	3.39	22	29, 71, 6, 75, 85, 95, 55, 42E, 53 (2)	8.33	0
1030	43	22.28	72.88	132	W57 (43)	24.57	72.09
W57	59	30.57	100	134	1030 (43)	30.57	94.91
2009	1	0.52	1.69	7	W57, 6, 75, 1030, 18042, 3A, 54 (7)	8.33	100
18042	1	0.52	1.69	7	W57, 6, 75, 1030, 2009, 3A, 54 (7)	8.33	100

Note. For interpretation see Table 4.

the risk of cross contamination.

Fig. 1 shows the number of sensitive *Staphylococcus aureus* strains to each phage, and Tables 4, 5 and 6 show the results of the behaviour evaluation of the phages at RTD, 100 RTD or when using reversed phage typing. For the elaboration of Fig. 1 and Tables 2-6 all lytic reactions (++ , + and ±) were considered.

At RTD, phages 75 and 77 were the most active because they lysed 22 and 15 strains, respectively. The rest of the phages showed lesser typeability rates, lysing between 1 (phages 29, 79, 3A, 3C, 55 and 71) and 11 (phages 53 and 54) strains. The specificity index varied between 20 and 100, and the percentage of strong reactions between 0 and 100. Only phage 95 allowed for the increase in the number of typeable strains in four of them.

At 100 RTD, phages with greater activity were 84 and 95, with 40 (70.17%) and 14 (24.56%) strains lysed, respectively. At the same time, these phages presented the greater specificity index. Phage 84 also showed the greater

percentage of strong reactions.

Using reversed phage typing, phages W57, 1030 and 6 were the most active, with 59 (100%), 43 (72.88%) and 35 (59.32%) sensitive strains, respectively. These phages showed a high specificity index and percentage of strong reactions.

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