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STAPHYLOCOCCAL MASTITIS: PHAGE TYPES AND PATTERNS OF *S. AUREUS*

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ABSTRACT

GIESECKE, W. H., VAN DEN HEEVER, L. W. & DU TOIT, I. J. Staphylococcal mastitis: phage types and patterns of *S. aureus*. *Onderstepoort J. vet. Res.* 39 (2) 87-96 (1972).

Phage typing of 187 isolates of *S. aureus* showed that a small proportion of udder infections was caused by "human" strains of *S. aureus* and the majority by "bovine" strains. A total of 35 different phage patterns was determined.

The majority of *S. aureus* isolates (78,3%) lyzed by phages were resistant to one or more antibiotics at high test levels.

INTRODUCTION

Staphylococcus aureus Rosenbach, 1884 (syn. *S. pyogenes*) is a ubiquitous micro-organism whose pathogenicity is associated with the production and liberation of a host of substances, including a coagulase, a penicillinase and haemotoxins.

In cows *S. aureus* causes galactophoritis and interstitial forms of mastitis which may be acute to peracute, serofibrinopurulent, and accompanied by fever and malaise. More commonly, however, the inflammatory process is insidious and chronic. On occasions the tissue reaction to infection is inapparent.

Strains of *S. aureus* responsible for the various forms of mastitis are not obviously different when subjected to conventional morphologic and biochemical tests. However, differences may be demonstrated by subjecting *S. aureus* to lysis by various phages.

S. aureus may be typed by the use of phages of the "International Phage Series" (IPS), which originate from human sources (Blair & Williams, 1961), human phages adapted to lyze bovine staphylococci, and phages originating from bovine sources (Smith, 1948; Seto, Kaesberg & Wilson, 1956; Seto & Wilson, 1958; Coles & Eisenstark, 1959 a, b, c; Nakagawa, 1960 a, b, c; Davidson, 1961 a, b, c; Pargaonker, Coles & Eisenstark, 1962; Lohrbacher, 1967). These may be used either on their own or in combination as in the "Davidson Phage Series" (DPS) (Davidson, 1964). Phage typing in epidemiological studies has demonstrated that *S. aureus* with phage patterns 52A/80, 52A/80/81, 29/52A/80, 29/80/81/92D, and 80/81 cause serious infections in man and bovines (Reid & Wilson, 1959). Staphylococci of Phage Group I and II usually cause pyogenic inflammations in man (Anderson & Williams, 1956; Milch, Kalman & Baranyai, 1961; Popowici, Alexenco & Viane, 1967) and staphylococci of Group III commonly occur as wound infections (Barnum & Fuller, 1956). In the Republic of South Africa (R.S.A.), staphylococci of Group I have been isolated from a breast-abscess and infected tonsils while staphylococci of Phage Group III have been isolated from sputum, tonsils, wounds, blood, pus, ulcers and bedsores (Koornhof, H. J., S.A.I.M.R., personal communication, 1971).

As to *S. aureus* from bovine mastitis, certain phage patterns belonging to Phage Group I have been reported as the cause of recurrent acute mastitis (Edwards & Rippon, 1957). However, other phage types were also isolated and those found in acute mastitis did not differ from staphylococci isolated from normal milk (Barnum & Fuller, 1956; Edwards & Rippon, 1957; Reid & Wilson, 1959; Solomon, Sanclemente & Drury, 1961; Götze, 1967; Nyhan & Archer, 1967; Popowici *et al.*, 1967). Although mastitogenic staphylococci may be isolated from various sites on dairy cows (Spencer & Lasmanis, 1952; Edwards & Rippon, 1957; Morrison, Fair & Kennedy, 1961; Renk, 1961; Wilson & Davidson, 1961; Davidson, 1961 a, b, c; Pulverer & Entel, 1967) - and each coagulase positive *Staphylococcus* establishing itself in a herd was shown to be a potential mastitis hazard - a mastitic cow shedding the pathogenic bacteria for long periods constitutes the most important source of staphylococci (Schalm & Woods, 1953; Davidson, 1961 a; Wilson, 1961; Sharpe, Neave & Reiter, 1962; Wilson, 1963; Edds & Sanders, 1966; Marica, Pirau, May & Elias, 1967). Spread of the staphylococci within the dairy herd occurs through hand or machine milking, the same phage types being found in mastitic udder secretions, on udders, skin and teats and in the pharynx of dairy cows as well as on the hands and in the pharynx of milking personnel (Spencer & Lasmanis, 1952; Thörne & Wallmark, 1960).

S. aureus from bovine sources has been identified as the cause of severe infections in man (Eckert, 1959; Pagano, Farrer, Plotkin, Brachman, Fekety & Pidcoe, 1960; Wallace, Quisenberry & De Harne, 1960; Slanetz & Bartley, 1962; Wallace, Quisenberry, Tanimoto & Lynd, 1962) and the possibility exists that cattle serve as an additional host for antibiotic-resistant staphylococci (Zinn, Anderson & Skaggs, 1961). These organisms present an ever increasing therapeutic problem in the control of bovine mastitis and there is apparently an increasing incidence of carriers in the human population (Price, Neave, Rippon & Williams, 1954; Munch-Petersen, 1960; Bonin, 1966). Generally it appears that increase of resistance of *S. aureus* to various antibiotics runs parallel to an increasing resistance to phage lysis. Penicillin-resistant staphylococci frequently belong to Phage Group III. The smallest proportion of resistance has been encountered with staphylococci of Phage Group IV (Price *et al.*, 1954; Munch-Petersen, 1960; Loken & Hoyt, 1962; Engelbretsen, 1967; Kopp, 1967; Nyhan & Archer, 1967; Nygard, Egeland & Elfland, 1968; Koiranen, 1969).

These findings are supported by data obtained in the R.S.A., where it was found that the majority of penicillin-resistant strains examined belonged to Phage Groups I and III whereas there were few penicillin-resistant strains in Groups II and IV (Koornhof & Robinson, 1967). This phage group-antibiotic relationship, in-

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TABLE 1 Relationship of antibiotic resistance to the phage group of *S. aureus* isolates (H. J. Koornhof, S.A.I.M.R., personal communication, 1971)

Phage Group	Resistant to Penicillin 41%	Resistant to Benzyl-Penicillin and Tetracyclines 32%	Multiresistant including Erythromycin 14%	Multiresistant including Chloramphenicol 2%
Group I	36%	36%	27%	27%
Group II	8%	8%	10%	10%
Group III	34%	41%	44%	60%
Miscellaneous (81 and/or 187)	4%	4%	5%	0%
Unidentifiable	18%	11%	14%	3%

cluding other antibiotics, is summarized in Table 1.

In addition to its important role as a cause of infection in man and animal, strains of *S. aureus* producing a heat-stable enterotoxin are of particular significance as an aetiological factor responsible for acute food-poisoning of man characterized by vomiting, nausea, abdominal pain, cramps, diarrhoea, faintness and occasional fatalities. Usually, recovery is rapid and complete but no immunity is produced (Hobbs, 1962; Kaplan, Abdusallam & Bijlenga, 1962; Mossel, 1962; Hull, 1963; Cruikshank, 1965).

Although subclinically or clinically affected or latent carriers amongst man and dairy cows are the usual sources of staphylococcal contamination of food, strains of *S. aureus* isolated from contaminated foods generally possess phage patterns which differ from those of *S. aureus* isolated from pyogenic conditions. While *S. aureus* of Phage Groups I and II cause food poisoning only under exceptional circumstances, the strains usually responsible for this condition belong to Groups III or IV (Anderson & Williams, 1956; Ortel, 1958 a, b; Pöhn & Kienitz, 1960), the commonest being those of Phage Type 42. The latter strains are also frequently isolated from bovine mastitis (Allison, 1949; Drysdale, 1950; Coles & Eisenstark, 1959 c) and from fresh milk and processed dairy products (Allison, 1949; Hauge, 1951; Worseck, 1956; Coles & Eisenstark, 1959 a; Galton & Steele, 1961; Abo-Elnaga & Kandler, 1965). From the limited data available this also appears true of outbreaks of food poisoning in the R.S.A. (Koornhof, H. J., S.A.I.M.R. personal communication, 1971). However, no data have been published on the phage patterns of *S. aureus* responsible for bovine mastitis in this country.

MATERIAL AND METHODS

A total of 187 hemolytic and coagulase-positive isolates of *S. aureus* was subjected to phage-lysis. From farm A, a producer-distributor, 163 strains were obtained during herd examinations. From six other herds (farms B to G), all fresh milk producers, an additional 24

TABLE 2 Summary of data relevant to Phages used

Phage number	Reference***	RTD used	Source of Phage		Phage series			Lytic Group
			Human	Bovine	IPS	DPS	Not allocated	
29	1	10^{-3}	+		+	+		I
52	1	10^{-5}	+		+			I
52A	1	10^{-3}	+		+	+		I
79	1	10^{-4}	+		+			I
80	1	10^{-4}	+		+			I
3A	1	10^{-4}	+		+	+		II
3B	1	10^{-4}	+		+			II
3C	1	10^{-4}	+		+			II
55	1	10^{-6}	+		+			II
71	1	10^{-5}	+		+			II
6	1	10^{-4}	+		+	+		III
7	1	10^{-4}	+		+			III
42E	1	10^{-5}	+		+			III
47	1	10^{-5}	+		+			III
53	1	10^{-4}	+		+	+		III
54	1	10^{-4}	+		+			III
75	1	10^{-5}	+		+	+		III
77	1	10^{-3}	+		+	+		III
83A	2	10^{-5}	+		+			III
42D	1	10^{-3}	+		+	+		IV
81	1, 3	10^{-4}	+		+			I/III*
187	1	10^{-4}	+		+			M
AC 1	4	10^{-4}		+		+		I
102	5	10^{-4}		+		+		IV
107	5	10^{-5}		+		+		IV
1363-14	6	10^{-6}		+		+		IV
S 1	7	10^{-5}		+		+		M**
S 6	7	10^{-5}		+		+		M
883	8	10^{-4}		+		+		M
105	5	10^{-5}		+			+	IV
129-16	6	10^{-4}		+			+	IV
P42D-E193	6	10^{-5}		+			+	IV
88A	6	10^{-6}		+			+	IV

*Strains lyzed by Phage 81 only belong to Phage Group I (Koïranen, 1969)

**M = Miscellaneous

***[1 = Blair & Williams, 1961; 2 = Blair & Parker, 1967; 3 = Parker, 1962; 4 = Coles (According to Bonin, 1966); 5 = Davidson, 1961c; 6 = Smith, 1948; 7 = Seto *et. al.*, 1956; 8 = Nakagawa, 1960c]

TABLE 3 Frequency of Phage Lysis of *S. aureus* Isolates

Source of *S. aureus* Isolates		Frequency of Phage Lysis								
		Total number of *S. aureu* strains subjected to phage-typing	*S. aureus* typed at RTD (%)		*S. aureus* typed at 1 000 × RTD (%)		*S. aureus* not lyzed (%)		Total number of *S. aureus* lyzed at RTD and 1 000 × RTD (%)	
		IPS + MDPS	IPS	MDPS	IPS	MDPS	IPS	MDPS	IPS	MDPS
Herd A	Dry cows with clinical or subclinical mastitis	33	0(0)	29(37,8)	4(12,1)	2(6,1)	29(87,8)	2(6,1)	4(12,1)	31(93,9)
	Lactating cows with cell counts/ml of milk:									
	$<300 \times 10^3$	46	0(0)	31(67,4)	4(8,7)	11(23,9)	42(91,3)	4(8,7)	4(8,7)	42(91,3)
	300×10^3 to 500×10^3	24	0(0)	10(41,7)	7(29,2)	12(50,0)	17(70,8)	2(8,3)	7(29,2)	22(91,7)
	$>500 \times 10^3$	60	0(0)	39(65,0)	7(11,7)	18(30,0)	53(88,3)	3(5,0)	7(11,7)	57(95,0)
Herds B to G	Secretions of lactating cows with clinical mastitis and cell counts/ml of milk $>500 \times 10^3$	24	3(12,5)	18(75,0)	1(4,2)	5(20,8)	20(83,3)	1(4,2)	4(16,7)	23(95,8)

TABLE 3a Phage patterns obtained by lysis of *S. aureus* at RTD

Source of *S. aureus*	Phage patterns obtained by typing with					
	IPS Pattern	F	P.G.	MDPS Pattern	F	P.G.
From Herd A:						
1. Dry cows with clinical or subclinical mastitis	0	0	0	102/105/129-16/P42D-E193/88A	9	IV
				102/129-16/P42D-E193/88A	8	IV
				102/105	3	IV
				105/129-16/P42D-E193/88A	1	IV
				102/105/129-16	2	IV
				102	2	IV
				102/129-16	3	IV
				102/P42D-E193	1	IV
2. From milk-like secretion with cell count/ml $<300 \times 10^3$	0	0	0	102/105/129-16/P42D-E193/88A	11	IV
				102/129-16/P42D-E193/88A	6	IV
				102/105	2	IV
				105/129-16/P42D-E193/88A	2	IV
				102/105/129-16	1	IV
				102	4	IV
				102/129-16	4	IV
				102/P42D-E193	1	IV
3. From milk-like secretion with cell count/ml 300×10^3 to 500×10^3	0	0	0	102/129-16/P42D-E193	2	IV
				102/105/129-16/P42D-E193/88A	4	IV
				102/129-16/P42D-E193/88A	2	IV
				102/105/129-16/P42D-E193	1	IV
				102	1	IV
4. From milk-like secretion with cell count/ml $>500 \times 10^3$				102/105/129-16/P42D-E193/88A	12	IV
				102	3	IV
				102/129-16	5	IV
				102/105/129-16	3	IV
				102/105/129-16/P42D-E193	2	IV
				102/129-16/P42D-E193/88A	10	IV
				102/129-16/P42D-E193	1	IV
				129-16	1	IV
				S6/105/129-16/P42D-E193	1	IV/M
				102/105	1	IV
5. *From Herds B - G*						
Secretions of lactating quarters with clinical mastitis and cell count/ml of $>500 \times 10^3$	80/81	1	I	42D/102/107/1363-14	1	IV
	77/42D	1	III/IV	77/42D/1363-14	1	III/IV
	52A/80/81	1	I/III	102/107	4	IV
				102/105/129-16/P42D-E193/88A	3	IV
				102/105	1	IV
				102	2	IV
				102/1363-14	1	IV
				102/P42D-E193	1	IV
				S6	1	M
				102/129-16/P42D-E193	2	IV
				52A/107/1363-14/105/129-16/P42D-E193/88A	1	I/IV

F = Frequency of phage pattern concerned
P.G. = Lytic group of phages concerned
0 = Zero

strains were isolated from sporadic cases of severe clinical mastitis affecting lactating cows. All strains examined were isolated from single colonies in pure culture from fore-milk quarter samples by established techniques (Giesecke, Nel & Van den Heever, 1968).

The identity of the isolates was confirmed by Dr. M. E. Stiles* who also performed the phage typing according to standard methods (Blair & Williams, 1961) employing a multiple phage dispenser (Bonin, 1966). Details of phages, Routine Test Dilution (RTD) and lytic grouping systems are summarized in Table 2.

An additional 1 000 × RTD was used in the typing of strains not lyzed at 1 × RTD.

As it is internationally accepted that confluent lysis on less than 50 plaques should not be included in the pattern (Blair & Williams, 1961), phage patterns reported below only reflect the numbers of the phages which gave such reactions when tested at 1 × RTD or 1 000 × RTD. Other strains were considered unidentifiable. The phages lyzing an isolate by ++ reaction (Blair & Williams, 1961) combine to form the phage pattern of the strain concerned. Strains which were lyzed by one or more phages of the same lytic group were assigned to this group. Where the isolate was lyzed by phages belonging to different lytic groups, the strain was allocated to the mixed group concerned.

Because phages which have not been allocated to any internationally accepted series were also used in typing by means of Davidson phages, this combination is referred to as the Modified Davidson Phage Series (MDPS).

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RESULTS

1. *Phage lysis and patterns*

Analysis of the results, as summarized in Table 3, shows that of a total of 187 *S. aureus* isolates tested, only 3 (1,6%) and 23 (12,3%) could be typed by means of

IPS at RTD and 1 000 × RTD whilst 127 strains (67,9%) were lyzed by means of MDPS at RTD and 48 strains (25,7%) at 1 000 × RTD.

No *S. aureus* from Herd A were lyzed by IPS at RTD, whilst 3 strains isolated from clinical cases of mastitis in Herds B to G were lyzed at RTD.

Diseased dry udders in Herd A yielded the highest proportion of isolates which could be typed by means of MDPS at RTD, i.e. 87,8%, whereas strains obtained from lactating cows in the same herd were lyzed to varying degrees ranging from 41,7% to 67,4%.

From Table 3a it is clear that with the exception of Phage Pattern S6/105/129-16/P42D-E193 of lysis Groups IV/M all isolates fall into Group IV.

S. aureus with basically the same phage patterns was isolated from "normal" as well as abnormal udder-secretions of dry and lactating cows in Herd A. The same strains of *S. aureus* were isolated most frequently from clinical cases of mastitis occurring in lactating cows in Herds B to G, indicating that no *S. aureus* of a specific phage pattern was related to mastitis in dry or lactating cows or to different degrees of mastitis as determined by cell counts of milk. At RTD there was no *S. aureus* with Phage Pattern 80/81 isolated from Herd A, whereas *S. aureus* of Phage Group I was recovered from two lactating cows with clinical mastitis amongst animals in Herds B to G. The numerous patterns obtained at RTD from *S. aureus* isolated from Herds B to G, such as Patterns 80/81, 77/42D, 52A/80/81, 102/107, 102/1363-14, S6, 52A/107/1363-14/105/129-16/P42D-E193/88A, suggest that a large variety of *S. aureus* with different phage patterns may be found when examining more representative numbers of isolates. One may also find more evidence of the relative specificity of a certain *S. aureus* population affecting dairy cows in a particular herd. Prevalence of such strains should, however, not be expected as a rule.

Retyping at 1 000 × RTD those strains which were not lyzed at RTD resulted in an overall increase in the number of identifiable isolates (Table 3) and an increase in the variety of phage patterns (Table 3b). This observation applies to both the isolates from all the herds.

By comparison it is apparent that phages of the lytic Group IV lyzed most of the isolates at RTD (121 isolates = 64,7%) (Table 4). The predominant phage patterns which emerged were:

102/105/129-16/P42D-E193/88A - 38 strains;
102/129-16/P42D-E193/88A - 25 strains;
102/129-16 - 12 strains;
102 - 12 strains;

The presence of *S. aureus* Phage Type 42D in Herd A, from which "certified" raw milk is sold, was confirmed only after lysis of the isolate at 1 000 × RTD.

From a total of 43 strains (Table 5) which were lyzed (3 at RTD and 40 at 1 000 × RTD) by Phage 42D alone (13 strains) or in combination with other phages (30 strains) (Table 4), the majority were isolated from Herd A where they affected both dry and lactating

TABLE 3b Phage patterns obtained by lysis of *S. aureus* at 1 000 × RTD

Source of *S. aureus*	Phage patterns obtained by typing with					
	IPS Pattern	F	P.G.	MDPS Pattern	F	P.G.
From Herd A:						
1. Dry cows with clinical or subclinical mastitis	42D	2	IV			
	29/77/42D	1	I/III/IV	42D	2	IV
	77/42D	1	III/IV			
2. From milk-like secretion with cell count/ml $<300 \times 10^3$	42D	2	IV	102/105/129-16/P42D-E193/88A	3	IV
	53	1	III	102	2	IV
	42E/53/42D	1	III/IV	P42D-E193	2	IV
				53/102	1	III/IV
				52/42D/102/129-16/P42D-E193/88A	1	I/IV
				42D/102/1363-14/S6/129-161/P42D-E193/88A	1	IV/M
				129-16	1	IV
3. From milk-like secretion with cell count/ml 300×10^3 to 500×10^3	42D	2	IV	42D/P42D-E193	2	IV
	83A/42D	1	III/IV	102/105/129-16/P42D-E193/88A	3	IV
	77/42D	3	III/IV	77/42D	1	III/IV
	29/77/42D	1		77/42D/P42D-E193	3	III/IV
				102/105	1	IV
				105/129-16	1	IV
				102/129-16/P42D-E193/88A	1	IV
4. From milk-like secretion with cell count/ml $>500 \times 10^3$	42D	3	IV	102	4	IV
	77/42D	4	III/IV	102/129-16	2	IV
				105/129-16/P42D-E193/88A	2	IV
				102/105	2	IV
				42D/P42D-E193	2	IV
				77/42D/P42D-E193	4	III/IV
				102/107/S6/105/129-16	1	IV/M
				105/129-16	1	IV
From Herds B-G						
Secretion of lactating quarters with clinical mastitis and cell counts/ml $>500 \times 10^3$	77	1	III	42D	2	IV
				77/42D	1	III/IV
				102/105	1	IV
				1363-14	1	IV

F = Frequency of phage patterns concerned
P.G. = Lytic group of phages concerned

TABLE 4 Summary of frequency of phage patterns of *S. aureus*

Lysogenic phage pattern		Lytic group	Number of isolates lyzed at	
Serial No.	Pattern		RTD	1 000 × RTD
1	80/81	I	1	—
2	52A/80/81	I/III	1	—
3	52A/107/1363-14/105/129-16/P42D-E193/88A	I/IV	1	—
4	52/42D/102/129-16/P42D-E193/88A	I/IV	—	1
5	29/77/42D	I/III/IV	—	2
6	77/42D	III/IV	1	5
7	77/42D/1363-14	III/IV	1	—
8	42E/53/42D	III/IV	—	1
9	83A/42D	III/IV	—	1
10	77	III	—	1
11	53/102	III/IV	—	1
12	77/42D/P42D-E193	III/IV	—	2
13	102/105/129-16/P42D-E193/88A	IV	38	8
14	102/129-16/P42D-E193/88A	IV	25	—
15	103/105	IV	7	4
16	105/129-16/P42D-E193/88A	IV	3	3
17	102/105/129-16	IV	6	—
18	102	IV	12	5
19	102/129-16	IV	12	2
20	102/P42D-E193	IV	3	—
21	102/129-16/P42D-E193	IV	5	—
22	102/105/129-16/P42D-E193	IV	3	—
23	129-16	IV	1	1
24	42D/102/107/1363-14	IV	1	—
25	102/107	IV	4	—
26	102/1363-14	IV	1	—
27	42D	IV	—	13
28	P42D-E193	IV	—	2
29	42D/P42D-E193	IV	—	4
30	105/129-16	IV	—	2
31	1363-14	IV	—	1
32	105/129-16/P42D-E193/S6	IV/M	1	—
33	42D/102/1363-14/S6/129-16/P42D-E193/88A	IV/M	—	1
34	102/107/S6/105/129-16	IV/M	—	1
35	S6	M	1	

TABLE 5 Frequency of lysis of *S. aureus* from herd A by individual phages

Lytic group	Phages		Lysis of *S. aureus* at	
	IPS	MDPS	RTD	1 000 × RTD
I	29	29		2
	52			1
	52A	52A	2	
	79			
	80		2	
		AC1		
II	3A	3A		
	3B			
	3C			
	55			
	71			
III	6	6		
	7			
	42E			1
	47			
	53	53		3
	54			
	75	75		
	77	77	2	20
	83A			1
	81		2	
IV	42D	42D	3	40
		102	119	23
		105	60	15
		107	6	1
		1363-14	4	2
		129-16	97	17
		P42D-E193	81	24
		88A	69	11
M	187			
	S1			
	S6		2	2
	883			

cows (Table 3b). On two occasions strains of Phage Type 42D were also isolated from severely mastitic lactating cows in Herds B to G (Table 3b).

2. *Phage patterns in relation to antibiotic sensitivity*

The majority of isolates lyzed by phages of the MDPS were resistant to one or more antibiotics at the test-levels employed (78,3%) (Table 6). Although the number of strains that were examined is too small to warrant specific conclusions, there is a tendency amongst the twelve isolates not lyzed by phages of the MDPS towards a relatively higher incidence of multiple drug resistance. This is indicated by the number of strains resistant to two (four strains) and more antibiotics (five strains) at the test levels employed.

Regarding the relation of drug sensitivity to source of isolate, relatively more resistant strains were isolated from clinically diseased lactating quarters (21 out of 24 strains) and from clinically or subclinically diseased dry quarters (28 out of 33 strains) than from quarters with apparently normal milk and a cell content/ml ranging $<300 \times 10^3$ to $>500 \times 10^3$ (98 out of 130 strains). *S. aureus* isolates from milk with cells in excess of 500 000/ml (71 out of 84 strains) were more frequently resistant to antibiotics than strains from milk with a lower cell content (48 out of 70 strains). *S. aureus* resistant to two or more antibiotics were isolated more frequently from severe clinical mastitis affecting lactating cows in Herds B to G (18 out of 24 strains).

No conclusion can, however, be drawn regarding the specificity of certain phage patterns, lytic groups, individual phage types or lysogenicity at RTD or 1 000 × RTD in relation to varying degrees of resistance (Tables 7, 8), because:

(a) in some instances the number of *S. aureus* of certain

TABLE 6 Comparison of drug sensitivity and MDPS lysis of *S. aureus* isolates

Source of cultures			Pattern of sensitivity obtained				
	(A) Chlortetracycline 50 μg		+	+	+	+	+
	(C) Chloramphenicol 50 μg		+	+	+	+	+
	(P) Penicillin 5 units		+	—	±	—	—
	(S) Streptomycin 25 μg		+	+	+	—	—
	(T) Oxytetracycline 50 μg		+	+	+	+	—
Herd A	*S. aureus* isolated from secretion of clinically diseased dry cows	Identifiable	5	11	0	1	14
		Unidentifiable	0	0	0	1	1
	S. aureus isolated from milk with a cell count/ml $< 300 \times 10^3$	Identifiable	15	17	1	7	2
		Unidentifiable	1	1	0	1	1
	S. aureus isolated from milk with a cell count/ml 300×10^3 to 500×10^3	Identifiable	5	13	0	1	3
		Unidentifiable	0	0	0	1	1
	S. aureus isolated from milk with a cell count/ml $> 500 \times 10^3$	Identifiable	10	32	0	0	15
		Unidentifiable	0	1	0	1	1
Herds B to G	*S. aureus* isolated from secretions of clinically diseased lactating cows with a cell count/ml $> 500 \times 10^3$	Identifiable	3	3	0	5	12
		Unidentifiable	0	0	0	0	1
Total	Of 175 *S. aureus* typable by means of the MDPS (93,6%)		38 21,7%	76 43,5%	1 0,6%	14 8,0%	46 26,2%
	Of 12 *S. aureus* not lyzed by means of the MDPS (6,4%)		1 8,3%	2 16,7%	0 0	4 33,3%	5 41,7%

\+ = sensitive
— = resistant

TABLE 7 Relationship between phage patterns, lytic groups, RTD and drug sensitivity

Phage pattern* serial no.	Lytic group	RTD					1 000 × RTD.				
		**A—	+	+	+	+	—	+	+	+	+
		C+	+	+	+	+	+	+	+	+	+
		P—	—	+	—	±	—	+	+	+	+
		S—	—	+	+	+	—	—	+	+	+
		T—	+	+	+	+	—	+	+	+	+
1	I		1								
2	I/III	1									
3	I/IV				1						
4	I/IV								1		
5	I/III/IV						1		1		
6	III/IV						2	1		2	
7	III/IV		1								
8	III/IV								1		
9	III/IV									1	
10	III							1			
11	III/IV									1	
12	III/IV						1			1	
13	IV	13	3	13	9		2	1		3	
14	IV	7	3	2	13						
15	IV	5			2		3			1	
16	IV			3			1		2		
17	IV	2			3	1					
18	IV	1	6		5				3	2	
19	IV	5	1		6					2	
20	IV	1			2						
21	IV			1	4						
22	IV			2	1						
23	IV				1					1	
24	IV				1						
25	IV	4									
26	IV	1									
27	IV								11	2	
28	IV								1	1	
29	IV						1		3		
30	IV								1	1	
31	IV							1			
32	IV/M				1						
33	IV/M									1	
34	IV/M									1	
35	M	1									

*see Table 4 ** See Table 6

TABLE 8 Relationship between individual phages, RTD and drug sensitivity

Lytic Group	IPS	MDPS	RTD					1 000 × RTD				
			*A— C+ P— S— T—	+ + — — +	+ + + + +	+ + — + +	+ + ± + +	— + — — —	+ + — — +	+ + + + +	+ + — + +	+ + + + +
I	29 52 52A 79 80	29 52A AC_1	 1 1	 1	 1 	 	 	1 	 	1 1 	 	
II	3A 3B 3C 55 71	3A 	 	 	 	 	 	 	 	 	 	
III	6 7 42E 47 53 54 75 77 83A 81	6 75 77 	 1 2	 1 	 	 	 	 9 	 3 	 1 1 2 	 2 6 	
IV	42D 	42D 102 105 107 1363-14 129-16 P42D-E193 88A	1 44 18 4 1 28 22 22	1 14 6 1 7 6 6	 19 15 21 21 18	1 41 20 2 2 40 32 23	 1 1 1 	12 6 6 3 8 3	3 1 1 1 1 1 1	13 11 2 4 8 3	12 5 6 1 1 9 7 4	
M	 	187 A1 S6 883	 	 	 	 2	 	 	 	 	 2	

* See Table 6

phage patterns and phage types at RTD or 1 000 × RTD is too small (Tables 7, 8);

(b) where the number of isolates of a certain phage pattern or individual phage type is numerically adequate, the distribution of strains resistant or sensitive to antibiotics indicates that strains with the same phage pattern or lyzed by the same individual phage at RTD or 1 000 × RTD vary considerably in sensitivity to antibiotics (Tables 7, 8). Generally it appears that antibiotic-resistance is related less frequently to phage type than previous exposures to antibiotic mastitis remedies (Tables 6).

However, in view of the above findings, it appears noteworthy that *S. aureus* with Phage Pattern 80/81 was resistant against both penicillin and streptomycin (Tables 4, 7, 8) at the test levels employed and the strain with Phage Pattern 52A/80/81 was resistant against penicillin, streptomycin and tetracyclines (Tables 4, 7, 8). Strains of *S. aureus* lyzed by Phage 42D alone or in combination with other phages showed no consistency in their sensitivity to the antibiotics tested (Tables 4, 8).

DISCUSSION

Phage typing of 187 isolates of *S. aureus* by means of IPS and MDPS has provided data on South African strains which differ materially from those reported elsewhere. Whereas 98,4% of our strains were not lyzed by IPS at RTD, this figure was much lower in other countries: in the U.S.A., the average percentage of strains lyzed was 39,6 (Coles & Eisenstark, 1959 a; Mann, 1960; Loken & Hoyt, 1962; Slanetz & Bartley, 1962; George, Russell & Wilson, 1962; Jones & Bennett, 1965), whilst in Australia it was 38,3% (Frost, 1962), in Britain it amounted to 35,5% (Davidson, 1964), in Germany to 74,2% (Bonin, 1966) and in Finland to 42,7 (Koiranen, 1969). Due to the inconsistent results obtained from phage typing of *S. aureus* by means of "bovine" phages such as those in the DPS or modified systems thereof, a comparison of results obtained by typing with DPS is rather limited.

In our investigations 127 strains (67,9%) were lyzed at RTD whereas the corresponding figure for Britain is 86,8% (Davidson, 1964), for Germany it averages 80,4% (Bonin, 1966; Gedek, 1966; Kopp, 1967) and for Finland it is 91,7% (Koiranen, 1969). Although the numerous factors affecting the results of phage typing make direct comparison a hazardous undertaking, the smaller proportion of isolates lyzed by means of the IPS and MDPS indicates the need for more extensive study of the *S. aureus* responsible for udder infections and for phages which would result in more efficient typing procedures.

S. aureus lyzed by "bovine" phages of Group IV clearly predominated. This is in general agreement with the findings of Mann (1960), Coles & Eisenstark (1959 c), Fleming & Paton (1961), Loken & Hoyt (1962), Bonin (1966) and Koiranen (1969). The individual phage types which were found to be predominant by various authors, however, vary considerably. Thus, in Germany, Bonin (1966) found that Phages 102, 1363-14

and 107 lyzed 43,3% 34,3% and 34,0% respectively of his *S. aureus* isolates, while Gedek (1966) found that Phages 102 and 107 lyzed 64,2% and 69,7% of his strains. In Finland, Koiranen (1969) reported that Phage 1363-14 lyzed 62,0% of his isolates of *S. aureus* while Phage 107 lyzed 32,9%, Phage 102 lyzed 25,5% and Phage 42D lyzed 20,6%. In our studies Phages 102, 129-16, P42D - E193, 88A and 105 lyzed 63,6%, 51,9%, 43,3%, 36,9% and 32,1% of *S. aureus* isolates respectively.

Consideration of the phage type patterns which were most frequently encountered in our series shows that the Pattern 102/105/129-16/P42D - E193/88A lyzed 20,3% of isolates while Pattern 102/129-16/P42D-E193/88A lyzed 13,3%.

Within the limited material available, no correlation existed between the *S. aureus* strains isolated from different types of mastitis and lysis by the various phages. The same applies to the antibiotic resistance of various strains of *S. aureus*.

Conclusions

1. From data obtained in this study it may be concluded that phage typing of *S. aureus* isolated from infected milk-glands gave a lower typability at RTD than reported for both the IPS and the DPS from the U.S.A., Australia, Britain, Germany and Finland.

2. With the exception of Phage 102, the individual phages possessing the most predominant lytic activity towards *S. aureus* differed from those reported from Germany and Finland.

3. No correlation was found to exist between strains of *S. aureus* isolated from the various types of mastitis and their typability by different phages.

4. A relative specificity of *S. aureus* existed within a herd. However, different phage types found in various herds suggest that a certain phage pattern may not always predominate.

5. The greater frequency of isolation of multi-resistant *S. aureus* from clinically diseased udders than from apparently healthy quarters with milk of normal cell content, suggests that antibiotic resistance is related to a lesser degree to phage types than to previous exposure to antibiotic mastitis remedies. This is supported by an analysis of the antibiograms in relation to the source of strains, specificity of phage patterns, lytic groups, individual phage types and typability at RTD or 1 000 × RTD.

6. From the fact that 93,6% of the strains of *S. aureus* fell into the "bovine" MDPS-types at RTD and 1 000 × RTD whereas only 13,9% were "human" strains typable by IPS at RTD and 1 000 × RTD, it appears that cows in the herds concerned were rarely infected from human sources. In the few instances where such sources are indicated by the typing and known pathogenic strains are involved, public health hazards must be considered. This emphasizes the need for high standards of hygiene in milk production to ensure good keeping and aesthetic qualities, to prevent the spread of udder disease within the herd and particularly to prevent transfer of staphylococcal disease between cows and man. It also emphasizes the need for routine examination of herds and handlers. Undetected sources of *S. aureus* present a health hazard to the community because of possible drug resistance and the preformation of heat stable enterotoxin in food.

Summary

Phage typing of 187 isolates showed that only 3 (1,6%) and 23 (12,3%) of *S. aureus* strains were lyzed by phages of the IPS at RTD or 1 000 × RTD respectively. By means of the MDPS, 127 strains (67,9%) were typable at RTD and 48 strains (25,7%) at 1 000 × RTD. Results obtained suggest a considerable difference between South African strains of *S. aureus* and those examined elsewhere. The results also indicate that a small proportion of udder infections was caused by "human" strains of *S. aureus* and the majority by "bovine" strains.

A total of 35 different phage patterns was determined with distinct predominance of Patterns 102/105/129-16/P42D-E193/88A (38 strains), 102/129-16/P42D-E193/88A (25 strains), 102/129-16 (12 strains), and 102 (12 strains). Data suggest that certain strains are specific in a particular herd, but this should not be taken as a rule. Basically there was no difference in the phage patterns of strains isolated from normal as well as abnormal udder secretions of dry and lactating cows in a herd. A larger variety of patterns was, however, obtained from isolates from 6 other herds including Strains 80/81, 52A/80/81, and 42D either alone or in combination with other phages.

The majority of *S. aureus* isolates (78,3%) lyzed was resistant to one or more antibiotics at the high test levels employed. There was a tendency for a higher incidence of multiple drug resistance amongst the strains not lyzed by phages. No conclusions could be reached regarding specificity of certain phage patterns, lytic groups, individual phage types, or typability at RTD or 1 000 × RTD relative to antibiotic resistance. From data obtained it appears that antibiotic resistance is less related to phage types and patterns than previous exposure to antibiotics.

Data are presented in 8 tables.

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