An evaluation of two methods of bacteriocine typing of organisms of the genus *Proteus*

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SYNOPSIS An assessment of two simple methods of typing *Proteus* isolates using bacteriocines is described. The methods chosen were those of Cradock-Watson and Al-Jumaili. It has been shown that the use of liquid bacteriocine preparations is more satisfactory than methods involving proteocine production in solid media. For example, no non-typable isolates were encountered and a large number of strains were demonstrated. This method is therefore proposed for use in a routine diagnostic laboratory.

Previously described bacteriocine typing systems for use with *Proteus* isolates (Cradock-Watson, 1965) have proved unsatisfactory. Indeed, of 252 isolates tested by this method, only 139 described as *Proteus mirabilis* and one described as *Proteus vulgaris* were shown to produce bacteriocines (55.5%). However, a higher proportion (75%) of isolates tested by the Central Public Health Laboratory (Colindale) (CPHL) were found to be typable (Pitt, personal communication). A typing scheme based on the bacteriocine sensitivities of unknown isolates to standard proteocine preparations has now been developed and tested (Al-Jumaili, 1975) and the purpose of this work is to compare the epidemiological value of these two previously described systems.

Materials and Methods

SOURCE OF ISOLATES

Two hundred and four isolates of *Proteus* were supplied by T. L. Pitt, Cross-infection Reference Laboratory, Colindale. Two hundred of these, which had been identified specifically as *P. mirabilis*, had been originally obtained during an investigation into an outbreak of *Proteus* infection in a neonatal unit in Boston City Hospital, details of which are described by Burke *et al* (1970). These included three isolates taken from the same nurse (Nurse A) suspected of causing the outbreak, and strains taken from six infants admitted by that nurse.

The other four were gentamicin resistant isolates sent to the CPHL from the Central Middlesex

Hospital. They originated from four patients in the same surgical ward. Two were blood specimens, one catheter urine, and the fourth from a wound swab. These had all been identified as *P. vulgaris*.

METHOD OF TYPING

The method of typing was that described by Al-Jumaili in which the sensitivities of the unknown isolates to 12 standard proteocine preparations are determined (Al-Jumaili, 1975). All isolates had been previously investigated using the method of Cradock-Watson.

Results

The results of typing the 204 isolates by the method of Cradock-Watson are shown in table I. The results of typing the same 204 isolates by the method of Al-Jumaili are shown in table II. Only patterns 1 to 33 were demonstrated, and all the isolates were found to be typable.

A comparison between the Al-Jumaili patterns

Cradock-Watson Type	Number	Approx. %
1	37	18
2	17	8.3
3	12	5.8
4	22	10.8
5	11	5.4
6	17	8.3
9	20	9.8
Non-typable	46	23
Miscellaneous and atypical	22	10.8

Table I Results obtained on typing 204 isolates of *Proteus* by the Cradock-Watson method

Sensitivity Pattern Numbers	Number	Approx. %
1	7	3.4
2	20	9.8
3	14	6.7
4	10	4.9
5	3	1.4
6	5	2.4
7	20	9.8
8	2	0.9
9	4	1.9
10	5	2.4
11	19	9.3
12	7	3.4
13	10	4.9
14	3	1.4
15	4	1.9
16	5	2.4
17	3	1.4
18	3	1.4
19	5	2.4
20	7	3.4
21	8	3.9
22	1	0.5
23	1	0.5
24	1	0.5
25	3	1.4
26	1	0.5
27	2	0.9
28	1	0.5
29	11	5.3
30	15	7.4
31	1	0.5
32	1	0.5
33	1	0.5
Non-typable	None	

Table II Results obtained on typing 204 isolates of *Proteus* by the Al-Jumaili method

Al-Jumaili Sensitivity Pattern	Cradock-Watson Types							Non-typable	Miscellaneous and Atypical	Total
	1	2	3	4	5	6	9			
1								2	5	7
2							18	2		20
3	13								1	14
4		1	3		6					10
5			2					1		3
6	3							2		5
7				1				14	5	20
8									2	2
9				3				1		4
10					5					5
11						17			2	19
12	7									7
13	4	4						2		10
14	1							2		3
15	3								1	4
16	1		2					2		5
17			2						1	3
18	1						1	1		3
19				3				2		5
20	1	1	1					1	3	7
21								8		8
22									1	1
23							1			1
24								1		1
25	3									3
26								1		1
27								2		2
28								1		1
29		11								11
30			1	15						16
31								1		1
32									1	1
33			1							1
Total	37	17	12	22	11	17	20	46	22	

Table III Number of isolates with different Al-Jumaili patterns within Cradock-Watson types

and the Cradock-Watson types is given in table III. The number of isolates with different Al-Jumaili patterns within Cradock-Watson types is illustrated. Table IV shows the number of patterns into which the Al-Jumaili method subdivides each Cradock-Watson type. Table V shows the results of the tests on the three isolates taken from nurse A and the strains from the six infants admitted by her. Finally, the typing results of the four gentamicin resistant isolates sent from the surgical ward of the Central Middlesex Hospital are shown in table VI.

A further 33 isolates originally obtained from CPHL were provided by Dr R. H. George, Virology Department, Birmingham University. These had been previously typed by the Cradock-Watson method, by bacteriophage, serologically, by the Dienes phenomenon, and by a resistogram method. These too were typed by the Al-Jumaili method.

The 33 isolates were thus known to comprise 21 serologically distinct types (de Louvois, 1969) and divided into 10 sensitivity patterns using bacteriophage, three isolates being untypable (CPHL, unpublished data). The same 33 isolates had been typed using the Cradock-Watson method on two occasions with identical results, giving nine types with two untypable isolates (George, unpublished results; CPHL, unpublished results). In addition, 26 types had been demonstrated using the Dienes

	Cradock-Watson Types							Non-typable	Miscellaneous and Atypical
	1	2	3	4	5	6	9		
<i>Al-Jumaili Sensitivity Pattern Numbers</i>	3	4	4	7	4	11	2	1	1
	6	13	5	9	10		18	2	3
	12	20	16	19			23	5	7
	13	29	17	30				6	8
	14		20					7	11
	15		30					9	15
	16		33					13	17
	18							14	20
	20							16	22
	25							18	32
								19	
								20	
								21	
								24	
								26	
								27	
								28	
								31	
<i>Total</i>	10	4	7	4	2	1	3	18	10

Table IV *Al-Jumaili sub-divisions within Cradock-Watson types*

Patient	Source	Ward	Ref. Lab. No.	Cradock-Watson Type	BJ											
					1	2	3	4	5	6	7	8	9	10	11	12
Nurse A (1)	Vagina	Mat 1	868	4	-	-	-	+	-	+	-	-	-	-	-	-
Nurse A (2)	Vagina	Mat 1	869	Non-typable	-	-	-	-	-	+	-	-	+	-	-	+
Nurse A (3)	Hand	Mat 1	870	Non-typable	-	-	-	-	-	+	-	-	+	-	-	+
Child 1	Umbilicus	Mat 1	834	Non-typable	-	-	-	-	-	+	-	-	+	-	-	+
Child 2	Rectum	Mat 1	860	Non-typable	-	-	-	-	-	+	-	-	+	-	-	+
Child 3	Rectal	Mat 1	861	Non-typable	-	-	-	-	-	+	-	-	+	-	-	+
Child 4	Umbilicus	Mat 1	838	Non-typable	-	-	-	-	-	+	-	-	+	-	-	+
Child 5	Umbilicus	Mat 1	854	4	-	-	-	+	-	+	-	-	-	-	-	-
Child 6	Rectal	Mat 1	856	4	-	-	-	+	-	+	-	-	-	-	-	-

Table V *Proteocine typing of isolates taken from Nurse A and six infants she admitted, using both methods*

Patient	Source	Ref. Lab. No.	Cradock-Watson Type	BJ											
				1	2	3	4	5	6	7	8	9	10	11	12
G	Blood	2965	2	-	-	-	+	+	+	-	-	-	-	+	-
B	Blood	2966	2	-	-	-	+	+	+	-	-	-	-	+	-
C	Catheter urine	2967	2	-	-	-	+	+	+	-	-	-	-	+	-
S	Wound swab	2968	2	-	-	-	+	+	+	-	-	-	-	+	-

Table VI *Proteocine typing of four gentamicin resistant strains from the Middlesex Hospital*

phenomenon as a marker (Kashbur *et al*, 1974), and by the resistogram technique the 33 isolates had been shown to consist of 28 different strains (Kashbur *et al*, 1974). The Al-Jumaili system demonstrated 18 proteocine sensitive patterns with no untypable isolates (table VII).

Discussion

In the selection of a *Proteus* typing method for routine use it was felt that bacteriocine typing was

the method of choice, as far as reliability, reproducibility, and consistency are concerned. It is apparent, moreover, that methods using liquid bacteriocine preparations are more successful than those relying on proteocine preparation in solid media. This is exemplified by the present comparison between the existing Cradock-Watson and Al-Jumaili typing schemes.

The main advantages of the Al-Jumaili system over the Cradock-Watson method are:

1 the 100% typability obtained using the

Ref. Lab. No.	Serology		CW Proteocine Typing	Al-Jumaili Proteocine Typing Sensitivity Patterns												Phage Type	Resistogram Type	Dienes Type	
	Q	H		1	2	3	4	5	6	7	8	9	10	11	12				Code
747	3	1	5	+	-	-	-	-	-	-	-	+	-	+	-	4012	2/9	B(C)DEF(G)K	D1
347	3	2	1	-	-	-	+	+	-	-	+	-	-	+	-	0622	19/28	(A)BDE(F)GK	D2
348	3	2	1	-	-	-	+	+	-	-	+	-	-	+	-	0622	19/28/+	(A)BDE(F)GK	D2
699	3	2	3	-	-	-	-	-	+	-	-	-	-	-	+	0101	19/28/+	ABEFK	D3
753	3	2	3	-	+	-	-	-	+	-	-	-	-	-	-	2100	8/19/20/28	BCEF(G)K	D3
759	3	2	3	-	-	-	-	-	+	-	-	-	-	-	+	0101	31	BCEF(G)K	D3
837	5	2	4	-	-	-	+	+	-	-	-	-	-	-	+	0601	8/19/20/27	B(C)DFHK	D4
836	5	3	6	+	-	-	-	-	+	-	-	+	+	-	-	4114	28/29/31	B(D)EK	D5
742	6	2	3	-	-	-	+	+	-	-	-	-	-	-	-	0600	8/19/20/27	BCF(G)K	D6
750	10	2	3	-	+	-	-	-	+	-	-	-	-	-	-	2100	28/29/31	(A)BCDEFG	D7
775	10	3	NT	-	+	-	-	-	+	-	+	-	-	-	-	2120	8/19/20/28	CD(E)FGK	D8
700	10	4	8	-	-	-	-	-	-	-	+	-	-	-	-	0020	31	BEK	D9
713	11	1	5	+	-	-	-	-	-	-	-	+	-	+	-	4012	8/19/20/28	(A)BCDEFK	D10
341	11	2	3	+	-	-	-	-	-	-	-	+	-	+	-	4012	NT	BCDEGK	D11
766	19	2	3	-	-	-	-	-	+	-	-	-	-	-	+	0101	19/28	BCE(F)(G)K	D3
850	19	2	1	-	+	+	-	+	+	-	-	-	+	-	-	3304	8/19/20/28	BCD(G)K	D12
708	20	1	13	-	-	-	+	+	-	-	-	-	-	+	-	0602	28/29/31	ABCDEF	D13
608	23	1	2	-	+	-	-	-	+	-	-	-	-	-	-	2100	19/27/28/+	BCDEFGK	D14
706	23	1	2	-	+	-	-	-	+	-	-	-	-	-	-	2100	8/19/20/27	AC(D)HK	D15
859	23	NT	6	-	-	-	-	-	+	-	-	+	-	-	+	0111	28	ABCDEFHGHK	D16
762	24	3	1	+	-	+	-	-	-	-	-	-	-	-	-	5000	NT	ABCE	D17
687	26	1	1	-	-	-	+	+	+	-	-	-	-	+	-	0702	8/19/20/28	ABCDEF(G)K	D18
568	26	1	1	-	-	-	+	+	+	-	-	-	-	+	-	0702	19/27/28	BF(G)K	D18
352	26	2	1	-	-	-	+	-	-	+	-	-	-	-	-	0440	19/20/28	BCF(G)K	D19
704	26	NT	NT	-	-	-	-	-	+	-	-	+	-	-	+	0111	8/19/20/28	ABDE	D20
614	28	2	5	-	-	-	+	+	+	-	-	-	-	+	-	0702	9/+	ABDEFK	D21
760	28	NT	5	-	-	-	+	+	+	-	-	-	-	+	-	0702	8/19/20/28	BDEFK	D22
705	28	NT	9	+	-	+	-	-	-	-	-	+	-	-	-	5010	2/9	ABCDEK	D23
567	30	2	1	-	+	+	-	+	+	-	-	-	-	-	-	3300	26W	AB(D)EFH	D24
572	30	2	1	-	+	+	-	+	+	-	-	-	-	-	-	3300	26W	(A)B(C)DEF	D24
612	30	2	1	-	+	+	-	+	+	-	-	-	-	-	-	3300	26W	BCD(E)F(G)K	D25
854	NT	2	4	-	-	-	+	+	+	-	-	-	-	-	-	0500	8/18/20/28	ABCDEFHGHK	D26
856	NT	2	4	-	-	-	+	-	+	-	-	-	-	-	-	0500	31	AB(C)EFGHK	D26
																	8/19/20/28		
																	31/+		

Table VII Typing of 33 *Proteus* isolates by proteocine sensitivities and other methods

Al-Jumaili method. This compares with approximately 75% typability achieved by the Cradock-Watson method.

2 the larger number of strains demonstrated by the Al-Jumaili method as compared with the Cradock-Watson method. The 204 isolates typed by this method were shown to be divisible into 33 distinct proteocine sensitivity patterns whereas the Cradock-Watson method reveals only nine types, a miscellaneous group, and non-typable organisms. A total of 48 sensitivity patterns has been demonstrated elsewhere (Al-Jumaili, 1975) using 1000 *Proteus* isolates.

3 the greater specificity of the Al-Jumaili method. It can be seen that the Al-Jumaili system subdivides

strains of *Proteus* which appear to be identical when typed by the Cradock-Watson system (table IV). Thus, where the Cradock-Watson type is frequent, for example, types 1 and 4, the Al-Jumaili system subdivides these types respectively into 10 and four sensitivity patterns. Thus a higher degree of discrimination between apparently similar isolates of *Proteus* is achieved using the Al-Jumaili method.

Also, the large number of untypable isolates are differentiated, in this instance, by the Al-Jumaili method into 18 separate strains on the basis of their sensitivity patterns. On the other hand, where the Cradock-Watson type is infrequent, for example, CW type 6, identical results are obtained with the Al-Jumaili method (table IV). This obviously

indicates a degree of specificity for the Al-Jumaili system at least as good as that for the Cradock-Watson method. The remaining Cradock-Watson types (2, 3, 5, 9, and miscellaneous and atypical) were also shown to be capable of subdivision into more specific groupings—four in type 2, seven in type 3, two in type 5, three in type 9, and 10 in the atypical and miscellaneous group. The Cradock-Watson non-typable group was found to consist of 18 different strains. Moreover, with the exception of those isolates falling into the Cradock-Watson non-typable or miscellaneous groups, only five of the Al-Jumaili patterns (4, 13, 20, 16, and 18) were subdivisible by the Cradock-Watson system. Al-Jumaili pattern 4 occurred in Cradock-Watson types 2, 3, and 5; pattern 13 occurred in Cradock-Watson types 1 and 2; pattern 16 occurred in Cradock-Watson types 1 and 3; and pattern 18 occurred in Cradock-Watson types 1 and 9. The remaining 28 patterns were not encountered in more than one Cradock-Watson type. Also, the most commonly occurring Al-Jumaili pattern (pattern 2) accounted for under 10% of the total isolates typed.

4 the greater simplicity of typing using liquid proteocine preparations. Although Dienes' typing and resistogram typing gave a larger number of recognizable strains than the Al-Jumaili system (using a small sample of 33 isolates) both methods are laborious and are not necessarily reproducible and consistent. The Al-Jumaili system, on the other hand, is easy to execute, and once the standardizing proteocine preparations have been obtained the typing may be performed on large numbers of *Proteus* isolates routinely with a minimum of effort and materials. Also results may be obtained within 18 hours of isolating a strain of *Proteus*.

As bacteriophage and the Cradock-Watson methods of typing *Proteus* appear to be unsatis-

factory, it appears that the method described offers a simple and reproducible alternative to serology as the epidemiological tool of choice for the study of the spread of *Proteus* infections.

The results of the tests on nurse A and the six infants from the Boston Hospital seem to support this. It was discovered that nurse A carried two different types of *P. mirabilis*, and it can be seen that the six children carried either one or the other of these same two types (table V). This concurs with the results obtained by the Cradock-Watson method on the same isolates.

In addition, four strains of gentamicin resistant *Proteus* obtained from the surgical ward of the Middlesex Hospital were found to have an epidemiological relationship as they all have a similar sensitivity pattern. This was also shown by the Cradock-Watson results. In addition they were all found to be *P. vulgaris*.

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