Correlation between "Methicillin Resistance" and Serotype in Staphylococcus

"METHICILLIN resistance" has been found in a number of strains of staphylococcus isolated in French hospitals. Chabbert and Baudens[1] found in 1961 that ten of eighty-two strains investigated (12 per cent) were resistant to methicillin. In 1962 they found twenty-one resistant strains out of 127 (17 per cent), and in 1963 twenty-six out of 134 (19 per cent)[2,3]. Courtieu *et al.*[4] found a similar picture in their investigation of hospitals in other parts of France. In 1964 they found thirty-one resistant strains out of 216 (14 per cent). The frequency found in France seems to be different from that found among strains isolated in England and the United States[5–8].

Serological typing of staphylococcus has been carried out in France by the slide-agglutination technique since 1950 [9–12]. Using this technique, Pillet *et al.* have developed the work of Cowan[13], Christie and Keogh[14] and Hobbs[15] to show that the predominant serotype of staphylococcus in French hospitals before 1960 was serotype III. They then began to find a new type of hospital staphylococcus emerging, namely, type 14. Since 1963/64 this type is replaced by yet another, type 18, which is now prevalent. Although it is easy to obtain specific monovalent sera of types III, 14 and 18, there are antigenic relationships between strains of type III and 14 and also between strains of type 14 and 18. Furthermore, staphylococci belonging to serotypes III, 14 and 18 are usually lysed by group III phages.

We have therefore attempted to determine the correlation between agglutination serotype and methicillin resistance for various strains of staphylococcus.

We have investigated the serotype of ninety-nine strains isolated in 1965 from ten hospitals in Paris. Twenty-one of these strains belonged to serotypes I and I–II, twenty-eight to serotype III, seven to serotype 14, and forty-three to serotype 18.

The minimum inhibitory concentrations were determined using the agar streak method, using an inoculum consisting of one-hundredth of an overnight culture, on trypticase soy agar with 5 per cent added sodium chloride. This proportion of sodium chloride allows the phenotypic expression of methicillin resistance[16].

The results obtained with methicillin are shown in Table 1 and those with cephaloridin in Table 2. Both tables clearly show that twenty out of twenty-one strains of serotypes I and II and seven out of seven of serotype 14 are inhibited in these conditions by 8 μg/ml. or less of methicillin and by 2 μg/ml. or less of cephaloridin; these strains are sensitive. Conversely, the forty-three strains of serotype 18 are inhibited by 16 μg/ml. or more of methicillin and 4 μg/ml. or more of cephaloridin. These strains can be classified as resistant. The twenty-eight strains of serotype III are inhibited by various concentrations.

These results show clearly that all the strains belonging to serotype 18 are "methicillin resistant". But the resistant strains may also belong to serotype III. This probably means that there is no linkage between the genes responsible for serotype and resistance. A greater frequency of mutants resistant to β-lactam ring antibiotics (penicillins and cephalosporins) would be observed in this serotype. In any case, "methicillin resistant" staphylococcus serotype 18 has shown a particular ability to be selected in French hospitals. It would be interesting to investigate its prevalence in other countries.

Table 1. NUMBER OF STRAINS INHIBITED BY DIFFERENT CONCENTRATIONS OF METHICILLIN

		Methicillin Minimum inhibitory concentrations (μg/ml.)							
Serotype	No.	2	4	8	16	32	64	128	256
III	21	3	16	1	1				
III	28		7	4	3	–	3	6	5
14	7		3	4					
18	43				2	2	15	15	9

Table 2. NUMBER OF STRAINS INHIBITED BY DIFFERENT CONCENTRATIONS OF CEPHALORIDIN

		Cephaloridin Minimum inhibitory concentrations (μg/ml.)								
Serotype	No.	0·12	0·25	0·5	1	2	4	8	16	32
I	21	7	5	5	1	2			1	
III	28	4	1	7	2				9	
14	7		1	4	2					
18	43						2	9	31	1

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MICROBIOLOGY

Mutation of Bacterial Cells by Controlled Desiccation

PREVIOUS work has established reasonably well that the reorientation or removal of water molecules bound to macromolecules may result in the loss of viability and infectivity of bacterial cells and virus particles[1]. During the above studies when auxotrophic cells were held at certain levels of relative humidity (RH), prototrophic mutants were found, apparently produced as a direct result of desiccation. Later the mutation of bacterial cells by ultra-violet light was found to be more easily achieved if the cells were partially desiccated at 40 per cent RH (ref. 2), indicating that bound water molecules played some part in the mechanisms responsible for mutation. Cells or virus particles held at RH levels between 80 and 30 per cent contain from 35 g water/100 g of cell solids (35 per cent water) to 3 per cent water[1]. Because 30–40 g water/100 g of solid is just sufficient to hydrate fully protein RNA and DNA, desiccation below 80 per cent RH affects only the quantity of bound water in the cell. The apparent mutation of cells from desiccation suggested that water molecules were, in part, responsible for maintaining the biological integrity of DNA, and therefore further studies were warranted. It is the purpose of this paper to present the results of preliminary investigations.

Cells of *Escherichia coli B* were grown at 37° C in 'Bacto' brain–heart infusion broth, supplemented with 0·5 per cent yeast extract. After 12 h or 48 h of growth the cells were gathered by centrifugation, washed once in de-ionized water and resuspended in 20 ml. of water or 5 per cent w/v *i*-inositol. The latter compound was used because it had been found previously to prevent the death of cells by desiccation[1]. Oxygen free nitrogen gas was bubbled through the cell suspension for 1 h and the cells were then atomized with nitrogen in a rotating steel drum which contained nitrogen. In the drum the temperature was maintained at 25° C and the relative humidity was preset by spraying water free of oxygen into the drum,