Relationship of staphylococcal toxins and enzymes with serological and phage types

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SYNOPSIS A study has been made of 523 strains of staphylococci on the basis of biological properties, phage typing, and serology. The value of serology in the identification of pathogenic staphylococci has been assessed.

The recognized parameters of pathogenicity of staphylococci such as coagulase production or pathogenicity in animals are known to have a high degree of correlation with their toxigenic and enzymatic composition. The pathogenic staphylococci are also susceptible to the typing phages used (Blair, 1962; Fisk, 1942). The method of division of staphylococci into serological types is yet another approach to the classification of these organisms (Cowan, 1938 and 1939; Christie and Keogh, 1940; Hobbs, 1948). This method may be of importance for the subdivision of coagulase-negative, phage-insensitive staphylococci. On the basis of his comprehensive studies on Gram-positive, catalase-positive cocci, Baird-Parker (1963 and 1965) proposed a classification of these organisms into three genera, namely, *Staphylococcus*, *Micrococcus*, and *Sarcina*; the genus *Staphylococcus* could be further divided into seven subgroups. The subgroup I accommodated coagulase-positive organisms associated with human pathological lesions. The organisms of subgroup II were encountered from human and pig surfaces, and were coagulase-negative. But in view of the biological and technical limitations of the coagulase test, he considered it inadvisable to rely solely on this test for the identification of *Staphylococcus aureus*. In the present study some coagulase-negative strains isolated from human pathological sources were examined from the point of view of a possible association of certain serotypes with their capacity to produce known toxins and enzymes.

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Materials and Methods

Five hundred and twenty-three staphylococcal strains were isolated from clinical lesions of in- and outpatients of a Calcutta hospital. The strains were tested for coagulase, hyaluronidase, alpha, beta, and delta haemolysins, lipase, gelatinase, and phosphatase activities and Müller's phenomenon, according to the methods reviewed by Elek (1959), except for leucocidin and fibrinolysin, which were detected according to the methods as described by McLeod (1963) and Vogelsang, Wormnes, and Østervold (1962) respectively. Phage typing was done according to the method of Blair and Williams (1961), using the basic set. Serological typing of the coagulase-negative strains was determined following the method described by Stern and Elek (1957), using Cowan's type I, II, and III strains (NCTC nos. 8530, 8531, and 8532 respectively).

Results

ASSOCIATION OF DIFFERENT TOXINS AND ENZYMES AND OTHER BIOLOGICAL CHARACTERS WITH COAGULASE-POSITIVE STAPHYLOCOCCI ISOLATED FROM HUMAN LESIONS

Out of a total of 523 strains studied 502 were coagulase-positive and the rest negative. The incidence rate of various toxins and enzymes among the former were as follows: phosphatase (98.4%), mannitol (97.6%), and glucose fermentations (95.9%); gelatinase (95.4%), leucocidin

No. of Strains	Sero-typable	Haemolysins			Leuco-cidin	Hyaluro-nidase	Fibrino-lysin	Phos-phatase	Lipase	Gelati-nase	Müller's Phenome-non	Fermentation	
		Alpha	Beta	Delta								Glucose	Mannitol
13	Yes	6	—	1	3	9	7	11	9	10	—	11	2
8	No	—	—	—	—	—	—	2	—	1	—	—	2

Table Production of different toxins and enzymes by coagulase-negative strains of staphylococci

(92.4%), Müller's phenomenon (91.6%), hyaluronidase (91.2%), alpha haemolysin (90.2%), lipase (81.4%), and beta-haemolysin (16.1%). It appeared that the frequency of occurrence of the individual biological characters was a variable feature of this group.

ASSOCIATION OF COAGULASE-NEGATIVE STRAINS WITH THE DIFFERENT TOXINS AND ENZYMES

The association of various toxins, enzymes, and other biological characters with 21 coagulase-negative strains was studied and found to be as follows: alpha haemolysin (42.8%), fibrinolysin (33.3%), phosphatase (33.3%), lipase (42.8%), gelatinase (52.3%), mannitol (61.9%) and glucose fermentations (9.5%), delta haemolysin (4.7%), and leucocidin (14.2%). These strains had neither beta-haemolysin nor exhibited Müller's phenomenon.

CORRELATION OF DIFFERENT BIOLOGICAL CHARACTERS WITH SEROLOGICAL TYPES OF THE 21 COAGULASE-NEGATIVE STRAINS

The 13 coagulase-negative but serologically typable strains exhibited a distribution pattern of toxins and enzymes somewhat similar to that of the coagulase-positive strains. The relationship of these products with toxins and enzymes of coagulase-positive staphylococci was demonstrated by their neutralization with specific staphylococcal antitoxins prepared for this purpose (Table). On the other hand, the remaining eight coagulase-negative, serologically non-typable strains exhibited little capacity to elaborate these biological products. Of these 13 coagulase-negative sero-typable strains, six belonged to group I, five to group II, and two to group III in the Cowan classification (1939).

RELATIONSHIP OF THE DIFFERENT BIOLOGICAL CHARACTERS WITH THE STAPHYLOCOCCAL PHAGE TYPES

The 21 coagulase-negative strains were all non-typable with the basic set of phages. Of the remaining 502 strains, those belonging to phage group I had the highest correlation with gelatinase activities (100%), group II the highest correlation with gelatinase and phosphatase activities (100%), group III with phosphatase

(98%), the mixed type with delta haemolysin and gelatinase (100%), and the non-typable variety with phosphatase (100%).

Discussion

There is very little doubt about the pathogenic status of staphylococci that are coagulase-positive and which, in addition, possess the capacity to produce many other toxins and enzymes together with phage susceptibility. Besides this clear-cut group, there are others which are encountered in pathological materials from human cases, and the status of these organisms in relation to the previous group as well as their pathogenicity needs to be assessed. Studies of numerous workers (Elek, 1959) on the association of single or groups of characters with staphylococci considered to be pathogenic, either on account of the clinical history and nature of the lesion, or based on the results of animal pathogenicity, appear to have been carried out with a view to using the highly correlative characters as nearly equivalent substitutes for the more difficult proof of staphylococcal pathogenicity. In such an approach, in the absence of a character as important as coagulase production, other highly correlative characters may be relied upon.

The present studies showed that a high but varying percentage of coagulase-negative but sero-typable strains produced enzymes and toxins which were comparable to those of the coagulase-positive strains. In contrast, the coagulase-negative serologically untypable strains elaborated very few enzymes and toxins, and the pathogenicity of these strains, in spite of their isolation from human pathological sources, remains doubtful.

The study of the different phage groups with the biological characteristics of staphylococci showed that phage group I had the highest correlation with leucocidin, hyaluronidase, gelatinase, and Müller's phenomenon, which is in agreement with the observations of Fodor, Rozgonyi, and Csépe (1963) and Tauraso and White (1963). The finding in respect of phage group II having maximum correlation with alpha and beta haemolysins, lipase and phosphatase, and phage group III with fibrinolysin, and the mixed type with the delta haemolysin, does not support the observations of Solomon and San Clemente (1963) or Cannon and Hawn (1963).

It appears, therefore, that studies on the composite nature of biological characters of staphylococci are helpful in evaluating the status of coagulase-negative staphylococci. The serotyping is an additional adjunct in such a situation.

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