

**PHAGE TYPING OF STAPHYLOCOCCUS AUREUS STRAINS
OF AIN SHAMS UNIVERSITY HOSPITALS**

THESIS

**Submitted for Partial Fulfilment of Master Degree
in Clinical and Chemical Pathology**

BY

**NEVINE NABIL KASSEM
(M.B.,B.Ch.)**

SUPERVISORS

**Prof. ISLAH HASSAN EL FALAKY
*Prof. of Clinical Pathology
Ain Shams University***

**Dr. IBRAHIM KHALIL ALY
*Lecturer of Clinical Pathology
Ain Shams University***

**FACULTY OF MEDICINE
AIN SHAMS UNIVERSITY
CAIRO - EGYPT**

1985

ACKNOWLEDGEMENT

*I wish to express my profound gratitude and sincere appreciation to **Professor ISLAH HASSAN EL FALAKY**, for her valuable help, great care and encouragement. Her unfailing advice and constructive supervision were of great importance in bringing this work to light.*

*My thanks are also extended to **Dr. IBRAHIM KHALIL**, for his generous help.*

*I owe a debt of gratitude to the technicians in the bacteriology lab. of our department especially **Mr. MAGDY RIAD**, for their sincere help and unlimited patience.*

*Finally, I wish to thank my husband **Dr. ADEL EL ETRIBY**, for his personal support, encouragement and understanding.*



CONTENTS

	<u>PAGE</u>
INTRODUCTION AND AIM OF THE WORK	1
REVIEW OF LITERATURE	5
Historical Aspects of Bacteriophages	5
General Properties of Phages	10
Bacteriophage Typing	19
Bacteriophage Typing of Staphylococcus Aureus	22
Prevalence of Staph. aureus Phage Patterns in Hospital Acquired Infections	36
MATERIAL AND METHODS	55
RESULTS	70
DISCUSSION	90
SUMMARY	106
REFERENCES	108
ARABIC SUMMARY	

.....

INTRODUCTION

INTRODUCTION
AND AIM OF THE WORK

Hospital - acquired infection is not a new entity. It has been recognized over the years as a risk associated with all hospital admissions. It includes infection from one person to another - cross infection - and infection from one tissue to another in the same person - auto infection (Williams et al., 1966).

In the recent years, there has been a considerable increase in the number of patients susceptible to hospital acquired infection and there are numerous references documenting the role of staphylococcus aureus in these cases (Blair and Carr, 1958; Jevons and Parker, 1964; Taidi et al., 1971; Marraro and Mitchell, 1975; Altomeier et al., 1982).

Staphylococci were relatively unimportant bacteria. But since the late 1940s, it has been a common knowledge that the wide use of antibiotics in hospitals and the spectacular medical and surgical advances led to the selection and emergence of resistant strains of staphylococcus aureus (Aylife et al., 1979).

Hospital acquired infections due to antibiotic-resistant strains of staphylococcus aureus present a problem that has become increasingly serious in recent years. It has been demonstrated by numerous investigators that such strains become well established in the hospital community especially in newborn babies, surgical patients patients with diabetes and other chronic diseases; many authors felt "the staphylococcal plague" had begun. (Fekety, 1964).

The isolation and identification of this microorganism from clinical sources generally present no difficulty to the bacteriologist. Strains of staphylococcus aureus may be differentiated into several types either by serotyping (Cowan, 1939) or by phage typing (Williams and Rippon, 1952). Due to technical difficulties and less satisfactory results obtained by serotyping, phage typing has proved to be an invaluable tool for the epidemiological surveillance and control of these pathogenic organisms. The basic set of typing phages used for this purpose is chosen so that as many as possible of the important strains pathogenic for man, and particularly those causing endemic sepsis in hospitals show characteristic pattern of lysis (Fisk, 1942 a,b).

In the course of time, however, the strain responsible for sepsis in hospitals change, and the new ones may not be typable with the phages in current use. On several occasions, since 1950, the appearance of a new organism has made it necessary to introduce a fresh phage into the basic set of typing phages (Blair and Williams, 1961).

Several hundred different phage types of staph. aureus were distinguished according to the pattern of susceptibility of strains to an internationally recognized set of about 23 standard phages (De Saxe et al., 1982).

Bacteriophage typing has shown that in the majority of instances, the staphylococcus that are responsible for cross infection in any institution are confined to a relatively limited number of strains (Williams, 1959; Ward et al., 1977).

The aim of this work is to detect the prevalence of different phage type strains causing infection in Ain Shams University Hospitals.

For this purpose, 56 staphylococcus aureus strains were randomly isolated from the inpatients of the different departments and were typed according to the standard method of Blair and Williams (1961).

*REVIEW
OF
LITERATURE*

REVIEW OF LITERATURE

HISTORICAL ASPECTS OF BACTERIOPHAGES

The first discovery of the existence of viruses which attack bacteria was reported in 1915 by the English bacteriologist Frederick Twort who described a curious degenerative change in colonies of a staphylococcus isolated from calf lymph (Twort, 1915). These bacterial colonies appeared as contaminants on his culture plates during his attempts to cultivate virus in a cell-free system. Upon further incubation some of these colonies changed their appearance and underwent what twort referred to as "glassy transformation".

Twort made some very interesting observations about such phenomenon which were:

- 1- Attempts to obtain viable bacteria from these colonies failed, indicating that the constituent bacteria have been killed.
- 2- Examination of the glassy area revealed only minute granules and no bacteria.
- 3- After filtration of the glassy material through a Chamberland candle, it retained its ability to cause the glassy transformation.

- 4- Such transformation could be conveyed to fresh cultures for an indefinite number of generations.
- 5- The affected colonies would not grow on any medium.
- 6- Lastly, if a pure culture of micrococcus was touched with a small portion of one of the glassy colonies, the growth at the point touched started to become transparent and gradually made the whole colony transparent.

So, Twort concluded that the cause of the glassy transformation was an infectuous, filterable agent that killed bacteria and in the process multiplied itself.

He could have very logically concluded that he was dealing with a bacterial virus. Yet he did not do so, instead he suggested several possible explanations of his findings, of which one was that: the micrococcal disease was caused by a virus "acute infectuous disease of micrococci", so we get a remarkable discovery faced by its discoverer with uncertainty (Duckworth, 1976).

Two years later, 1917, a Canadian bacteriologist, Felix d'Herelle, had independently reached the same conclusion. On various occasions he had noticed circular

clear spots in bacterial cultures growing on the surface of nutrient agar. The spots were devoid of bacteria. In d'Herelles 1917 report, proof is supplied that the lytic agent is living and can replicate. This was based on his observation that the agent could be passaged repeatedly and the demonstration that the clearings on an agar surface were produced by the agent in lysed cultures. As a result d'Herelles coined the word "bacteriophage", which means eater of bacteria in Greek. Bacteriophage, bacterial virus and phage are used synonymously - the latter being most commonly used (Ritchie, 1983).

D'Herelle's view of the bacteriophage as a filtrable self reproducing virus parasitic on bacterial cells, in spite of being correct, met with considerable opposition in the early years. D'Herelle had not acknowledged Twort's published work. In 1911, he was faced with the Twort phenomenon but he asserted that this was not the same as his bacteriophage. This no doubt exacerbated the disagreement between d'Herelle and his critics particularly, as Gratia 1921, was soon to show that the glassy transformation of staphylococcus was identical in nature with d'Herelle's bacteriophage lysis. Staphylococcus is therefore, the first gram positive bacterium for which an observation of transmissible autolysis has been made (Gratia, 1921).

In the 1920's, the potential use of these lytic agents in the treatment and prophylaxis of bacterial diseases led to isolation of bacteriophages against bacteria of anthrax, cholera, diphtheria, gonococcus and a host of others. In 1925, Arrow Smith isolated an agent capable of lysing staphylococci. (Lipton and Weissbach, 1969).

A suggestion that specific phages might be used for typing was made by Williams and Timmins in 1938 (Williams and Rippon, 1952). But the starting point for the development of the present method of bacteriophage typing of staphylococcus aureus was Fisk's isolation from strains of staph. aureus of a series of bacteriophages that were apparently type-specific rather than species-specific. These phages were obtained by picking the plaques that developed when spot inocula of several strains were grown on the surface of an agar culture of a single strain (Fisk, 1942 a,b).

Wilson and Atkinson in 1945, isolated 18 phages by Fisk's cross culture method and with purification and propagation of the phages to relatively high titre, devised a method suitable for routine use (Wilson and Atkinson, 1945).

During the following years, workers in various countries became interested in typing staphylococci and in 1953, the International Subcommittee on phage typing of staphylococci was formed to supervise the development of the method and to select a basic set of phages for use by laboratories throughout the world.

The recommendations of the Subcommittee on the standard methods to be used for the propagation and testing of phages and for routine typing were published by Blair and Williams (1961).

The composition of the international basic set of typing phages has evolved over the years to keep up with changes in the prevalence of the strains causing human infections in different countries. Existing phages are replaced with new phages that appear to be more generally useful (de Saizy et al., 1981).

The international basic set of staph. aureus typing phages is:

Lytic group I: 29, 52, 52A, 79, 80
" " II: 3A, 3C, 71, 71
" " III: 6, 42F, 47, 53, 54, 75, 75
83A, 84, 85
Miscellaneous: 81, 84, 85, 86