STUDIES ON SOME LOCAL STRAINS OF *SALMONELLA TYPHOSA*

By

ISIS AZIZ MIKHAIL
B.Sc.

Thesis
Submitted in Partial Fulfilment of the Requirements
for the Degree of Master of Science

Department of Botany
Faculty of Science
Ain Shams University
Cairo, Egypt.

May, 1972

This investigation was suggested and supervised by Dr. Sabry H. Elwan, Assist.Prof. of Bacteriology, Department of Botany, Faculty of Science, Ain Shams University to whom the author offers her thanks.

Thanks are also due to Prof. Dr. Mohamed Saber Naim, Head of the Botany Department, Faculty of Science, Ain Shams University, for his encouragement.

The author wishes to express her sincere appreciation to Dr. John E. Sippel, Ph. D., Head of Bacteriology Department, NAMRU-3, for the willing instructions extended by him.

Thanks are also due to Dr. Refat Hablas, Director, Bacteriology Section, Central Public Health Laboratories for his great help in studying the phage types.

The author is most grateful to Dr. Anwar Hassan, Director, Abbassia Fever Hospital, for generously offering the facilities which made this study possible.

Sincere gratitude is due to Dr. J. F. Vieu, "Chief de Service des Bacteriophages, Institut Pasteur, Paris," in whose labcratory the phage types of the strains were made.

The author is also grateful to A. Iman Abdel Megid, Lab. technician NAMRU-3; for his sincere aid.

Special thanks are due to Mr. Berge H. Sek-Zenian, Photographer, NAMRU-3, for developing and producing of the photographs.

Many thanks to NAMRU-3 Library Staff for offering facilities.

Last but not least, the author is greatly indebted to the Director, NAMRU-3 for the help and facilities put at her disposal to accomplish this study. This investigation was supported by the U.S. Navy Work Unit No. MR005.20.01.0261A.

CONTENTS

Page

PART I

INTRODUCTION 1
Salmonellosis 1
Biochemical Reactions 9
Nutritional requirements 15
Resistance to Radiation 19
Sensitivity to Antibiotics 24
Selection of Antibiotic-Resistant Strains 25

GENERAL METHODS 27
Material 27
Growth curves of representative strains 29
Biochemical Reactions 30
Nutritional Requirements 32
Development of Resistance to Ultraviolet-Radiation of Salmonella typhi strains 35
Determination of Susceptibility of Salmonella typhosa strains to Antibiotics 42
Selection of Penicillin-resistant Strains of Salmonella typhi. 53

PART II

RESULTS 59
Growth Curves 59
Nutritional Requirements 72
Biochemical Reactions 75
Minimal Inhibitory Concentrations 78
Resistance to Radiation 81
Penicillin G Selectivity 83
Serology and Phage Typing 85

Page

PART III

PREFACE 88

MATERIAL AND METHODS 89

Section A:

Salmonella typhi strains sensitive to ultraviolet radiation and penicillin G 90

Section B:

Salmonella typhi strains sensitive to ultraviolet-irradiation and partially resistant to penicillin G 91

Section C:

S. typhi strains sensitive to ultraviolet-irradiation and resistant to penicillin G .. 96

Section D:

S. typhi strains resistant to ultraviolet-irradiation and partially resistant to penicillin G 102

Section E:

S. typhi strains resistant to ultraviolet-irradiation and highly selected by penicillin G 108

PART IV

DISCUSSION 124

SUMMARY 137

REFERENCES 144

ARABIC SUMMARY

PART I

INTRODUCTION

The aim of this thesis is to investigate the characteristics of local strains of the typhoid microbe with reference to their responses to various antibiotics. Since variants or mutants are likely to occur in microbes in vivo due to one factor or another, an attempt was made to treat the studied strains by ultraviolet irradiation. This was to determine the characteristics of possible variants or mutants of the local strains. Therefore, it is felt convenient in a historical review of a thesis of the present nature to deal with the following topics of the genus Salmonella and particularly Salmonella typhosa (S. typhi):

1- Salmonellosis.

2- Biochemical Reactions.

3- Nutritional Requirements.

4- Resistance to Radiation.

5- Sensitivity to Antibiotics.

6- Selection of Antibiotic-resistant strains.

1- Salmonellosis:

The genus Salmonella: Salmonellosis has been the subject of many studies throughout the world since 1885

when Salmon and Smith isolated the first member of the genus Salmonella from swine. The definition of the genus Salmonella formulated by White (1929) and amended by the Salmonella Subcommittee (1934) was again modified by the committee (1949), as follows:

"A large genus of serologically related, Gram-negative and non-sporing bacilli; 0.4 to 0.6 x 1 to 3 microns in usual dimensions but occasionally forming short filaments; showing, with certain exceptions, a motile peritrichous phase in which they normally occur; in fact adhering to the pattern of S. typhi in staining properties and morphology; rarely fermenting lactose or sucrose; liquefying gelatin or producing indol. They regularly attack glucose with, but occasionally without, gas production. All the known species are pathogenic for man, animals, or both". The currently accepted definition of the genus Salmonella by Edwards and Ewing (1967) is given in the following terms:

"Usually motile but non-motile forms occur, produce acid and gas from glucose, maltose, mannitol and sorbitol (except that in S. typhosa and S. gallinarum no gas is produced). Lactose, sucrose and salicin are not attacked.

Do not clot milk, form indol or liquefy gelatin, reduce trimethylamine oxide to trimethylamine. All of the known species are pathogenic for warm-blooded animals, including man, causing food infections and enteric fever. A few are found in reptiles. Some or all may also live in decomposing foods".

Serotypes and antigenic formula: More than 1000 serotypes belonging to this genus are known to date. Serologic studies are hampered by the complex antigenic structure of salmonellae that must be considered in the diagnosis of the different Salmonella types. Distribution of these antigenic components forms a basis for a natural scheme of classification, and this has been recognized im The Kauffmann-White-Schema illustrated by Kauffmann (1966).

The antigenic formula consists of three parts, describing the somatic (O) antigen, the flaggellar phase 1 (H) antigen (more specific) and the phase 2 (H) antigen (less specific). The three parts are separated by colons, and the components of each part by commas. In addition to the (H) and (O) antigens some strains, notably the typhoid bacillus, contain a further somatic antigen originally

described by Felix and Pitt (1934 a,b) as the (Vi) antigen. S. typhosa has the antigen o structure 9, 12, Vi:d, -.

Typhoid fever: S. typhosa is the causative organism of typhoid fever in man. Kent and Hassan (1970), stated that typhoid fever has been with us and recognized at least from the days of Hippocrates. The disease has remained a scourge of mankind for many hundreds of years. During the Middle Ages it ravaged Europe in epidemics. In the Middle of the 19th. century, Great Britain was struck by up to 50,000 cases per year. It has thus remained in many parts of the world as a serious disease with high morbidity and significant mortality.

The distribution by type varies greatly by region and time. The more complete knowledge of the relative prevalence of different Salmonella serotypes, by time and place, is becoming of increasing importance, both from a national and international standpoint.

In Egypt, Perry et al. (1929) made an investigation of some acute bacillary intestinal infections in man. It was observed that the most prevalent infection was due to (Bacillus typhosus) which had accounted for 69% of cases.

Gillmore et al. (1952), claimed the same findings, with a percentage of 79.5 due to Salmonella typhosa. Hector, Hassan and Abdel Wahab (1970), noted that 4000 to 8000 patients were diagnosed as Enteric Fever every year in each of the two Fever Hospitals in Cairo, namely Abbassia and Embaba; 75% of the causative isolates were Salmonella typhi and 25% were due to Salmonella paratyphi A; they stated that these results showed to agree with that of Kamal (1957). Accordingly, in Egypt, Salmonella typhi and Salmonella paratyphi A infections are reportable as the predominant types of human infections.

The type determination moreover helps the epidemiologist and public health responsible to find, trace and break chains of transmission between human-beings, between them and animals and between animals and them to prevent further spread of Salmonellosis.

Strain identification of various bacterial species by the use of specific bacteriophages has been shown by numerous authors to be an important epidemiologic tool. Phage typing of Salmonella typhi is a model of its kind (Nicolle, 1964). It may be useful to summarize here briefly the story of the development of the phage-typing method.

Phage typhi: In 1934, Felix and [illegible], of the Lister Institute of London, discovered a new antigen of the typhoid bacillus. This antigen presented properties different from those of other antigens already known for the same bacillus. They considered it as the only, or at least, the main factor responsible for the virulence of the typhoid bacillus; hence the name Vi antigen that they gave it (Vi being the first two letters of the word virulence).

Shortly thereafter, in 1936, Craigie and Brandon in Canada, Scholtens in Holland, and Sertic and Boulgakov (1937) in France, one group independently from the other, ascertained that some bacteriophages act on most of the "Vi positive" strains of S. typhi, but that they were always inactive against the "Vi negative" bacilli. From this they concluded that the Vi antigen must be the specific receptor for these particular phages and for this reason named them "Vi phages".

Craigi and Yen (1938a,b) noticed that one of their Vi phages, Vi phage II, possessed exceptional powers of adaptation. Upon growing it on a not very sensitive but not

completel refractory to positive strain of S. typhi, it became extremely active on that strain and in the same time on a few others; for instance those isolated in the same epidemic location. Its lytic activity did not undergo a significant rise for other strains coming from different locations. Therefore, the Vi phage 11 adapted to a Vi positive strain of S. typhi has, become, in some way, specific of a whole group of typhoid bacilli.

Thanks to the creation, by Craigi and Felix (1947), Felix (1955), Anderson (1962), of an International Committee for Enteric Phage Typing affiliated with the International Association of Microbiologic Societies and of an International Reference Laboratory entrusted with the preparation of the adapted phages and of their distribution of the members of the Committee and thanks also to the research of several of its members, the number of phage types of S. typhi was increased from 24 in 1946 to almost 90 today.

What is the use of S. typhi phage typing, and, in general what is the use of phage typing methods in applied epidemiology? If a person suffers from typhoid fever or any other transmissible bacterial disease, or if he is germ carrier, then the bacilli that are isolated from this person

during his illness or carrier state must necessarily belong to the same phage type, no matter what the source of the culture is (blood, stool, urine, bile, pus, cerebrospinal fluid, etc.) and no matter what treatment he has received (with the exception of treatment by bacteriophages, which may sometimes alter phage types).

If this patient or this carrier infects persons in his surroundings, no matter what the number of these contacts is (small school epidemics, barracks epidemics, or large epidemics caused by the contamination of drinking water or food) the phage type will be the same for all; the patient, the carrier, and the infected persons. Hence it follows that, by means of phage typing, one can establish the relationship between cases, trace the original source of infection, point out the responsibility of a carrier for beginning a focus of infection, and having all the facts, be able to take the appropriate measures in the case of each focus or each epidemic and thereby stopping its spread or even succeed in achieving the eradication of the illness in a given area (Nicolle, 1970).

Phage typing throws light on epidemiological research that would often, without it, remain confusing. One cannot

seriously study an epidemic of typhoid fever without using this method.

2- Biochemical Reactions:

The biochemical reactions characteristic of Salmonella were discussed in the definition of the genus. With a few exceptions, Edwards and Ewing 1967 stated that, the biochemical reactions of the individual types are so similar that they are of little value in differentiation. They suggested that there are a few distinctive reactions which are valuable diagnostic aids in distinguishing certain types.

For Salmonella typhi the author stated that the most distinctive biochemical reactions are the relative differences in fermenting arabinose and xylose. Trehalose is fermented promptly. Dulcitol is fermented late and irregularly positive. The species produces H_2S and does not produce gas. Simmons' Citrate, l-tartrate and I-tartrate are not attacked whereas D-tartrate is attacked after one or two days. Mucate is attacked late and it is irregularly positive. Decarboxylase reactions described by Moller (1955) was tested by Edwards and Ewing (1967) and Martin (1970). They stated that lysine decarboxylase reaction is positive by