Clinical Bacteriology

STOKES

CLINICAL BACTERIOLOGY

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LONDON

EDWARD ARNOLD (PUBLISHERS) LTD.

FOREWORD

Of the many disciplines in clinical pathology, bacteriology is perhaps the least easy to codify in fixed routines. Every patient with a suspected infection is a new biological problem that both the clinician and the bacteriologist can solve only by following their noses wherever their investigations lead them; and any attempted identification of the infecting microbe in the laboratory may lead the bacteriologist along unexpected paths. Clinical bacteriology is an exploratory art that demands flexibility of mind and technique, and the latest methods are less important to the (would-be) practitioner than a set of uniformly good procedures with which to explore the common and the not-too-rare infections of man, and with which he stands a reasonable chance of discovering new ones.

The methods in this book will go far to meet these requirements. They are well tried. Many indeed had their beginning in 1940–44 in the work of Dr. Stokes and other former colleagues of mine in the Emergency Pathological Services of the London Sector 4, and much has been added to keep pace with advances in post-war medicine; and they have an added merit in being subordinated to an all round picture of the practice of clinical bacteriology in a hospital department.

The work of those war years, when the treatment of war wounds and the difficulties of epidemic control in the emergency hospitals stimulated the study of cross-infection, also bears fruit in an innovation—Chapter on Hospital Epidemiology. The inclusion of such a chapter very properly implies that the hospital bacteriologist today must be more than the explorer of each single clinical problem as it arises; as a watchdog in hygiene and as a hospital epidemiologist, he has a function that is almost equally important.

Dr. Stokes insists a little on the academic approach. If it is academic to bear in mind the better established principles of bacteriology and immunology and to define the limits of, and justifications for, the hodge-podge of techniques that make up the practice of those sciences, then in attempting to do these things the book may be said to have an academic background; but to my mind they are equally the hallmarks of good practice.

A. A. Miles.

PREFACE

Examination of bacteriological specimens differs from other investigations in clinical pathology in that no complete set of rules can be laid down to guide the laboratory worker. The value of the result depends as much on the knowledge and judgement of the person who examines the cultures at each stage as on his technical accuracy. If it were possible to insist on the isolation in pure culture and identification of each type of colony appearing on every culture the task of the pathologist responsible for maintaining a high standard of work would be simple. For reasons of speed and economy, however, this is impossible, with the result that it is very difficult for the pathologist to ensure that each specimen receives adequate attention and to decide how far each examination shall proceed. This book is an account of how these problems, and others encountered in the routine laboratory of a large general hospital, are met.

Most of the methods recommended are well known. They have been tested both experimentally and in routine use in this laboratory. They are described dogmatically for the sake of clearness and reasons for preferring them are given, but no claim is made that they are the best available. The reader will have no difficulty in finding descriptions of alternative methods elsewhere. If he so wishes he can compare the various culture media by the methods described in Chapter 10.

The approach to the investigation of infection is frankly academic. I make no apology for this because I believe that by regarding each investigation as a separate problem to be pursued, as far as is required, in essentially the same manner as a research project, knowledge of infectious disease will grow and the best interests of the individual patient are served. It is possible to do this without elaborate laboratory equipment, numerous staff or delayed reports, by keeping the purpose of each investigation in mind and limiting it to essentials.

I hope the book will prove useful not only to pathologists and bacteriological technicians but also to clinicians, who wish to know what help they can reasonably expect from the laboratory, to those who teach nurses how to collect specimens for culture and to Resident Medical Officers and others responsible for the protection of patients from pathogenic bacteria in the hospital environment.

It is impossible to acknowledge properly the originators of all the methods quoted. Many of them are unknown to me; those that are known are mentioned in the text.

I am greatly indebted to Professor A. A. Miles; first for his teaching in the application of academic bacteriology to routine investigation; second for allowing me to use his *Practical Notes on Elementary and Clinical Bacteriology*, written in 1944 for University College Hospital Medical School, and finally for his kindness in writing the foreword. For his encouragement and for reading part of the manuscript I wish to thank Professor Wilson Smith. I am also very grateful to Dr. R. W. Riddell and Dr. J. R. May, for reading the proofs; to Dr. J. H. Hale and Dr. J. H. Humphry for reading parts of the manuscript, and to Dr. Robert Blowers, Dr. G. A. James, Mr. R. A. Bono and Mr. L. J. Jeffries for help in testing methods in the laboratory.

I would also like to thank H.M. Stationery Office for permission to reproduce Table 37, and the photographic department of U.C.H. Medical School for the photographs.

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CHAPTER 1

THE PRACTICE OF CLINICAL BACTERIOLOGY

Clinical bacteriology is the study of specimens taken from patients suspected of infectious disease to find, first, if there is any change in kind or distribution of the normal flora and, second, if the abnormal bacteria found are the cause of the disease. In most cases it is fairly easy to answer the first of these questions. The second is often difficult and sometimes impossible to solve; it may be approached in two ways. The question is asked, "We have found microbe A, is it causing disease D in this patient?" We may use the statistical argument that in many previous cases A has been found, to everyone's satisfaction, to be the cause of D; therefore the chance of it being so in this case is very great and the assumption may safely be made. There are many pitfalls in the use of this argument for individual cases because no two patients are exactly alike and there is also wide variation in virulence between strains of the same species of microbe. For example, it has been established beyond reasonable doubt that *Strept. pyogenes* causes sore throat, and if we use this argument we shall assume that in all cases of sore throat when this microbe is found it is the cause of the infection. But this is not so; healthy people are sometimes carriers of *Strept. pyogenes* and such a person may develop diphtheria, in which case if we say that microbe A (*Strept. pyogenes*) is the cause of disease D (sore throat) we may be at fault.

"Pathogen" and "Saprophyte"

It is clear from this that the terms pathogen and saprophyte are not precise. *Strept. pyogenes* is undoubtedly a pathogen when it causes fatal septicaemia, but it is apparently a saprophyte when it is found in the throat of a healthy person. Similarly achromobacteria, normally regarded as saprophytes, have been known to cause a fatal septicaemia.

The presence of a pathogenic microbe in human tissues results in a variety of conditions, ranging from healthy carriage to the moribund state; the factors which determine whether clinical signs of infection will develop and, when this happens, at what stage

the relation of host to parasite will reach equilibrium are not well understood. Some acute infectious diseases produce lasting specific immunity and in these it is easy to explain the lack of signs of infection which follows subsequent contamination by the causal microbe. In others it is known that the pathogen needs special conditions before infection can be established, which explains why the presence of virulent *Cl. welchii* in a wound is not always followed by signs of gas gangrene. Specific immunity and special growth conditions are not however the only factors concerned, and often when there is an unusual response to the presence of bacteria in the tissues we have to assume without evidence, other than the condition we are attempting to explain, that the resistance of the patient or the virulence of the strain is abnormal.

It follows then that by pathogen we mean a microbe which is often dangerous and by saprophyte one which is seldom or never dangerous. This leads us in the practice of clinical bacteriology to adopt the following rules:

1. Never without good reason dismiss a microbe as a contaminant because it is not an accepted pathogen.
2. Never without good reason accept a microbe as the *necessary* cause of a disease merely because it is an accepted pathogen.

Koch's postulates in Clinical Bacteriology

Another way of tackling the problem "Is microbe A causing disease D"? is to apply Koch's postulates:

1. The microbe should be found in all cases of the disease, distributed in the body according to the lesions observed.
2. The microbe should be grown artificially in pure culture for several subcultures.
3. The pure culture should reproduce the disease in a susceptible animal.

Postulate 1 must be modified for the single case to read, "The microbe should be constantly found associated with the lesions during the course of the disease."

We may add that the causal role of a microbe is strengthened if it can be shown that the serum of an infected animal contains a high antibody level which is specifically protective against infection by the microbe, particularly if a rise in titre during infection and a fall in titre after recovery can be demonstrated. It may also be

strengthened by showing the development of a specific allergic reaction to a preparation of the microbe injected intradermally.

This method of approach is much more satisfactory than the statistical argument, but it is often impossible to carry out since many human infections cannot be recognizably reproduced in animals. It is worth while to test the antibody level in the patient's serum but a negative result does not exclude its causal role. A positive result indicates that the microbe, or another with similar antigens, has been present in sufficient quantity to stimulate antibody formation. Even if the titre can be shown to rise during infection and fall afterwards it does not finally prove that A is causing D. Remember, for example, the positive proteus agglutination with serum from patients suffering from rickettsial diseases.

Two Types of Evidence

There are two types of evidence which may be gained from laboratory investigations. First, simple tests may be made which are in themselves of little significance but which when taken into consideration with the history and physical signs may help to establish the diagnosis. If urine from a patient with symptoms of renal tract infection contains acid-fast bacilli it is probable that the patient has renal tuberculosis and the demonstration of the bacilli adds weight to the diagnosis. Similarly, if Gram-negative diplococci are found in a smear from the urethra of a woman with symptoms of acute infection, the finding adds weight to the diagnosis of gonorrhoea. But neither of these findings when considered alone have much significance because they are occasionally seen in specimens taken from healthy people. The diagnosis in these cases is essentially a clinical one.

The second type of evidence is based on the isolation in pure culture and identification of the organism concerned. In the case just considered this could be reported not as "acid-fast bacilli seen" but as "*Myco. tuberculosis* present". This is a piece of evidence which is significant by itself without the support of clinical findings and if the patient has no other signs of tuberculosis the presence of the organism still needs an explanation.

Circular Arguments

It is very important that there should be no confusion between the two types of evidence; "acid-fast bacilli seen" is never synonymous with "*Myco. tuberculosis* present".

The first type of evidence is often useful in the diagnosis of infection in individual patients but can never lead to advance in our knowledge of infectious diseases, and unless its limitations are clearly understood its use may lead to much confusion of thought. The clinician says, " I think this is disease D which we know is caused by microbe A. Can this microbe be found ? " The bacteriologist sees a microbe resembling A and says, " The patient has disease D, therefore this must be A." This is an obvious example of a circular argument which is quite invalid. The same type of argument is very common in more subtle forms. For example, a patient may have symptoms suggesting glandular fever and when a Paul-Bunnell test is made a low titre of heterophile agglutinins is present which disappears after absorption. This result is insignificant because it occurs from time to time in normal serum. The bacteriologist may be tempted in borderline cases of this kind to stretch a point in view of the clinical findings and say that in this case the result can be considered to be weakly positive ; if he does so he falls into the trap of a circular argument. The clinical signs are an indication that the test should be repeated later to see if it becomes positive, but they must not be allowed to influence its interpretation. Laboratory tests are often repeated when the results fail to fit the clinical findings but very seldom when they satisfy clinical expectations. It is illogical to use a test for diagnosis of a particular infection and *at the same time* use the clinical findings to gauge its reliability.

Before tests are adopted for routine use they should be extensively tried out on *known* positive and negative material and their value and limitations established. When this has been done the experimental stage is over and it then remains for the bacteriologist to see that the conditions of the test are observed and that proper controls are included. If there is any doubt about its reliability when handled in a routine laboratory, duplicate tests should be set up in *all* cases irrespective of the clinical findings, and if this reveals variation of results which cannot be overcome the test is useless and must be discarded.

Another false argument frequently encountered is, " This colony resembles A which is commonly found in site S. The specimen came from S therefore the microbe must be A." Part of the work of a clinical bacteriologist is to recognize changes in the distribution of the human flora. He must not therefore assume that there are no changes and use this as evidence in the identification of microbes.

Legitimate Use of Clinical Findings

It may now seem that it would be better if the bacteriologist had no knowledge at all of the clinical condition so that he is not in a position to be biased; but information about the patient's clinical state is very valuable if legitimately used. When laying down rules for routine investigation they should be made to cover as far as possible all known eventualities and, no matter what the clinical findings are, nothing should be omitted from this routine. If, however, the patient's condition suggests infection by a certain group of organisms special methods may be employed from the outset, which may make it possible to isolate and identify the bacteria more quickly than by the routine method alone. For example, a wound swab may be sent from a patient suspected of gas-gangrene; the routine method will be followed, but in addition a Nagler plate will be inoculated and if *Cl. welchii* is present it will be possible to identify it by this method next day, whereas if the routine method alone had been used the bacterial diagnosis would not have been possible for 48 hours at the earliest.

Avoidance of False Reasoning

False reasoning along the lines indicated may be avoided either by fully identifying all the microbes found, which is not usually possible in a routine laboratory, or by adopting the following procedure. All bacteria isolated from sites which are normally sterile are assigned to their genus on laboratory evidence alone and if it is useful to proceed further the species is also named. If the laboratory identification has stopped short at the genus this is made plain in the report. For example, a microbe identified by colonial and microscopic morphology and by a positive satellitism test is reported as an " haemophilus type of organism "; if necessary, tests for the utilization of X and V growth factors may be made and the species finally identified, but in most cases this delays the report and serves no useful purpose. Haemolytic streptococci on the other hand must be fully identified and grouped because the result is important both in the prognosis and treatment of the individual case and from the point of view of hospital epidemiology; they are therefore reported by name.

Specimens from sites which have a normal flora are treated somewhat differently. It is rarely necessary to identify all the microbes cultured. The routine procedure is to exclude all known

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pathogens using the best selective methods available and reports are sent which make it clear that this has been done. For example, the report of a faeces culture reads, " No organisms of the Salmonella or dysentery groups isolated." This tells the clinician what he needs to know without giving him any misleading information and is to be preferred to the type of report which states, " Cultures yield *Bact. coli*, *Strept. faecalis* and *Proteus vulgaris*." Naming the species is at first sight impressive, but a full identification of them cannot be made without delay and it misleads the clinician who may be led to believe that those are the only viable bacteria in the specimen. " No pathogens isolated " or " Cultures yield normal flora only " are further common variations ; they assume that we know which of the faecal flora are pathogens under all conditions and are therefore undesirable ; as recent work on infantile diarrhoea has shown, it is hard to say what species can be considered part of the normal faecal flora.

The amount of work necessary for satisfactory identification of the microbes frequently encountered in clinical bacteriology and the way in which different investigations may be reported will be considered later but the guiding principles for avoiding unsound reasoning in routine work are the same in all cases and may be stated thus :

1. The report must be based on laboratory evidence only.
2. All microbes named must be isolated in pure culture and identified by biochemical or serological tests.
3. When identification has proceeded as far as the genus only this must be made clear.
4. If identification falls short of the genus in the examination of cultures from sites with a normal flora, the microbe is considered to be insufficiently important for identification and is not mentioned in the report which concerns known pathogens only.

The Need for Speed

It may appear that the second of these rules is incompatible with speed and that it is more valuable for the experienced bacteriologist to use his " clinical " judgement of the appearance of microbes to send a quick report because, it may be argued, he will only fail to identify atypical strains which are infrequent, and from a practical point of view do not matter, since they are probably of low virulence. Speed in clinical bacteriology is of course very important and it is

perfectly legitimate for the bacteriologist to use his experience of morphological appearances to give the clinician a quick *preliminary* report, which may be very valuable in indicating lines of treatment or the need for isolation; but the investigation must never be permitted to stop at this stage. The idea that strains which appear atypical are comparatively harmless is unsound and epidemics have arisen because such strains have not been recognized. The preliminary report must therefore be checked to avoid this risk and also to ensure that both the clinician and the bacteriologist do not put too much faith in it. The great variation in appearance that occurs among different strains of the same species, and the unreliability of colonial and microscopic morphology as a final test of identity, can only be appreciated if it is checked by bio-chemical and serological methods; atypical strains will continue to be thought rare if this is omitted.

If bacteriological reports based on inadequate tests are entered in the case records much harm may be done. Since the report came from the laboratory it may be regarded by the clinician as accurate in the same way that a chemical estimation is accurate and the records with the bacteriological findings may be included in a survey of cases for research purposes with very misleading results. If the principles of reporting investigations listed above are followed, there is no danger of a misunderstanding about the value of the findings.

"Academic" and "Clinical" bacteriology

Since the discovery of sulphonamides and antibiotics a large proportion of routine work has been devoted to testing bacterial sensitivity to them, to find which is most likely to be successful in treatment. Assay of the drugs in body fluids is sometimes required to find if dosage is sufficient. Much of this work is new and there is still considerable difference of opinion about the value of different antibiotics in different infections and the best methods of giving them. These drugs are capable of causing gross morphological variation in bacteria growing in their presence which makes preliminary identification from colonial and microscopic appearance hopelessly unreliable. It is therefore more important than ever before that routine methods should be based on sound scientific reasoning and a knowledge of the factors which influence bacterial growth. It is sometimes thought that academic and clinical bacteriology are totally different and that the clinical bacteriologist need only

know a few tricks for the rapid identification of pathogens and may safely leave a more fundamental knowledge of bacterial behaviour and host-parasite relationship to his more learned colleague. If the bacteriologists in day-to-day contact with the material from human infections are insufficiently trained or fail to apply conscientiously the scientific method, they will fail to recognize departures from the accepted behaviour of host and parasite. Such observations may not be of great value in the treatment of individual patients but it is on them that the growth of our knowledge of infectious disease depends.

CHAPTER 2

GENERAL PROCEDURE

Delay in the diagnosis of acute infections may have disastrous consequences both for the patient himself, whose chance of recovery diminishes with every hour that the bacteria are allowed to flourish in his tissues, and also for other patients and staff who are thus exposed for an unnecessarily long time to the risk of infection.

The results of investigations of infected material and the speed with which they are obtained depend not only on laboratory methods but also on the manner in which the specimens are taken and the promptness with which they are transmitted to the laboratory. When sampling fluids such as urine and cerebrospinal fluid, which are normally sterile, extreme care must be taken to avoid contamination; greater precautions are necessary than are used in the operation theatre. Aseptic surgical technique prevents contamination of wounds by pathogens but takes little account of airborne saprophytes, many of which fall into the wound during the operation without harm to the patient; but the presence of a single contaminating organism in a culture may completely ruin the investigation. Specimens from sites such as fauces, vagina, or alimentary canal which have a normal flora may be taken with less stringent precautions but extraneous material should be excluded as far as possible.

Pathogens may not be cultivated if there is delay in sending the specimen to the laboratory. Delicate bacteria may die from lack of nutrition, lowered temperature and the action of enzymes. When saprophytes are present, either originally in the specimen, or introduced accidentally during sampling, they may survive and multiply at room temperature before cultures are made, gaining advantage in numbers over the pathogens which are subsequently outgrown.

Collection of Specimens

If reliable results are to be obtained the following rules for taking specimens should be observed:

1. All specimens must be labelled with the patient's name and the date and time of sampling, and must be accompanied by a signed form which states the name and age of the patient, the number of

the ward or department, the nature of the specimen and the site from which it was taken, the clinical diagnosis and duration of the illness, the examination required and the nature of any antibacterial treatment.

2. Specimens for culture must never be in contact with antiseptics or disinfectants. If the site must be cleaned, a dry sterile swab moistened with sterile saline and held in sterile forceps should be used, and the site should be dried before sampling.

3. All specimens for culture must be sent to the laboratory on the day of collection and with as little delay as possible; speed is essential in the following investigations:

(*a*) *N. meningitidis* and *N. gonorrhoeae* are very sensitive to exposure to air in the cold. Cerebrospinal fluid from patients with signs of meningitis must arrive warm and be incubated immediately. Negative results from specimens which have cooled are valueless. Swabs for the culture of *N. gonorrhoeae* should be seeded on warm blood agar at the bedside unless special medium for transport is available (page 272).

(*b*) *Eye swabs.* Lachrymal secretion contains lysozyme, an enzyme which rapidly kills bacteria, therefore swabs should be plated at the bedside.

4. *Urine.*

(*a*) Catheter urine should be taken with strict aseptic precautions into a sterile screw-capped bottle.

(*b*) "Clean urine" is suitable for microscopy and may be cultured if taken skilfully from male patients.

5. *Faeces.* Bedpans for collecting specimens for culture should be sterile to prevent accidental contamination by bacteria from another sample of faeces. A part of the specimen containing any abnormal material such as pus or mucus is placed in a clean screw-capped bottle or waxed carton. A warm specimen freshly passed into a warmed bedpan is necessary for examination for amoebæ. Rectal swabs are taken while the patient "bears down" by passing the swab through the anal canal. They are very satisfactory for the culture of dysentery bacilli provided they arrive in the laboratory while still moist. It is helpful to moisten the swab in sterile peptone water before sampling.

6. *Sputum*, for culture, should be collected into a sterile screw-capped bottle when the patient first wakes in the morning. If there is very little of it, it may be possible to collect a satisfactory sample

by placing the patient comfortably so that his head and shoulders are lower than his chest. If he remains thus for about ten minutes sputum may drain into the trachea and then he will be able to cough it into a conveniently placed container. Specimens for microscopic examination must be taken either into a container which has never previously been used, waxed cartons are suitable, or into one specially treated to destroy any dead acid-fast bacilli still adherent to it from previous specimens. It should be clean and dry ; it need not be sterile.

7. *Serous fluids :* pleural, pericardial synovial, ascitic. Two specimens are sent, one in a sterile screw-capped bottle for culture, the other citrated for a cell count. If culture for *Myco. tuberculosis* is required, a large volume of citrated fluid, about 200 ml. if possible, is sent in a sterile bottle.

8. *Cereobrospinal fluid.* Two samples are sent in sterile bottles, about 3 ml. each, one for culture (see rule 2) the other for cell count and chemical analysis.

9. *Blood.*

(*a*) For culture ; the samples are best taken by a member of the laboratory staff (see page 25).

(*b*) For serology ; although serological tests can be made on unsterile serum, samples should be taken with a dry sterilized syringe and needle and the blood should be delivered into a sterile container. This course avoids all danger of accidentally infecting the patient ; it also prevents lysis of the red cells which makes the serum unsuitable for the tests and is often caused by haemolytic aerobic sporebearers which multiply in the blood at room temperature.

10. *Tissue.* Biopsy or autopsy specimens should be sent in a *dry* sterile container. They must not be allowed to dry, therefore small samples are sent without delay ; saline and water are to some extent bactericidal and should not be added.

11. Whenever possible specimens should be sent early in the day so that there is time to examine them during normal working hours. If an investigation is required which needs immediate attention notice should be given at least an hour before the specimen is taken.

12. All specimens for culture are particularly dangerous to the nurses and laboratory staff who handle them. Leaking containers should be incinerated or autoclaved with their contents and another sample requested. If the examination is continued, not only is there

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