The Pattern of Bacteriological Cultures in a State Laboratory*

by Lim Swee Eng
M.B.B.D., D.C.P., D.Path.
and
Abdul Hamid bin Md. Hussain
A.I.M.H.L.T.
Institute for Medical Research
Perak Branch
Ipoh.

THE PERAK BRANCH OF the Institute for Medical Research (I.M.R.) at Ipoh serves as the Government Clinical Laboratory for all the 13 hospitals in the State of Perak. About a third of the specimens come from the Ipoh General Hospital, about 2 miles away, and the rest from other hospitals in the State, stretching from Parit Buntar in the north to Tanjong Malim in the south, about eighty miles away. I.M.R. Ipoh is also the only place in Perak where bacteriological cultures are done for the hospitals in the State.

The purpose of this paper is to survey the pattern of bacteriological isolates, the sensitivity pattern of the various pathogens and to identify the problems involved in running a bacteriological service for the State with hospitals upon to 80 miles away.

Material and Methods

Information of the various cultures done in 1970 were gathered from the records. Specimens for culture from the various hospitals were received by ambulance, taxi, rail or through the post with considerable delay in some cases.

Urine specimens were sent in sterile plain bottles and a semi-quantitative count (1) of the bacteria content was done using a standard loop delivering approximately 0.004 ml. urine and the growth obtained assessed as to its significance ($>$ 400 colonies) in conjunction with the smear and clinical findings. Swabs were sent in Stuart's transport medium. Blood for culture were sent in liquoid broth, those of suspected typhoid cases in bile salt medium which were kept for 48 hours before innoculation into blood agar and Desocycholate agar (DCA) respectively. Stools of gastroenteritis and suspected typhoid cases were conveyed in selenite encrichment medium and incubated for 24 hours before innoculation into DCA. Stools of "infants" (children below 2 years old) were also innoculated into Blood Agar and Robertson's cooked meat medium besides DCA. Identification of pathogens were by techniques similar to those recommended by Stokes (2). Antibiotic sensitivity was done on lightly stroked plates of Oxoid diagnostic sensitivity test agar using single antibiotic discs. For M. tuberculosis drug sensitivity testing was by the dilution method using H 37 RV as the control organism.

Results

Of the total 11,411 cultures done on various types of specimens, 1,543 or 13.5% were positive for a potential pathogen (see Table 1). Blood and Stool cultures accounted for about 50% of all cultures done but only for about 18% of positive isolations. On the other hand Urine, Pus and Swabs accounted for only 25% of all the cultures done but for 71% of all positive isolates.

Urine Cultures

1,393 cultures (12.2% of all cultures) were done on urine of which 477 (34.2%) were positive (Table 1) or 30.9% of all positive isolates. Of the 916 "negative" urine cultures 309 (22%) were contaminated specimens. From the records, there

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was no significant difference in the pattern of cultures among the various races and between the sexes or in the number of contaminated specimens between males and females. Coliforms were the most frequently isolated organism constituting 62% (297 of 477 positive cultures) of the urine isolates, followed by Staph. aureus (12%), Proteus (8%) and Pseudomonas (7%).

The sensitivity pattern of the various pathogens is shown by Table II. Nearly all of the organisms isolated were sensitive to Gentamycin (Garamycin).

Table I
Distribution of Cultures (1970)

Specimens	Total Number	% Total	Positive Cultures Number	% Specimens	% Positives	Negative Cultures Number	% Specimens	% Negatives
Urine	1393	12.2	477	34.2	30.9	916	65.8	9.3
Pus & Swabs	1471	12.9	620	42.1	40.2	851	57.9	8.6
Throat Swabs & Sputum	1672	14.7	67	4.0	4.4	1506	96.0	16.2
M. tuberculosis	693	6.0	91	13.1	5.9	602	86.9	6.1
Blood	2716	23.8	133	4.9	8.6	2583	95.1	26.2
Stools	3004	26.3	141	4	9.1	2873	95.3	29.1
C.S.F.	462	4.1	14	3.0	0.9	448	97.0	4.5
Total	11411	100%	1543	—	100%	9878	—	100%
% Total	100%	—	13.5%	—	—	86.5%	—	—

Table II
Sensitivity Patterns of 477 Urine Isolates

Antibacterial Drugs	Percentage Sensitive Coliforms (297)	Proteus (39)	Pseudomonas (32)	Staphylococcus (59)	Streptococcus (5)
Penicillin	—	—	—	9	25
Erythromycin	—	—	—	98	75
Cloxacillin	—	—	—	83	60
Ampicillin	56	87	3	88	100
Chloramphenicol	57	69	3	86	60
Streptomycin	42	51	15	59	25
Tetracycline	31	0	3	36	60
Nitrofurantoin	28	3	0	—	—
Sulphatriad	3	0	3	10	0
Cephaloridine	71	72	6	98	100
Septrin	68	46	—	93	—
Neomycin	85	92	81	—	—
Kanamycin	59	88	12	—	—
Polymyxin B	87	15	100	—	—
Gentamycin	100	100	97	100	—
Carbenicillin	—	—	100	—	—

The majority of Coliforms isolated were also sensitive to Polymyxin and Neomycin; Proteus to Neomycin and Kanamycin; Pseudomonas to Polymyxin and Carbenicillin (Pyopen); Staphylococcus to Erythromycin and Cephaloridine (Ceporan) and Streptococcus to Ampicillin (Penbritin) and Cephaloridine. The majority of organisms were resistant to Sulphatriad; Staphylococcus and Streptococcus were resistant also to Penicillin; Coliforms, Proteus and Pseudomonas to Tetracycline and Nitrofurantoin (Furadantin).

Pus and Swab Cultures

This constituted 12.9% of all cultures done (Table I). Of the total 1,471 examinations carried out, 620 (42.1%) were positive; this formed 30.2% of all positive cultures. 52% of pus specimens, 50% ear swabs, 24% vaginal swabs, 17% nasal swabs, 10% eye swabs and 8.3% unbilical swabs were positive. Of the 620 positive isolates, 499 (81%) were from pus specimens. The 851 "negative" cultures included 69 isolates of Staph. albus which were considered as commensuals. Staph. aureus was the most often isolated pathogen constituting 45.5% of all cultures followed by Coliforms (25%), Pseudomonas (14%) and Proteus (13%). Staph. aureus was also the main pathogen isolated from pus specimens and eye swabs.

Table III shows the sensitivty pattern of the isolates. The majority of organisms were again sensitive to Gentamycin and the gram-negative bacilli also to Neomycin. The majority of Coliforms isolated were in addition sensitive to Polymyxin and Kanamycin; Proteus to Septrin (Bactrim), Kanamycin, Cephaloridine and Ampicillin; Pseudomonas to Polymyxin and Carbenicillin; Staphylococcus to Erythromycin, Cephaloridine and Septrin; and Streptococcus to Erythromycin, Chloramphenicol, Penicillin and Tetracycline. The majority of organisms were resistant to Sulphatriad and the gram-negative bacilli also to Tetracycline. The majority of Staphylococcus were penicillin resistant and Pseudomonas were usually resistant to all antibiotics tested except Carbenicillin, Gentamycin, Polymyxin and Neomyxin.

Throat Swabs and Sputum Cultures

1,672 cultures (14.7% of the total cultures done) were performed, of which 67 (4%) were positive for a potential pathogen. 5.6% of throat swabs

Table III

Sensitivity Patterns of 620 Pus and Swabs Isolates

Antibacterial Drugs	Percentage Sensitive				
	Coliforms (153)	Proteus (79)	Pseudomonas (85)	Staphylococcus (282)	Streptococcus (21)
Penicillin	—	—	—	6	86
Erythromycin	—	—	—	98	95
Cloxacillin	—	—	—	96	—
Ampicillin	74	19	2	71	—
Chloramphenicol	60	84	6	89	95
Streptomycin	65	75	29	68	—
Tetracycline	48	6	5	66	86
Sulphatriad	10	0	0	7	—
Cephaloridine	59	89	4	99	—
Septrin	84	95	2	98	—
Neomycin	94	95	94	—	—
Kanamycin	89	86	6	—	—
Polymyxin B	97	11	94	—	—
Gentamycin	99	100	98	100	100
Carbenicillin	—	—	100	—	—

and 13% of sputum grew an organism, the majority being gram-negative bacilli. One case of diphtheria was positive out of 989 cultures done. No Pneumococcus nor Haemophilus was siolated.

All the Staphylococci isolated were resistant to Penicillin and Tetracycline and the majority to Sulphatriad; all were sensitive to Ampicillin Erythromycin, Chloramphenicol, Gentamycin, Cloxacillin and Cephaloridine. All the Streptococci were sensitive to Tetracycline, Erythromycin, Ampicillin and Chloramphenicol. The Pseudomonas were all sensitive to Neomycin, Polymyxin, Carbenicillin and Gentamycin, and the Coliforms to Gentamycin and Polyxin.

Cultures for M. tuberculosis

693 cultures (6% of all cultures) were done, of which 91 (13.1%) were positive. Specimens were of various types, the majority (49%) were sputum, followed by Cerebro-spinal fluid (17%), body fluids (12%), urine (9%), gastric lavage (4%) and pus (4%). Of the 91 positive cultures, most (89%) were sputum cultures. Positive cultures were also obtained from pus (4 cases), C.S.F., body fluids and curettings (2 cases each). 15 Guinea-pig innoculations were also performed, of which 2 were positive (1 each from curettings and knee tissue). 10% of the 602 negative cultures was positive on smear examination, nearly all being sputum specimens. 29% of positive cultures were negative on smear examination, the majority again being sputum specimens. All the positive cultures of C.S.F., pus, body fluids, and curettings were negative on smear examination.

73 cultures were tested for sensitivity against Para-Aminosalicylic Acid, Isoniazid and Streptomycin. There were no growth in 6 cases. The majority (about 69%) were sensitive to the three primary drugs.

Blood Cultures

There were 2,716 blood cultures (23.8% of all cultures done) and 133 (4.9%) were positive. Of the 2,716 cultures, 88% were clot and blood cultures for Salmonella organisms. 5.7% (28 out of 449 cultures) of blood cultures and 2.9% (55 out of 1,945 cultures) of clot cultures for Salmonella were positive. Of the 322 blood cultures, 50 (16%) were positive; the majority of organisms isolated being Staph. aureus or Coliforms. Staph, albus was isolated in 22 instances and these were regarded as contaminants.

All the organisms isolated were sensitive to Gentamycin. All the Staph. aureus were also sensitive to Cephaloridine, Cloxacillin and Septrin but resistant to Penicillin and Sulphatriad. The majority of Staphylococcus were sensitive to Chloramphenicol, Erythromycin and Ampicillin. One case of Streptococcus was resistant to Penicillin and Sulphatriad but sensitive to all the other antibiotics tested. The majority of Coliforms were sensitive to Neomycin and Polymyxin.

Stools Culture

3,004 cultures (26.3% of all cultures done) of stools were performed, of which 141 (4.7%) were positive. Of 750 cultures of "infant" stools (up to 2 years of age), 108 (14.4%) were positive; the majority (96%) being Staph. aureus or pathogenic E. coli in about equal proportions. 54 cases of pathogenic E. coli were isolated, of which 24 (44.4%) were of serological type 0128/B12, 11(20.4%) type 026/B6, 6(11%) type 0125/B15, 4 type 055/B12 and 3 each of types 0126/B16 and 0127/B8. In 467 cultures of gastro-enteritis cases above 2 years, 7 instances of Shigella organisms were isolated. Of 566 requests for Salmonella isolations, 26 (4.6%) were positive. 1,221 cultures of stools were done on pilgrims going to Mecca and all were negative for V. cholera.

The antibiotic sensitivity of 50 cases of Staph. aureus from "infant" stools were tested and the sensitivity pattern was similar to that of Staph. aureus isolated from pus specimens.

C.S.F. Cultures

14 positive isolations (3%) were obtained from a total of 462 cultures done. There were 7 cases of H influenzae, 5 of Pneumococci, 2 of M. tuberculosis and a case of Torulosis.

Frequency of Organisms Isolated

Of the total 1,543 positive cultures, Coliforms were the most commonly isolated, accounting for 569 isolates (36.9%), the majority coming from urine, pus and swabs specimens. Staphylococcus were the next most frequently isolated, comprising 424 isolates (27.5%), mainly from pus and swabs specimens. Pseudomonas was isolated 129 times (8.4%) from urine, pus, swabs and sputum specimens. There were 118 isolates (7.6%) of Proteus from urine, pus and swabs specimens. There were 109 isolations (7.1%) of Salmonella typhi from blood and stools, of which blood specimens accounted for 76% of positive isolates of Salmonella. M. tuberculosis isolates accounted for 6% (93 cases) of all positive cultures.

Discussion

The pattern of bacteriological cultures and isolates are for 1970 for I.M.R. Ipoh. The pattern will be different for different years as requests for

cultures change and techniques of isolation and identification are improved and as difficulties are identified and overcome. The pattern is also unique for I.M.R. Ipoh and is not comparable to a Hospital Laboratory where the laboratory is within the Hospital itself. Ipoh General Hospital is two miles away and bacteriological specimens come from as far as 80 miles away, sometimes involving a delay of up to 24 or even 48 hours. Nevertheless the isolation rate of 13.5% is comparable to that in a General Hospital laboratory in Adelaide (3) where the isolation rate was about 10%.

Blood and Stools specimens accounted for half of all specimens examined (5,720 specimens out of the total 11,411) but for only 18% of positive isolates. This is largely due to the fact that the majority of blood cultures (70%) done were clot cultures for Salmonella organisms from blood specimens sent for the Widal and Weil-Felix test, and that 40% of the stools specimens were from Mecca pilgrims for the screening of V. cholera. On the other hand urine, pus and swabs specimens formed only 25% of all specimens examined but accounted for 71% of pathogens isolated. This is also the pattern in the hospital mentioned in Adelaide (3) where urinary tract and "localised infections" comprised 68% of all isolates. In I.M.R. Ipoh Coliforms formed the majority (62%) of organisms isolated from urine specimens and Staph. aureus formed 46% of positive isolates from pus and swabs specimens. Coliforms and Staph, aureus are the common pathogens in urinary tract infections and localised infections respecitvely, and as they are also easily cultured from the specimens; urine being a good culture medium itself, account for the majority of pathogens isolated. Of the total 1,543 positive cultures from all types of specimens, E. coli formed 37% and S. aureus 28% of all pathogens isolated.

22% (309 out of 1,393) of all ruine cultures were contaminated. This rather high rate of contamination is partly explained by the delay of urine specimens in reaching I.M.R. Ipoh especially when the specimens are from outstation. Contaminated specimens usually yield a mixed growth. In cases of comtamination in which Coliforms predominate and the growth is profuse, extra care has to be taken in interpreting the results, as the long hours of delay enable the Coliforms to multiply several generations to give a "significant growth". Here, the presence of pus cells, the clinical history and repeated positive cultures must be taken into account. A common cause of contamination in urine is the insufficient care taken in its collection. A urine culture is ordered by the ward doctor who usually leaves it to the Staff Nurse to collect the urine who in turn may leave it to the Assistant Nurse or Attendant who makes the patient collect the urine himself without the help of specific instructions on the proper way of doing it.

44% of urine cultures did not yield any growth. In some of these the clinical picture was that of urinary tract infection, with pus cells in the urine. Such cases might have been treated with antibiotics. Often no information is given regarding this. The role of growth inhibitors in urine e.g. vegetable anti-bacterials like paciferrins, leaves and tubers of Arum species and tumeric spices (4) has to be considered as these are consumed by our local population.

Isolations from throat swabs and sputum were not satisfactory. Partly to circumvent the problem of drying and delay in the transportation, swabs were transported in Stuart's transport medium. We have since dispensed with the transport medium and swabs are now conveyed to I.M.R. Ipoh as such. The medium may itself disperse and dilute whatever organisms that may have been present on the swabs in the first place. The presence of detergents on inadequately washed rubber bunds of the screw caps of bottles, the inadequate surface area for culture of Diphtheria organisms on Loeffler's medium in Bijou bottles, the death of organisms due to delay in the transportation of specimens, the chemotheraphy that has already been administered, and improper bacteriological techniques are some of the problems that have been identified, which have contributed to the rather poor results. However, it is also interesting to note that the Hospital in Adelaide had only about 17% positive isolations from sputum specimens and that 31% were Gram-negative organisms (3).

The pattern of M. tuberculosis culture was interesting in that 10% of negative cultures had positive smears. The possibilities of this are non-viable M. tuberculosis present in the specimens, contamination, and acid-fast bacilli other than M. tuberculosis from e.g. the tap. 29% of positive cultures were at first seen to be negative on smear examination. This may be explained by inadequate smear examination or inadequate or non-representative material examined. The moral of this discrepancy would be to submit specimens for culture even though these specimens are microscopically negative. Repeated microscopic examinations should also be the rule.

Of the pathogenic E. coli that were isolated from stools of children up to 2 years old, serotype 0128/B12 was the commonest (44%) followed by 026/B6 (20%). Stools of adults were not tested for pathogenic E. coli. However a recent report

has implicated that certain strains of E. coli e.g. 015: H11 not previously recognised as enteropathogenic can cause acute diarrhoea and that a previously unrecognised serotype 0148:K:H28 is responsible for "travellers' diarrhoea" (5). These serotypes are different from that which cause diarrhoea in infants.

The sensitivity pattern of Urine, Pus and Swabs isolates are as shown in Tables II and III. In general, most organisms isolated were sensitive to Gentamycin and the gram-negative bacilli also to Neomycin. Pseudomonas were usually only sensitive to Polymyxin, Carbenicillin, Gentamycin and Neomycin and resistant to other antibiotics. Most pathogens were resistant to Sulphatriad, Staphylococcus to Penicillin, and the gram-negative bacilli to Tetracycline. Sulphonamide sensitivity testing is affected by "inhibitors" present in many laboratory media unless neutralised by lsyed horse blood (6). Oxoid Diagnostic Sensitivity Test Agar, which is used in I.M.R. Ipoh, does not contain inhibitors of Sulphonamides and other antibiotics.

As the frequency of organisms isolated depends on the type of specimens examined and as different organisms vary in their sensitivity to different antibiotics, it is essential that the causative pathogen be isolated, identified and its sensitivity determined. The principles of rational chemotherapy are adequately discussed in Garrod's book on Antibiotic and Chemotherapy (&). Suffice to say that when specimens e.g. urine, pus, ear and vaginal swabs are known to yield a variety of organisms, both gram-positive and gram-negative, it is important that culture and sensitivity be done, especially when infection by Pseudomonas is a possibility as they are usually sensitive to only certain antibiotics.

The present situation where I.M.R. Ipoh is the only place in Perak where bacteriological cultures can be done for all the hospitals in the State is naturally not ideal for a good bacteriological service in the State. The incidence of contaminated specimens and the frequency of isolates can be improved with hospital laboratories doing their own bacteriological cultures and where there is minimum delay in culturing the specimens obtained. The situation can be further improved with the services of a Bacteriologist in the State and with "refresher courses" for the Laboratory Technologists concerned.

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