

ETIOLOGY AND MANAGEMENT OF DIARRHOEAL DISEASES IN KARACHI

Pages with reference to book, From 211 To 213

Saleem Hafiz, Umaira Rauf, Birjees Qadr (Institute of Urology and Transplantation, Civil Hospital, Karachi.)

Yasmin Syed (Dr. Ziauddin Hospital Laboratory, Karachi.)

M. Shahabuddin (Department of Microbiology, University of Karachi, Karachi.)

ABSTRACT

The incidence of diarrhoeal disease are very high in our population and bacterial etiology amounts to 43.55% of all cases. Pathogen such as *Aeromonas hydrophila*, *Plesiomonas shigelloides*, *Yersinia enterocolitica* and *Campylobacter* are also present in significant numbers, they make up 50% of bacterial causes. All enteric pathogens can be easily isolated on commonly used media and could be precisely identified by simple biochemical tests. (JPMA 41: 211, 1991).

INTRODUCTION

Most diarrhoeal states are self-limiting and pose no special problem; they are often due to dietary indiscretions or mild gastro-intestinal infections. In Pakistan diarrhoeal disease is the commonest ailment which is encountered almost by every individual atleast once a year. Many infections of the gastrointestinal tract are spread by faecally contaminated water which is used for drinking, washing or cleaning teeth. The part of the gastrointestinal tract that invariably harbours microorganisms¹ is the large intestine, although faecal organisms can be recovered. It seems almost superfluous to list the organisms which can be encountered, many of them are ignored in search of enteric pathogens specially salmonellae and shigellae, which certainly donot represent the only microbes endowed with the potential of disease production. All members of the genus salmonella are capable of evoking the various clinical symptoms of salmonellosis and its complications. The various shigella can cause diarrhoea or the syndrome known as bacillary dysentery. *Vibrio cholerae* and related disease are encountered increasingly in many parts of the world. Enterotoxigenic and enteroinvasive *E. coli* are known to cause damage to the human intestine. Localized pathology of the large intestine may involve, *Aeromonas* spp., *Plesiomonas* species or *Yersinia enterocolitica*, the latter causing not only occasional gastroenteritis but also the symptoms of appendicitis. Haemorrhagic colitis is a recently recognized enteric infection due to *E. coli* strains of a specific serotype, 0157-H7. These strains cause a severe diarrhoea characterized by grossly bloody stools². Over the past 20 years, *Y. enterocolitica* has been clearly demonstrated to be a significant cause of gastroenteritis³. *Campylobacter jejuni* (*Helicobacter jejuni*) one of the recent additions to the list of enteric pathogen, appears to be by far the most important species from the standpoint of human disease. In humans the disease usually occurs as diarrhoea sometimes with blood in the stool; abdominal pain, fever, nausea and sometimes vomiting. *Aeromonas* spp. are widely distributed in stagnant and flowing fresh water, in salt waters which interface with fresh water and in sewage^{4,5}. *Aeromonas* spp. cause acute diarrhoeal disease of short duration, sometimes bloody or choleraform, occurring worldwiv and affecting any age⁶. *Plesiomonas Shigelloides* strains have been isolated from stool of diarrhoeic patients⁶.

It is a common practice in our local laboratories to culture stool of diarrhoeic patient and only look for *Salmonella*, *Shigella* and rarely enteropathogenic *E.coli*, hence, a great majority of diarrhoeal cases go undiagnosed. We embarked upon a study to workout simple practicable methods which would give a true spectrum of pathogens causing diarrhoea.

MATERIAL AND METHODS

Specimens

2000 stools samples received in the laboratory from patients of gastrointestinal symptoms were cultured for the presence of enteric pathogens including Salmonella sp., Shigella sp., enteropathogenic E.coli, Aeromonas species, Plesiomonas, Yersinia sp., Campylobacter and Vibrio cholerae.

Culture

Stool samples were cultured on to MacConkey's medium (OXOID) T.C.B.S. Cholera medium and Skirrow's⁷. Campylobacter medium. The inoculated plates were incubated as follows;

MacConkeys

Plates were incubated aerobically at 35-37°C for 24 hours, colonies were examined and processed to that given in Figure.

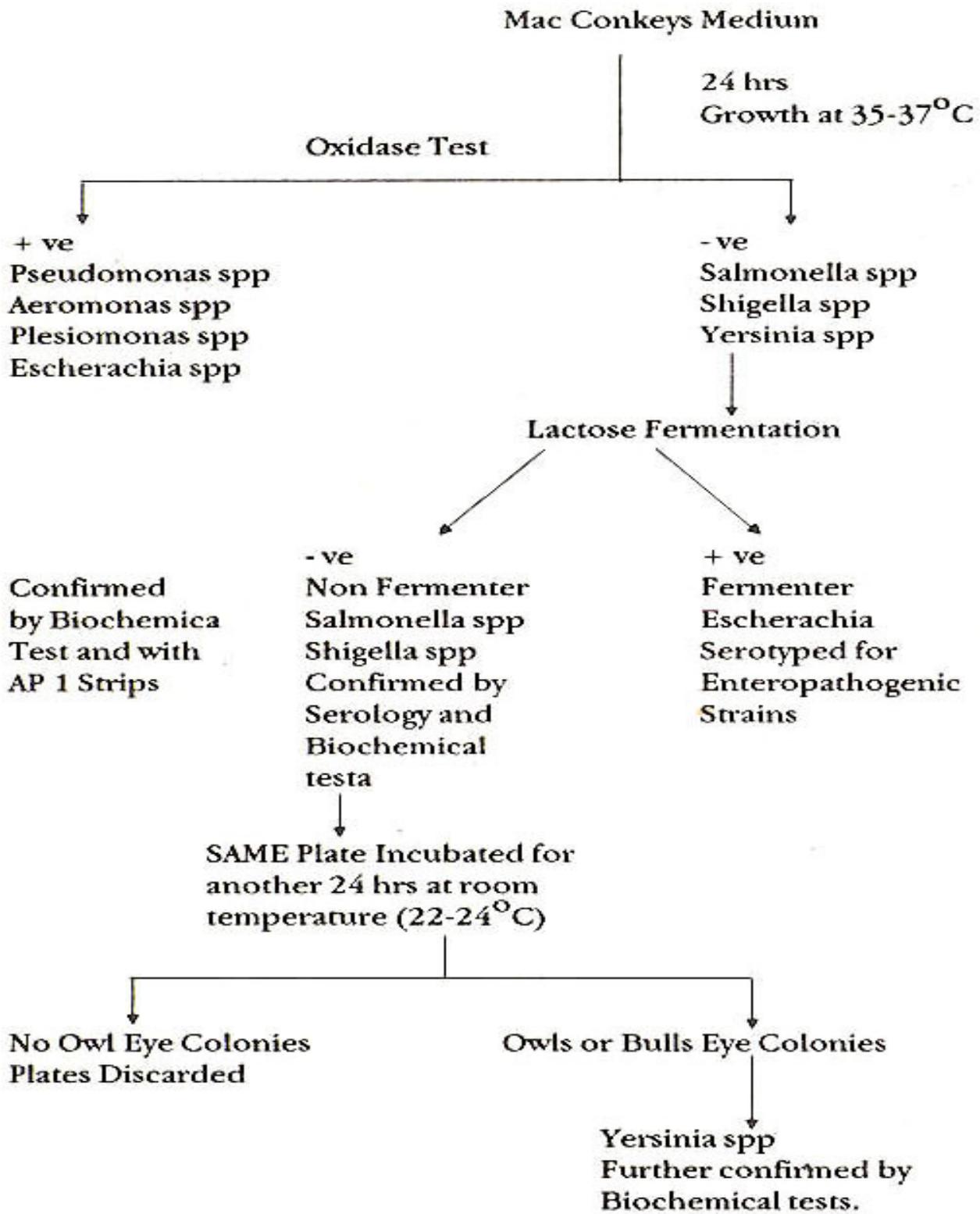


Figure. Processing growth on Macconkeys medium.

T.C.B.S. Plates:

Incubated aerobically at 35-37°C looked for the presence of typical vibrio colonies. Suspected colonies confirmed by biochemical and serological tests.

Campylobacter Medium Plates

Plates incubated at 42° to 43°C in a candle jar (5-10%CO₂) and plates examined after 24, 48 and 72 hours. Suspected colonies were confirmed by Gram stain, oxidase and biochemical tests.

Sensitivity test:

Sensitivity test was carried out by disc diffusion methods on oxoid sensitest agar.

Biochemical identification:

The isolates were inoculated in API enterobacteriaceae strips and the results compared with biochemical tests carried out in Triple Sugar Iron medium (T.S.I), Lysine Indole Motility (LIM) medium, citrate and urease medium alongwith catalase and oxidase test.

Results

TABLE I. Biochemical reactions the reactions of enterobacteriaceae & vibrio after 18-24 hours of incubaton at 35 °C.

| Organisms | T.S.I. | -AGAR | Gas | H ₂ S | Lim-Medium | | | Citrate | Urease | Catalase | Oxidase |
|--------------------------------|--------|-------|-------|------------------|------------|--------|----------|---------|----------|----------|---------|
| | Slant | Butt | | | Lysine | Indole | Motility | | | | |
| Escherichia | A(K) | A | + (-) | - | + (-) | + | + (-) | - | - | + | - |
| Shigella | K | A | - | - | - | d | - | - | - | Few(+) | - |
| Edwardsiella | K | A | + | + | + | + | + | - | - | + | - |
| Salmonella Typhi | K | A | - | + (-) | + | - | + | - | - | + | - |
| Salmonella Paratyphi (A) | K | A | + | - | + | - | + | - | - | + | - |
| Other Salmonella (sal.para(B)) | K | A | + | + (-) | + (-) | - | + (-) | d | - | + | - |
| Citrobacter | K(A) | A | + | d | - | d | + | + | d | + | - |
| Klebsiella | A | A | + | - | + | - | - | + | + | + | - |
| Enterobacter | A | A | + | - | d | - | + | + | + | + | - |
| Hafnia | K | A | + | - | + | - | + | - | - | + | - |
| Serratia | K/A | a | d | - | + | - | + | + | d | + | - |
| Proteus | K/A | A | d | + (-) | - | d | + | d | + | + | - |
| Morganella | K | A | d | - | - | + | + | + | + | + | - |
| Vibrio Parahaemolyticus | A | A | - | - | + | + | + | + | - | + | + |
| Pseudomonas Aeruginosa | K | A | - | - | + | + | + | - | - | + | + |
| Aeromonas Hydrophila | A | A | d | - | - | + | + | + | - | + | + |
| Plesiomonas Shigelloides | A | A | - | - | + | + | + | - | - | + | + |
| Campylobacter | | | | | | | + | - | + | d | + |
| Acinetobacter | A | A | - | - | - | - | - | - | - | + | - |
| Yersinia Enterocolitica | A | A | - | - | - | d | +* | - | (+)-Late | + | - |

Symbols
A = Acidic
K = Alkaline
+ Positive
() Occasional reactions
* Positive at 25°C

Table 1 summarizes the biochemical reactions of the total of 871 cultures which included Salmonella typhi (86) Salmonella paratyphi A (102), Sal. paratyphi B(48), enteropathogenic E.coli (207), Shigella sp (40), Aeromonas hydrophila (154), Plesiomonas shigelloides (175), Yersinia enterocolitica (43), Vibrio cholerae (4) and Campylobacter jejuni (12). Other bacteria normally isolated from faeces such as Citrohacter sp, Klebsiella sp, Edwardsiella, enterobacter hafnia, Serratia sp, Proteus sp, Morganella, vibrio para haemolyticus. A cinetobacter and pseudomonas aeruginosa were obtained as standard cultures from National type collection (NCTC) England and inoculated in the locally made TSI, LIM,citrate, and urease medium all of OXOID, The clinical isolates were also confirmed by Apl and serological tests. The reactions summarized in the table werefound to be reliable and reproducible and in agreement with the APJ and serological tests.

TABLE II. Breakdown of 200 Stool samples.

| Species Isolated | Total Isolates | % Isolates |
|---|----------------|--------------|
| Salmonella spp | 236 | 11.8 |
| Salmonella typhi | 86 | |
| Paratyphi A | 102 | |
| Paratyphi B | 48 | |
| Enteropathogenic E. coli | 207 | 10.58 |
| Shigella spp (Sh. Sonnei, Sh. dysenteriae and Sh. flexanari) | 40 | 2.0 |
| Aeromonas hydrophila | 154 | 7.7 |
| Plesiomonas shigelloides | 175 | 8.75 |
| Yersinia enterocolitica | 43 | 2.15 |
| Vibrio chlorae | 4 | 0.2 |
| Campylobacter jejuni | 12 | 0.6 |
| Total isolates | 871 | 43.55 |

Table II lists the isolates from 2000 stool samples yield of positive culture being 43.55%, of these only 24% of total were conventional pathogens i.e., Salmonella, shigella and Enteropathogenic E.coli. While almost equal number (20%) were found to be other bacterial enteric pathogens which are normally not reported and which includes Aeromonas hydrophila, Plesiomonas shigelbides and Yersinia enterocolitica.

TABLE III. Sensitivity pattern of enteric Isolates in %.

| Species | Ampicilin | Augmentin | Septan | Chloramphenicol | Ofloxacin | Ceftriaxone | Velosef | Carbenicilin | Tetracycline | Fosfomycin | Cefotaxime |
|--------------|--------------|--------------|--------------|-----------------|--------------|-------------|--------------|--------------|--------------|--------------|--------------|
| Salmonella | 60 | 73 | 53 | 80 | 99 | 98 | 96 | 90 | 87 | 92 | 92 |
| E.P.E.C. | 93 | 97 | 87 | 91 | 100 | 100 | 98 | 95 | 82 | 97 | 96 |
| Shigella | 68 | 73 | 54 | 83 | 100 | 100 | 83 | 83 | 78 | 80 | 92 |
| Aeromonas | Nil | 33 | 60 | 93 | 100 | 100 | 78 | Nil | 86 | 96 | 85 |
| Plesiomonas | 50 | 70 | 70 | 95 | 100 | 100 | 58 | 90 | 70 | 76 | 80 |
| Yersinia | 40 | 48 | 20 | 80 | 100 | 100 | 80 | 92 | 67 | 80 | 80 |
| Other | 50 | 72 | 45 | 80 | 100 | 100 | 80 | 92 | 80 | 96 | 88 |
| Total | 51.57 | 66.57 | 55.57 | 86 | 99.85 | 99.7 | 81.85 | 77.42 | 78.57 | 88.14 | 87.57 |

Table III gives the sensitivity pattern of all the enteric pathogens and it appears that the sensitivity varies with individual pathogen. On one hand enteropathogenic E.coli are sensitive to the commonly used antibiotics while there is increasing resistance among other enteric pathogen to the commonly used antibiotics.

DISCUSSION

The study clearly illustrates that diarrhoeal illnesses are very common in Karachi and bacterial etiology amounts to 43% of the total cases, apart from the conventional pathogens such as Salmonella, Shigella and Enteropathogenic E. coli. There is also the existence of other recently established pathogen such as Aeromonas hydrophila, Plesiomonas shigelloides, Yersinia enterocolitica, Vibrio cholerae and Campylobacter jejuni. The isolation of the pathogen is not difficult and certainly not a very costly affair, it can be achieved easily and is reproducible, simpler methods can be used. The antibiotic sensitivity pattern is quite alarming as the commonly used antibiotics seem to be losing their effectiveness while the recently introduced antibiotics are atleast for the present very effective although these antibiotics have certain limitations and it is better to be cautious in using them, their usage should be controlled.

REFERENCES

1. Rosebury, T. Microorganisms indigenous to man. New York, McGraw- Hill, 1962.
2. Riley, L. W., Remis, R. S., Helgerson, S. D., McGee, H. B., Wells, J. G., Davis, B. R., Herbert, R. C., Olcott, F. S., Johnson, L.M., Hargrett, N. T., Colitis Blake, P. A. and Cohen, M. L. Hemorrhagic associated with a rare *Escherichia coli* serotype. N. Engl. J. Med., 1983; 308: 681.
3. Weisfeld, A. S. *Yersinia enterocolitica*. *Ann. Microbiol. Newsi.*, 1981; 3:91.
4. Hazen, T. C., Filiermans, C. B., Hirsch, R. P. and Esch, G. W. Prevalence and distribution of *Aeromonas hydrophila* in the United States. *Appl. Environ. Microbiol.*, 1978; 36:731.
5. Schubert, R. H. Des vorkommen der Aeromonaden in oberirdischen Gewässern, *Gewässern. Arch. Hyg. Bakf.*, 1967; 150: 688.
6. Pitarangsi C., Echseverria, It., Whitmire, P., Tirapat, C., Formal, S., Dammin, C. J. and Tingtalapong, M. Enteropathogenicity of *Aeromonas hydrophila* and *Plesiomonas shigelloides* prevalence among individual with and without diarrhoea in Thailand. *Infect. Immun.*, 1982; 35: 666.
7. Skirrow, M. B. *Campylobacter enteritis: a 'new' disease.* *Br. Med. J.*, 1977; 2:9.