A STUDY OF SELECTED INTESTINAL BACTERIA FROM ADULT PILGRIMS

M. I. Huq
G. Kibryia

INTERNATIONAL CENTRE FOR
DIARRHOEAL DISEASE RESEARCH, BANGLADESH
Dacca, Bangladesh
August, 1978
(Reprinted February, 1980)

Scientific Report No. 15

A STUDY OF SELECTED INTESTINAL BACTERIA FROM ADULT PILGRIMS

M. I. Huq[1]
G. Kibryia[2]

CHOLERA RESEARCH LABORATORY
G.P.O. Box 128, Dacca - 2
Bangladesh

1/ Head, Microbiology Branch
2/ Senior Research Assistant, Microbiology Branch

PREFACE

The Cholera Research Laboratory (CRL) operates under a bilateral project agreement between the governments of Bangladesh and the United States of America. Research activities of CRL center on the interrelationships between diarrheal disease, nutrition, fertility and their environmental determinants. CRL issues two types of papers: scientific reports and working papers which demonstrate the type of research activity currently in progress at CRL. The views expressed in these papers are those of authors and do not necessarily represent views of Cholera Research Laboratory. They should not be quoted without the permission of the authors.

ABSTRACT

Increased interest on the normal intestinal flora in human population has led to many studies in recent years, various reports have also been published on the enumeration of bacterial flora in the gut during acute and convalescent stages of the disease. In this study qualitative enumeration of bacterial flora has been made on the normal healthy adult population of Bangladesh. Out of 850 people swabbed 560 (65.9%) showed pure growth of E.coli and 290 (34.1%) showed non lactose fermenting colonies along with others. Of these 290, 18 (5.88%) showed Shigella sp. and (0.7%) showed Sal typhi. Enteropathogenic E.coli was isolated from 1.6% of the samples.

INTRODUCTION

In recent years there has been an ever increasing interest in the normal human enteric flora. The development of both broad spectrum antibiotics and practical methods of maintaining animals in a germ free environment has stimulated renewed interest in the significance of bacteria commonly found in the intestinal tract. Recent investigations of the role of intestinal bacteria in nutrition, resistance to infection, shock etc. suggests that these micro-organisms constitute an important part of the host's environments. Various reports have been published on the enumeration of microbial intestinal flora in acute diarrheal diseases and in normal healthy individuals. (Pierce, N.F. et al. (1) and Gorbach S.H. et al. (2-3)). It is the purpose of this paper to report the results of some studies of bacterial population of normal Bengali adults. The population studied represents a sample of individuals coming from most of the thana head-quarters of the nineteen districts of Bangladesh. All had their rectal swab cultures done before their departure for Saudi Arabia for performing pilgrimage. All were normal adults in the age group of 40-60 years.

Though the main objective of culturing was to detect vibrios in their stool, a section of the people were cultured to enumerate the other bacterial flora (aerobic or anaerobic, pathogenic or non-pathogenic) present in the gut.

MATERIALS AND METHODS

Two groups of people are included in the study. The first group were 550 pilgrims who reported at the Haji camp at Chittagong in four different batches before their journey by sea to Saudi Arabia. The rectal swab was taken 1-3 days arter they showed up at the camp. This group had complete gram negative aerobic bacteriology.

Microbiological techniques: Two rectal swabs were taken from each individual. For the isolation of vibrios, the potassium tellurite impregnated pink swab was streaked onto Gelatin Agar, GA (4), Taurocholate Tellurite Gelatin Agar, TTGA (5) and dipped into Bile peptone enrichment media, TTP (6) in tubes of 5 ml. For the isolation of all gram negative members of family entero-bacteriacae the second plain cotton swab was streaked onto MacConkey and SS Agar, and the swab enriched into Selenite

F broth. All the plates and enrichment media were then sent to the main Laboratory at Dacca by Air where detailed bacteriological examination were carried out.

The second group included three hundred pilgrims travelling to Saudi Arabia by Air. These people were selected randomly from a total of 1,540 pilgrims reporting in 12 batches to the Haji camp 5 to 6 days before their departure. They were cultured at the main laboratory at Dacca and on these culture complete aerobic and anaerobic, gram positive and gram negative bacteriological investigations were performed. From this group of pilgrims three swabs were taken, one tellurite impregnated cotton swab and two white plain cotton swab. Like the first group the tellurite impregnated swab were streaked onto Gelatin Agar and Taurocholate Tellurite Gelatin Agar and enriched into Bile Peptone Media. One of the white swabs inoculated onto MacConkey and SS Agar plates and enriched onto Selenite F broth. The second white swab was inoculated onto two plain Blood Agar plate and one Neomycin Blood Agar plate (Blood Agar containing 40 mcg. Neomycin Sulphate per ml.). The Neomycin blood agar and one of the plain blood agar plate was immediately put into an anaerobic jar. After ensuring complete vacuum in the jar, hydrogen gas passed into the jar to make it complete anaerobic. A control plate of a Psendomonas sp. was inoculated into each jar. All the anaerobic isolates were streaked onto blood agar plates and incubated at 35°C aerobically to see whether they were strict or facultative anaerobes.

All the other plates and the enrichment broth incubated aerobically at 35°C. The TTP of enrichment broth for cholera was subcultured after 6 hours incubation onto GA and TTGA and the Selenite broth subcultured onto MacConkey and SS Agar. All the plates are read after 18-24 hrs. incubation. Colonies suspected to be vibrios from GA and TTGA plates and the non lactose fermenting colonies from MacConkey and SS Agar are picked and inoculated onto Kligler Iron Agar, (KIA), Motility Indole Urea (MIU) combined media and citrate media. Depending on the biochemical reactions suspected colonies were serologically confirmed by agglutination with respective antisera. Where necessary a complete set of biochemical tests were done to identify the organisms.

Sensitivity pattern of the pathogenic organisms isolated from these pilgrims were tested by routine Kirby Bauer method. Standard growth of the organisms were inoculated onto Muller Hinton medium and the BBL antibiotic discs containing certain concentration of antibiotic placed on the plates at certain distances. The zone size of inhibition if any, was measured and compared with the control.

It is worth mentioning here that excepting only 300 of the pilgrims who were cultured for both anaerobic and aerobic organisms (both Gram positive and Gram negative) all the 850 pilgrims were cultured for aerobic Gram negative organisms.

RESULTS

Table 1 shows the results from the 850 pilgrims examined for Gram negative organisms. Out of 850 samples 560 (65.9%) had pure growth of Escherichia coli only while the remainder had Klebsiella and non-lactose fermenting organisms as well.

TABLE 1

NUMBER AND PERCENTAGE OF GRAM NEGATIVE ORGANISMS ISOLATED (850 SAMPLES)

	Number	Percentage
No. of rectal swabs studied for Aerobic gram negative organism	850	100
No. of rectal swabs showing pure growth of Escherichia coli	560	65.9
No. of rectal swabs showing E.coli Klebsiella and Non Lactose Fermenter	290	34.1

Table 2 shows the number and percentage of non-pathogenic organisms isolated from the non-lactose fermenting colonies picked from cultures of 290 pilgrims. It may be seen that a large percentage (75.2) of E.coli fermented lactose when inoculated into Kligler Iron Agar within 24 hours of incubation through these E.coli showed non-lactose fermenting colonies on the isolation MacConkey plates.

Table 3 shows the pathogenic organism isolated from the 290 pilgrims who showed non-lactose fermenting colonies in their culture. It may be mentioned here that this figure represents the isolation from the total number of pilgrims (850).

TABLE 2

SHOWS THE NON PATHOGENS ISOLATED FROM NON LACTOSE FERMENTING COLONIES STUDIED (290 SAMPLES)

Name of Organism	No. of Isolations	Percentage of Isolations
Citrobacter	4	1.4
Enterobacter cloacae	10	3.4
Enterobacter aerogenecs	1	0.34
Proteus	30	10.20
Providencia	3	1.00
Serratia	1	0.34
Chromobacter	1	0.34
Esch.coli (late lactose fermenter)	208	71.8
Klebsiella (late lactose fermenter)	4	1.4
Serotype 1831	3	1.0
Pseudomonas	3	1.0
Plesiomonas	10	3.4
Alkalescence Dispar (A.D.) group	10	3.4

Table 3 (A) shows the serotypes of pathogenic E.coli isolated from the cultures of 850 pilgrims which had E.coli in the culture. It may be mentioned here that we tested 12 WRAIR (Water Reed Army Institute of Research, U.S.A.) Enteropathogenic E.coli antisera and from each plate 5 colonies were tested for agglutination with 12 antisera.

TABLE 3

NUMBER AND PERCENTAGE OF PATHOGENS ISOLATED FROM NON LACTOSE FERMENTING COLONIES STUDIED (290 SAMPLES FROM 850 PILGRIMS)

Name of Organism	Number of Isolations	Percentage of Isolations	Overall Percentage
Shigella shiga	3	1.03	0.35
Shigella flexneri	12	4.12	1.40
Shigella boydii	1	0.34	0.12
Shigella sonnei	2	0.7	0.24
Salmonella typhi	2	0.7	0.24

TABLE 3 (A)

SHOWS THE SEROTYPES OF PATHOGENIC E.COLI ISOLATED FROM (850 SAMPLES)

Serotype	Number of Isolations	Percentage of Esch. coli Isolated
O127/B8	5	
O26/B6	3	1.6%
O126/B16	4	
O55/B5	2	

Table 4 shows the number and percentage of gram positive organisms isolated from 300 pilgrims. The anerobic organisms are shown within bracket which are included in the total number of isolation.

Table 5 shows the antibiotic sensitivity pattern of the pathogens isolated from this group of pilgrims.

TABLE 4

SHOWS THE GRAM POSITIVE ORGANISMS ISOLATED FROM 300 RECTAL SWABS STUDIED

Name of Organism	No. of Isolations	Percentage of Isolations
Alpha-haemolytic streptococci	175(69)	58(23)
Beta-haemolytic streptococci	101	34
Staphylococcus albus	100	33
Staphylococcus aureus	30	10
Clostridium	(56)	19

Figures with bracket indicated anerobic isolations.

TABLE 5

ANTIBIOTIC SENSITIVITY PATTERN OF THE PATHOGENS ISOLATED

Name of the Organism	No. of Isolation	Sensitive						
		TE	AM	C	ST	K	SXT	FX
Sh. shiga	3	1	3	3	0	3	3	3
Sh. flexneri	12	7	12	12	0	12	12	12
Sh. boydii	1	1	1	1	0	1	1	1
Sh. sonnei	2	1	2	2	0	2	2	2
Pathogenic E.coli	14	9	12	14	0	14	14	14

DISCUSSION

The requirement of rectal swab cultures of Hajis gave us an unique opportunity to study a broad cross section of normal adult Bangladeshi's representing every segments of all the districts of the country. All these people were to be cultured for vibrios before their departure for pilgrimage. It may be seen that no vibrios have been isolated from any of the cultures. This may be due to two main reasons; either vibrios were completely absent in the community at that time or most of these pilgrims might have got prophylactic dose of tetracycline before they gave the rectal swab though everybody denied taking any medicines.

The findings of other pathogenic and non-pathogenic organisms are interesting. Among pathogenic organisms 2% of the samples harboured Shigella as against 70-80% found from cases reporting to the hospital. Salmonella typhi showed in only 0.24%. No other Salmonella serotypes were isolated from this group. Isolation of this small percentage of Salmonella leads us to two main conclusions; either we are to use more better enrichment media than Selenite F broth only or the incidence of Salmonella in non-clinical adult apparently healthy individuals are really less as against in many other developed countries of the world like U.K., U.S.A. where the incidence is higher.

Enteropathogenic E.coli appeared in only 1.6%. We had the limitation of using only 12 sera for E.coli. Obtained from Walter Reed Army Institute of Research. If more type specific antisera would be available we are sure the percentage of isolation would be higher than reported. One interesting group of organisms were isolated in this study along with non lactose fermenters. They resembled Shigella biochemically but did not agglutinate. Although their pathogenecity is not yet known, they have been described as degraded serotype 1831. The scattered percentage of other organisms seems to have been of no significant interest.

The appearance of tetracycline resistant Shigella Shiga and flexneri in the normal individuals are interesting. The complete resistance of all Shigella isolates to Streptomycin is also of interest.

Considering the world wide interest in the study of human enteric flora, this study may serve as a base line data for normal individuals of Bangladesh.

REFERENCES

1. Pierce, N.F., Banwell, J.G., Gorbach, S.L., Mitra, R.C., Mondal, A. and Manji, P.M. (1969). Bacteriological studies of convalescent carriers of cholera vibrios. IJMR 57, 706-12.

2. Gorbach, S.L., Neale, G., Levitan, R. and Hepner, G.W. (1970). Alterations in human intestinal microflora during experimental diarrhoea. Gut 11, 1-6.

3. Gorbach, S.L., Plant, A.G., Nahas, L., Weinstein, L., Spanknevel, G. and Levitan, R. (1970). Studies of intestinal microflora, Microorganisms of small intestinal and their relation to oral and faecal flora. Gastroenterology 53, No. 6. 856-867.

4. Smith, H., Land Goodner, K. (1958). Detection of Bacterial Gelatinases by Gelatin Agar plate methods. J. Bact. 76, 662-663.

5. Monsur, K.A. 1961. A highly selective Gelatin Taurocholate Tellurite medium for isolation of V. cholerae. Trans. Roy. Soc. Trop. Med. & Hyg. 55, 440-445.

6. Monsur, K.A. 1963. Bacteriological diagnosis of cholera under field conditions. Bull W.H.O. 28, 387-389.

CRL publications can be obtained from Publications Unit, Cholera Research Laboratory, G.P.O. Box 128, Dacca - 2, Bangladesh.

List of current publications available:

A. CRL Annual Report 1976

CRL Annual Report 1977

B. Working Paper:

No. 1. The influence of drinking tubewell water on diarrhea rates in Matlab Thana, Bangladesh by George T. Curlin, K.M.A. Aziz and M.R. Khan.

No. 2. Water and the transmission of El Tor cholera in rural Bangladesh by James M. Hughes, John M. Boyce, Richard J. Levine, Moslemuddin Khan and George T. Curlin.

No. 3. Recent trends in fertility and mortality in rural Bangladesh 1966-1975 by A.K.M. Alauddin Chowdhury, George T. Curlin.

No. 4. Assessment of the Matlab Contraceptive Distribution Project - implications for program strategy by T. Osteria, Makhlisur Rahman, R. Langsten, Atiqur R. Khan, Douglas H. Huber, W. Henry Mosley.

No. 5. A study of the field worker performance in the Matlab Contraceptive Distribution Project by Makhlisur Rahman, T. Osteria, J. Chakraborty, Douglas Huber and W. Henry Mosley.

C. Scientific Report:

No. 1. Double round survey on pregnancy and estimate of traditional fertility rates by A.K.M. Alauddin Chowdhury.

No. 2. Pattern of medical care for diarrheal patients in Dacca urban area by Moslemuddin Khan, George T. Curlin and Md. Shahidullah.

No. 3. The effects of nutrition on natural fertility by W. Henry Mosley.

No. 4. Early childhood survivorship related to the subsequent inter-pregnancy interval and outcome of the subsequent pregnancy by Ingrid Swenson.

No. 5. Household distribution of contraceptives in Bangladesh-the rural experience by Atiqur R. Khan, Douglas H. Huber and Makhlisur Rahman.

No. 6. The role of water supply in improving health in poor countries (with special reference to Bangladesh) by John Briscoe.

No. 7. Urban cholera study, 1974 and 1975, Dacca by Moslemuddin Khan, George T. Curlin.

No. 8. Immunological aspects of a cholera toxoid field trial in Bangladesh by George T. Curlin, Richard J. Levine, Ansaruddin Ahmed, K.M.A. Aziz, A.S.M. Mizanur Rahman, Willard F. Verwey.

No. 9. Demographic Surveillance System - Matlab. Volume One. Methods and procedures.

No. 10. Demographic Surveillance System - Matlab. Volume Two. Census, 1974 by Lado T. Ruzicka, A.K.M. Alauddin Chowdhury.

No. 11. Demographic Surveillance System - Matlab. Volume Three. Vital events and migration, 1974 by Lado T. Ruzicka, A.K.M. Alauddin Chowdhury.

No. 12. Demographic Surveillance System - Matlab. Volume Four. Vital events and migration, 1975 by Lado T. Ruzicka, A.K.M. Alauddin Chowdhury.

No. 13. Demographic Surveillance System - Matlab. Volume Five. Vital events, migration, and marriages - 1976 by Lado T. Ruzicka, A.K.M. Alauddin Chowdhury.

No. 14. Ten years review of the age and sex of cholera patients by Moslemuddin Khan, A.K.M. Jamiul Alam and A.S.M. Mizanur Rahman.

D. Special Reprint:

Management of cholera and other acute diarrhoeas in adults and children - World Health Organization.