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# BACTERIA IDENTIFIED IN DIARRHEAL STOOLS OF EARLY CHILDHOOD AND SENSITIVITY TESTS, METRO MANILA (1980-1982)

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## Introduction

Despite remarkable advances in medicine and public health, diarrheal diseases continue to be a major cause of morbidity and mortality in childhood throughout the world, but particularly in developing countries. In infancy, diarrhea is estimated to cause higher mortality than all other infections combined. WHO Assistant Director General, WW Furth, recently stated that diarrheal diseases accounted for five million deaths annually in children below the age of five years in the developing countries.

In the Philippines, the latest health statistics show that among the ten leading causes of infant mortality gastroenteritis and colitis was second in the list, resulting in 12,800 deaths under 5 years (1978).

The consensus is that for years to come, diarrheal diseases will remain a scourg of populations in developing countries. In view of this, there has been increasing interest in and concern for diarrheas. The World Health Organization (WHO has led in undertaking studies to provide measures to control and prevent these disorders. Researches on different aspects of diarrheal diseases have resulted in increased knowledge and information.

The causes of diarrhea in childhood are varied and may range from a trivial dietary indiscretion to severe or intractable conditions such as cholera or malabsorption syndromes. It is reported that about ten years ago or so, the etiology of diarrheas could be determined in only 20% to 30% of cases. Recent researches have however made it possible to identify the etiologic agent in 70-80% of diarrhea cases.

About the same period as the present report, also in Metro Manila, viral studies of stool specimens in gastroenteritis of children have identified rotavirus in 42% of diarrhea under 2 years and 29% in the age group 2-10 years. Possibly the two studies (bacterial and viral) may complement each other, as a contribution to efforts to determine the role of infections in the etiology of diarrheas in developing countries.

## Objectives

*General*

1. To study the role of bacteria in the etiology of diarrhea in infants and young children of a developing country.
2. To identify specific bacteria in diarrheal stools.
3. To perform bacterial sensitivity tests.
4. To complement current or on-going researches on other infectious agents of childhood diarrheas, such as viruses.

*Specific*

1. To determine what bacteria are commonly associated with diarrheas of infants and young children in Metro Manila.
2. To determine the distribution of bacterial infectious diarrheas per month.
3. To observe the frequency of pathogenic bacteria in different age groups.
4. To study sensitivity tests of identified bacteria in diarrheal stools, to current anti-microbials.
5. To determine changes in the pattern of bacterial infections of childhood diarrheas.

## Materials and Methods

Fresh diarrhea stool specimens were obtained from the patient into specimen bottles and transported immediately to the laboratory. Cultures were made within 15 minutes after receiving the specimen. The stool was streaked into one SS (Salmonelia Shigella) medium and one EMU (Eosin Methylene Blue) Media in petri dishes. After 18-24 hours, cultures were read and biochemical studies for further differentiation and antibiotic sensitivity tests were then performed. The Kirby Bauer, single disc agar diffusion method for sensitivity test were followed. Each organism was cultured in Trypticase soy broth until turbidity matching *Mc Farland* scale No. 5 were reached, where each ml. of broth contains 108 organisms. Then a cotton swab was used to inoculate the organism to a Muller-Hinton Agar plate.

Five minutes later, seven to ten antibiotic discs were placed in each plate with a distance of 25 mm between each disc and 9 mm away from the side. Readings were made 18 hours later, the zone size was measured and the degree of sensitivity assessed according to the Kirby Bauer table.Diagrams of the procedure are depicted in the Annexes (1a, 1b and 1c).

## Results and Discussion

Stool specimens from infants and children under 5 years old were included in this study. These were patients who were admitted for acute diarrhea in two

hospitals in Metro Manila. Among the staff of this research, two were involved in the study in both hospitals.

As it was not intended to compare the results of the two groups, the findings and data were tabulated and presented separately as Group A (3 year study, 1980 to 1982) with a total of 624 and Group B (1980 and 1981) with 1480 specimens. The data of Group A are presented in Tables 1 to 5 and those of Group B in Tables 6 to 10.

Table 1. Stool Cultures in 3 Years (CMCP)*

| | |
|---|---|
| 1980 | 157 |
| 1981 | 266 |
| 1982 | 201 |
| Total | 624 |

*Children's Medical Center Philippines.

In 1980 and 1981 the first three months of the year were peak months for infant diarrhea (Table 2 and Fig. 1).

Table 2. Stool Cultures By Month and Year

| | *1980* | *1981* | *1982* |
|---|---|---|---|
| January | 19.7% | 20.6% | 5.9% |
| February | 15.2 | 9.0 | 6.9 |
| March | 14.0 | 6. | 8.9 |
| April | 8. | 8. | 6. |
| May | 3. | 7.5 | 4. |
| June | 3. | 7. | 8.9 |
| July | 8. | 6.7 | 7.9 |
| August | 4. | 7.5 | 12. |
| September | 3. | 5.6 | 14. |
| October | 9. | 7.5 | 8.9 |
| November | 5. | 10. | 3.9 |
| December | 5. | 6.7 | 6.9 |

It is evident that in all the three years, three-fourths of the specimens were from infants below one year old. These were also the findings in Group B and in other studies of acute diarrheas.

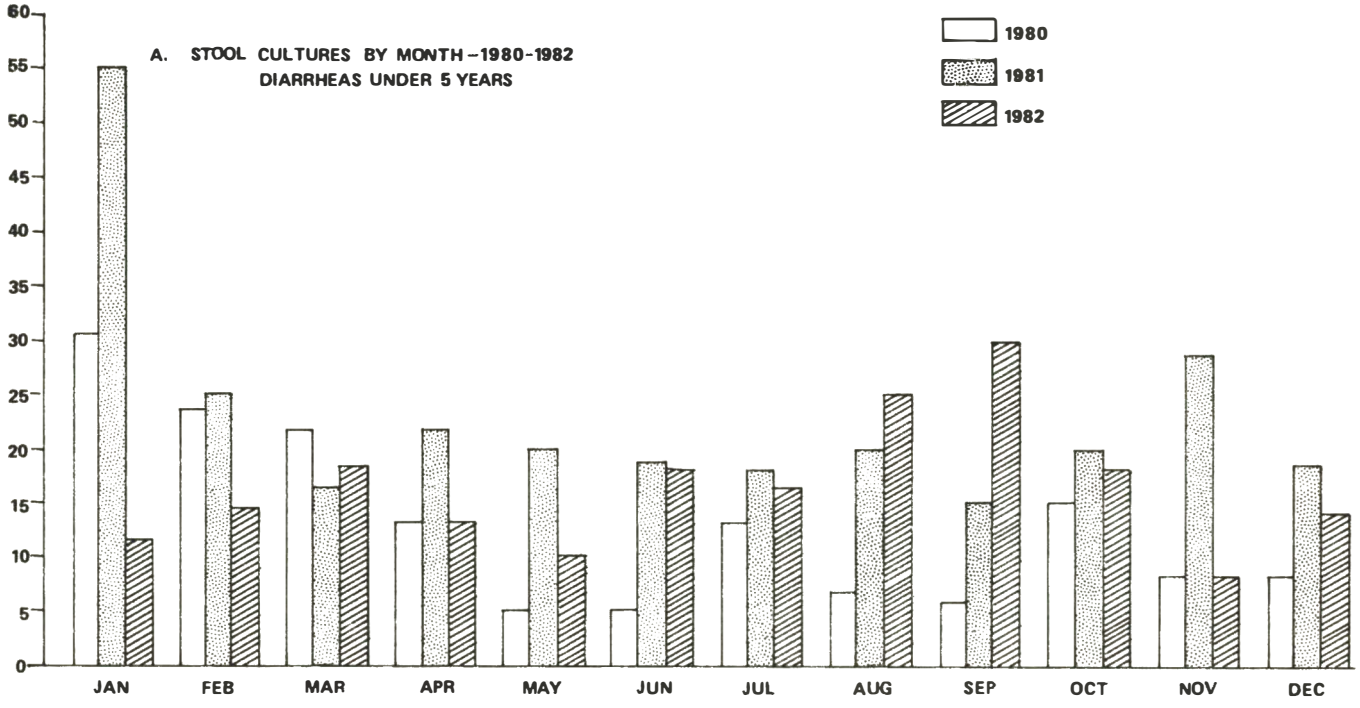


Figure 1. Group A. (CMCP)

Table 3. Diarrheal Stool Culture By Age

| | *1980* | *1981* | *1982* |
|---|---|---|---|
| 0 – 1 year | 75.0% | 77.8% | 76% |
| 1 – 2 years | 15 | 13 | 13.9 |
| 2 – 5 years | 9.5 | 9 | 9.9 |

In this series E. coli was the most common bacteria identified for all three years and were almost equal from year to year. (Table 4 and Fig. 2).

Table 4. Most Common Bacteria Identified Per Year, Children's Medical Center Philippines

| | *Organisms* | *1980* *No. 157* | *1981* *No. 266* | *1982* *No. 201* |
|---|---|---|---|---|
| 1. | E. coli | 33.75% | 31.5% | 33.8% |
| 2. | Enterobacter | 7.6 | 11.6 | 12.4 |
| 3. | Salmonella | 10.8 | 9.3 | 15.9 |
| 4. | Proteus | 23.5 | 34.9 | 15.4 |
| 5. | Arizona | 10.1 | 4.8 | 11.9 |
| 6. | Citrobacter | 4.4 | 9.0 | 6.4 |
| 7. | Shigella | 3.8 | 2.6 | 1.4 |
| 8. | Pseudomonas | 4.4 | 4.5 | 3.4 |

The above list is far from complete. Recent researches, in particular by the Scientific Working Group of WHO on Bacterial Enteric Infections, have given much attention to "Watery diarrheas" (Vibro Diarrhea and *E. coli*) and invasive pathogens (*Shigella, Campylobacter, Jejuni*) enteroinvasive (*E. coli, Salmonella* and *Yersinia enterocolitica*). The methods for their accurate identification have not been availed of in the present study. Serotyping of *E. coli* was not done as researches state there is lack of correlation of serotype with pathogenicity and is not a useful procedure.

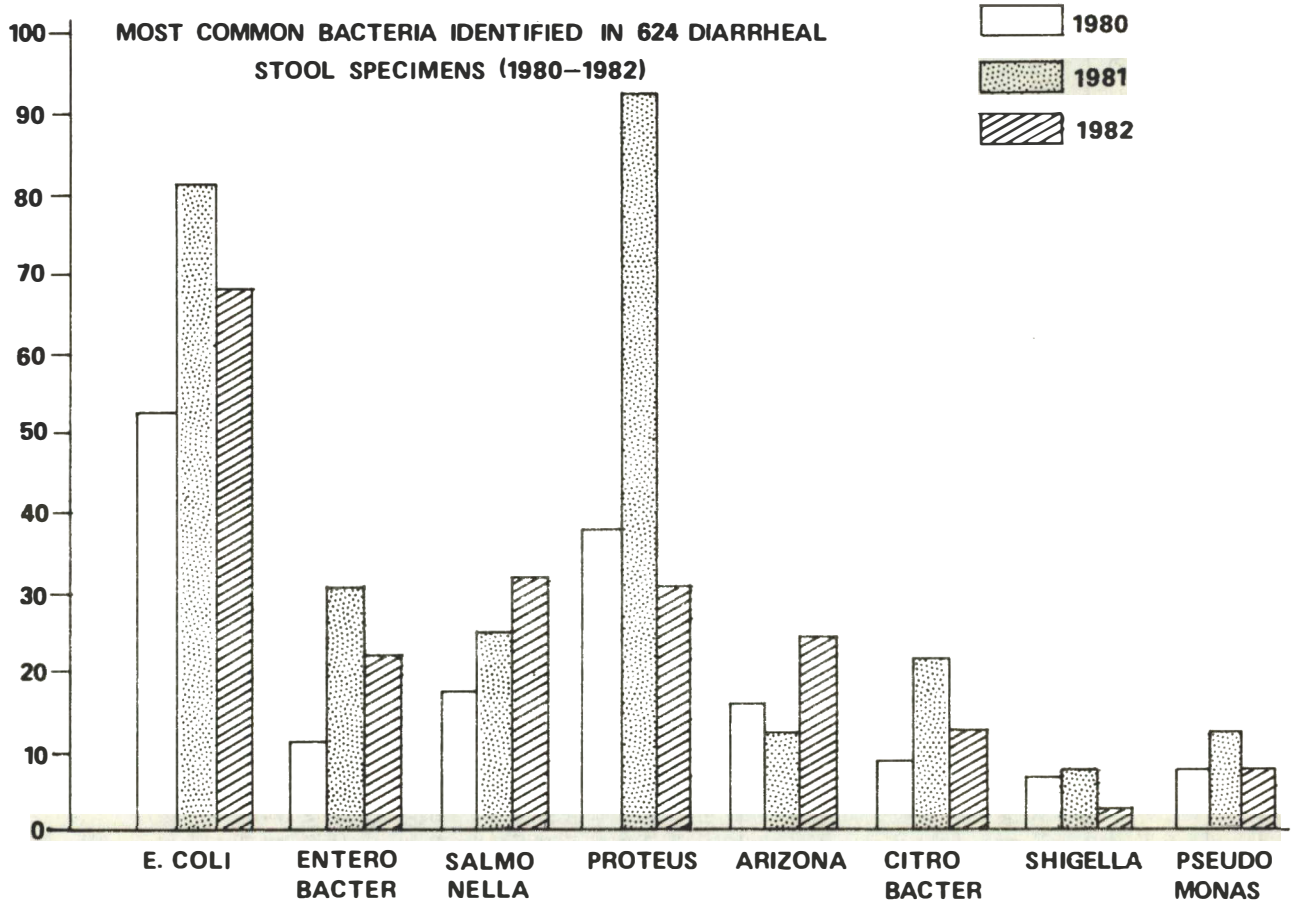


Figure 2. Group A (CMCP)

Table 5. Bacteria Identified in Different Age Groups Per Year From 1980 to 1982

| *(1980) Specimens Tested: 157* | *Number of Cases Positve For:* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Age Group* | *E. Coli* | *Proteus* | *Enterobacter* | *Salmonella* | *Pseudomonas* | *Citrobacter* | *Arizona* | *Shigella* |
| 0 – 1 year | 33 | 35 | 10 | 13 | 6 | 5 | 13 | 1 |
| 1 – 2 years | 15 | 2 | 1 | 1 | 1 | 2 | 2 | 2 |
| 2 – 5 years | 5 | 0 | 1 | 3 | 0 | 0 | 1 | 3 |

| *(1981) Number Tested: 266* | *Number of Cases Positive For:* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Age Group* | *E. Coli* | *Proteus* | *Enterobacter* | *Salmonella* | *Pseudomonas* | *Citrobacter* | *Arizona* | *Shigella* |
| 0 – 1 year | 55 | 79 | 28 | 21 | 9 | 19 | 10 | 2 |
| 1 – 2 years | 16 | 10 | 2 | 2 | 2 | 3 | 2 | 1 |
| 2 – 5 years | 15 | 4 | 1 | 2 | 1 | 2 | 1 | 4 |

| *(1982) Number Tested: 201* | *Number of Cases Positive For:* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Age Group* | *E. Coli* | *Proteus* | *Enterobacter* | *Salmonella* | *Pseudomonas* | *Citrobacter* | *Arizona* | *Shigella* |
| 0 – 1 year | 51 | 26 | 20 | 25 | 5 | 10 | 19 | 1 |
| 1 – 2 years | 9 | 5 | 4 | 3 | 1 | 3 | 4 | 0 |
| 2 – 5 years | 8 | 0 | 1 | 4 | 1 | 0 | 1 | 2 |

Table 8. Sensitivity Tests: Positive (P) and Resistance (R), Children's Medical Center, Philippines, 1982

| *Organisms* | | *Amika-cin* | *Amoxil-lin* | *Ampicil-lin* | *Cephalo-thin* | *Chloram-phenicol* | *Colymy-cin* | *Gentamy-cin* | *Kanamay-cin* | *Nalidixic Acid* | *Tobramy-cin* | *Cefota-xime* | *Netromy-cin* | *Sisomy-cin* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | P | 59 | 3 | 10 | 2 | 17 | 17 | 62 | 30 | 57 | 17 | 23 | 57 | 20 |
| E. Coli | R | 2 | 16 | 48 | 4 | 40 | 1 | 5 | 34 | 5 | 6 | 3 | 2 | 4 |
| | P | 26 | 6 | 4 | 34 | 2 | 15 | 12 | 2 | 18 | 3 | 18 | 26 | 9 |
| Salmonella | R | 4 | 10 | 23 | 1 | 23 | 5 | 23 | 19 | 8 | 14 | 1 | 1 | 14 |
| | P | 28 | 0 | 6 | 0 | 7 | 6 | 23 | 13 | 24 | 4 | 10 | 26 | 6 |
| Proteus | R | 2 | 7 | 19 | 2 | 18 | 8 | 8 | 18 | 6 | 4 | 0 | 1 | 3 |
| | P | 25 | 2 | 2 | 2 | 10 | 13 | 20 | 15 | 22 | 11 | 15 | 12 | 7 |
| Enterobacter | R | 2 | 16 | 23 | 3 | 12 | 2 | 3 | 10 | 3 | 7 | 1 | 19 | 11 |
| | P | 23 | 2 | 3 | 2 | 4 | 9 | 20 | 5 | 20 | 7 | 15 | 19 | 11 |
| Arizona | R | 1 | 11 | 20 | 0 | 2 | 3 | 4 | 19 | 4 | 4 | 0 | 3 | 1 |
| | P | 10 | 3 | 6 | 2 | 6 | 6 | 9 | 10 | 11 | 6 | 5 | 9 | 5 |
| Citrobacter | R | 0 | 2 | 6 | 0 | 7 | – | 2 | 4 | 1 | 0 | 0 | 0 | 1 |
| | P | 6 | 0 | 1 | 3 | 2 | 2 | 3 | 1 | 0 | 3 | 0 | 3 | 2 |
| Pesudomonas | R | 1 | 2 | 5 | 0 | 0 | 0 | 1 | 2 | 2 | 1 | 2 | 0 | 0 |

In the second hospital group of acute diarrheas of early childhood specimens and cultures were as follows:

Table 9. Stool Cultures By Year, Lungsod ng Kabataan (LnK)

| | |
|---|---|
| 1981 | 562 |
| 1982 | 918 |
| Total | 1,480 |

The last four months of 1981 were peak months for infant diarrheas while August to October showed a high incidence in 1982.

Table 10. Stool Cultures By Month, Lungsod ng Kabataan (LnK)

| *Month* | *1982* | | *1982* | |
|---|---|---|---|---|
| | *No.* | | *No.* | |
| January | 15 | 2.6 | 58 | 6.3 |
| February | 20 | 3.5 | 56 | 6 |
| March | 25 | 4.4 | 77 | 8 |
| April | 19 | 3.3 | 70 | 7.6 |
| May | 33 | 5.8 | 78 | 8 |
| June | 66 | 11 | 85 | |
| July | 96 | 17 | 75 | 8 |
| August | 64 | 11 | 95 | 10 |
| September | 59 | 10 | 111 | 12.7 |
| October | 53 | 9.4 | 96 | 10.4 |
| November | 44 | 7.8 | 83 | 9 |
| December | 68 | 12 | 34 | 3.7 |
| Total | 562 | | 918 | |

As in the first group and in fact in any study on acute diarrhea in children, infants under 1 year are most affected as shown in Table 11.

Again E, coli followed by Enterobacter, Proteus, and Salmonella take the lead among bacteria identified. The more recent and sophisticated procedures were not availed of so that this list does not include recently reported new pathogens like rotavirus, Canpylobacter jejune and E. coli types (enterotoxigenic and enteroinvasive).

## Summary and Conclusion

In 1980 to 1982, among 2,104 stool specimens of infants and young children who were confined in 2 hospitals in Metro Manila, cultures and sensitivity tests gave findings that are presented in this report.

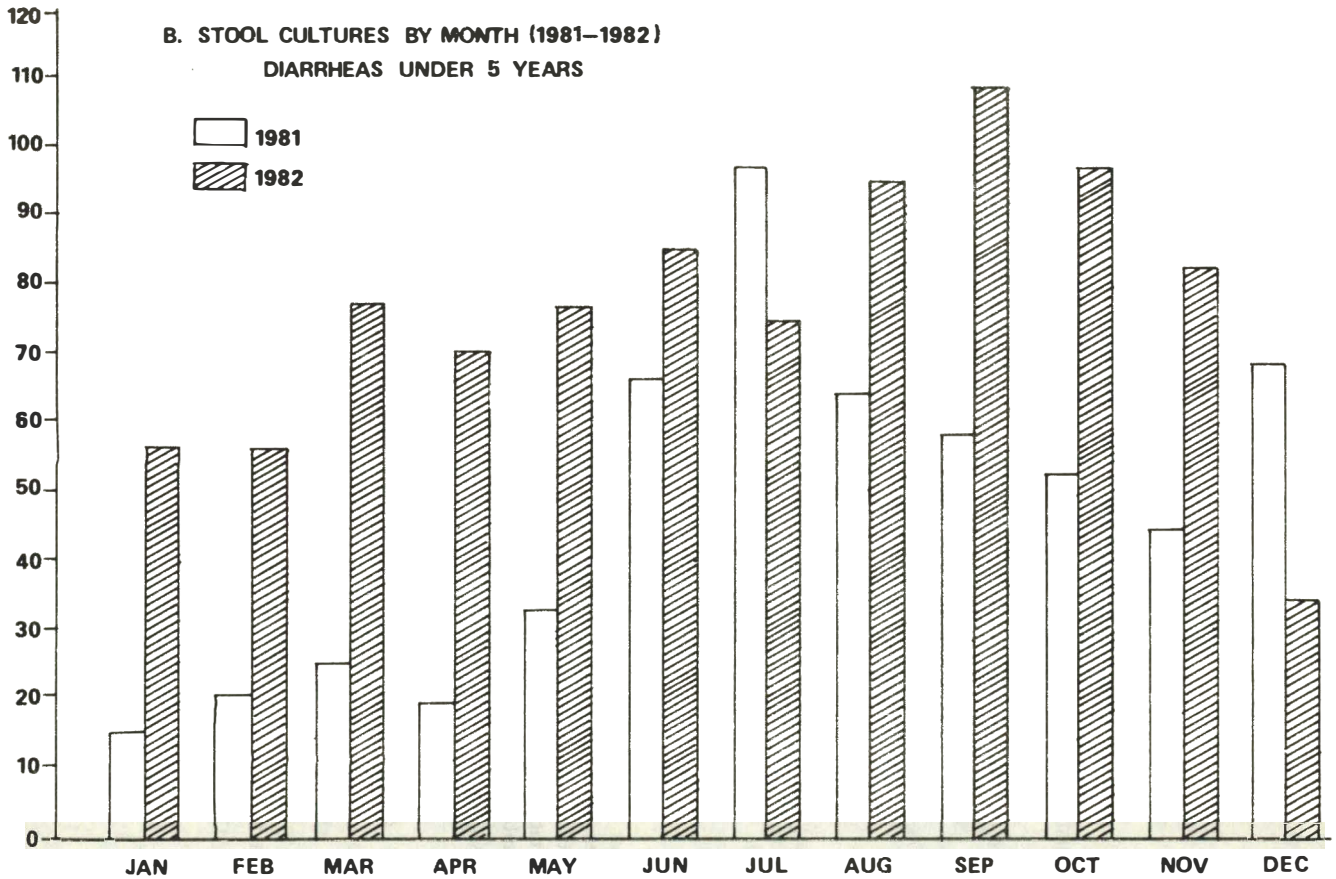


Figure 3. Group B (LNK)

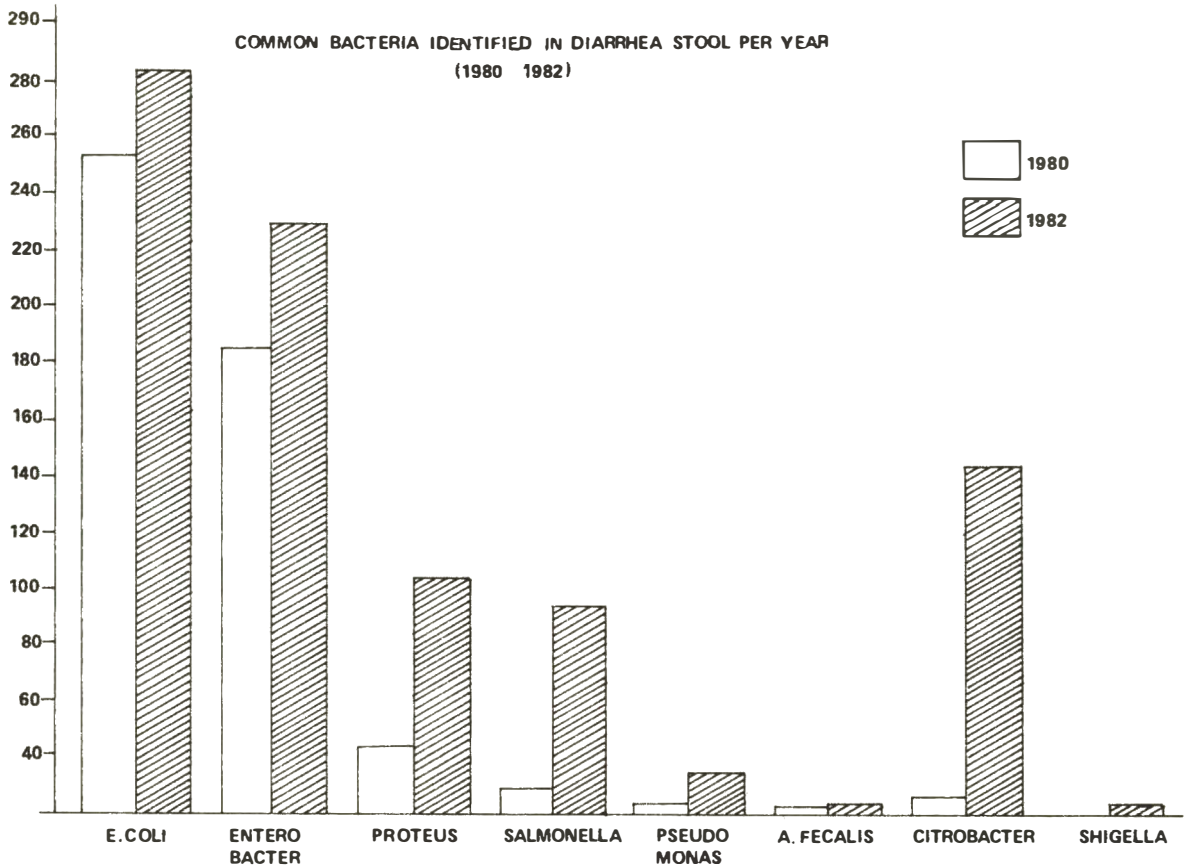


Figure 4. Group B (LNK)

Table 11. Stool Cultures By Age, Lungsod ng Kabataan (LnK)

| Age | 1981 No. | 1981 % | 1982 No. | 1982 % |
|---|---|---|---|---|
| 0 – 1 year | 368 | 65 | 626 | 68 |
| 1 – 2 | 110 | 19.5 | 144 | 15.6 |
| 2 – 5 | 84 | 14.7 | 148 | 16 |
| Total | 562 | | 918 | |

Table 12. Most Common Bacteria Identified Per Year, Lungsod ng Kabataan (LnK)

| Organisms | 1981 | 1982 |
|---|---|---|
| 1. E. coli | 253 | 282 |
| 2. Enterobacter | 186 | 230 |
| 3. Proteus | 43 | 104 |
| 4. Salmonella | 30 | 95 |
| 5. Pseudomonas | 17 | 33 |
| 6. Alkaligenes fecalis | 4 | 13 |
| 7. Citrobacter | 27 | 145 |
| 8. Shigella | 0 | 7 |

The main objective was to identify bacteria associated with diarrheas in early childhood and to determine antimicrobials to which such bacteria are sensitive or resistant. A viral study was not undertaken but, by coincidence, this was done about the same time at San Lazaro Hospital by a research team, also in Metro Manila.

In the first hospital (Group A), the study included 624 cultures and in the second 1480 (Group B), giving a total of 2,104. In the research staff, two members were involved in both groups.

Infants topped the list and constituted about 75% of the total patients in Group A and 65 to 68% in Group B.

The incidences by month showed high figures, either the first three months (Group A) or the last four months (Group B) of the year.

The most common bacteria identified in the first group were Proteus, E. coli and Enterobacter while in the second the order was E. coli, followed by Enterobacter, Proteus and Salmonella.

Sensitivity tests showed a significant number sensitive to Gentamycin, followed by Amikacin, Tobramycin and Kanamycin. Almost consistently, there

Table 14. Sensitivity Positive (P), and Resistance (R), Group B, 1981

| *Organisms* | | *Genta-mycin* | *Amika-cin* | *Kanamy-cin* | *Ampicil-lin* | *Cefoto-xim* | *Tobramy-cin* | *Chloram-penicol* | *Cephalo-thin* | *Neomy-cin* | *Carbeni-cillin* | *Colis-tin* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | P | 203 | 78 | 55 | 36 | 27 | – | – | – | – | – | – |
| E. coli | R | 36 | 8 | 98 | 136 | 1 | – | – | – | – | – | – |
| | P | 147 | 54 | 48 | – | – | 66 | 51 | – | – | – | – |
| Enterobacter | R | 22 | 6 | 57 | – | – | 3 | 72 | – | – | – | – |
| | P | 36 | 17 | – | – | – | – | 16 | – | – | – | – |
| Proteus | R | 7 | 3 | – | – | – | – | 14 | – | – | – | – |
| | P | 18 | 8 | – | – | 18 | – | – | 13 | – | – | – |
| Citrobacter | R | 6 | 1 | – | – | 0 | – | – | 2 | – | – | – |
| | P | 18 | – | – | – | 22 | 7 | – | – | 8 | – | – |
| Salmonella | R | 10 | – | – | – | 0 | 7 | – | – | 10 | – | – |
| | P | 12 | 10 | – | – | – | 12 | – | – | – | 9 | 5 |
| Pseudomonas | R | 5 | 1 | – | – | – | 2 | – | – | – | 5 | 4 |

was resistance to ampicillin, chloramphenicol and neomycin in the commonly identified bacteria. This was maintained from year to year in the 3-year study.

In 1982 when discs of new preparations became available for testing, cifatoxin and tobramycin showed the highest sensitivity with Gentamycin third or fourth in the list. On this year resistance was also high to ampicillin, chloramphenicol and kanamycin.

A viral study by another research team on a similar age group, also in Metro Manila, showed an incidence of rotavirus in 42% under 1 year and 29% for the age group 2-10 years old.

It is expected that a number of questions, complications and suggestions will arise from this simple study. Many lessons have been learned; perhaps some procedures may be disregarded while many omissions will surface out. All these will result in a more effective and efficient steps in the diagnostic work-up of diarrheas.

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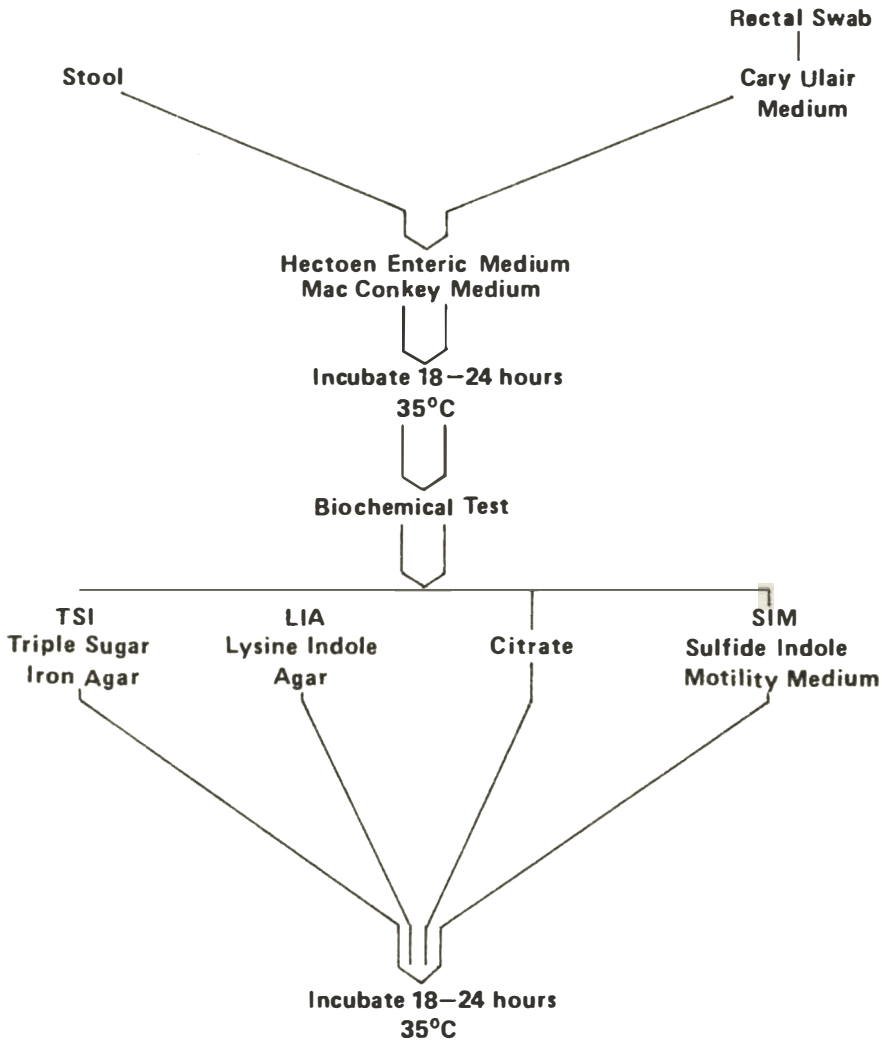
## ANNEX $I_a$

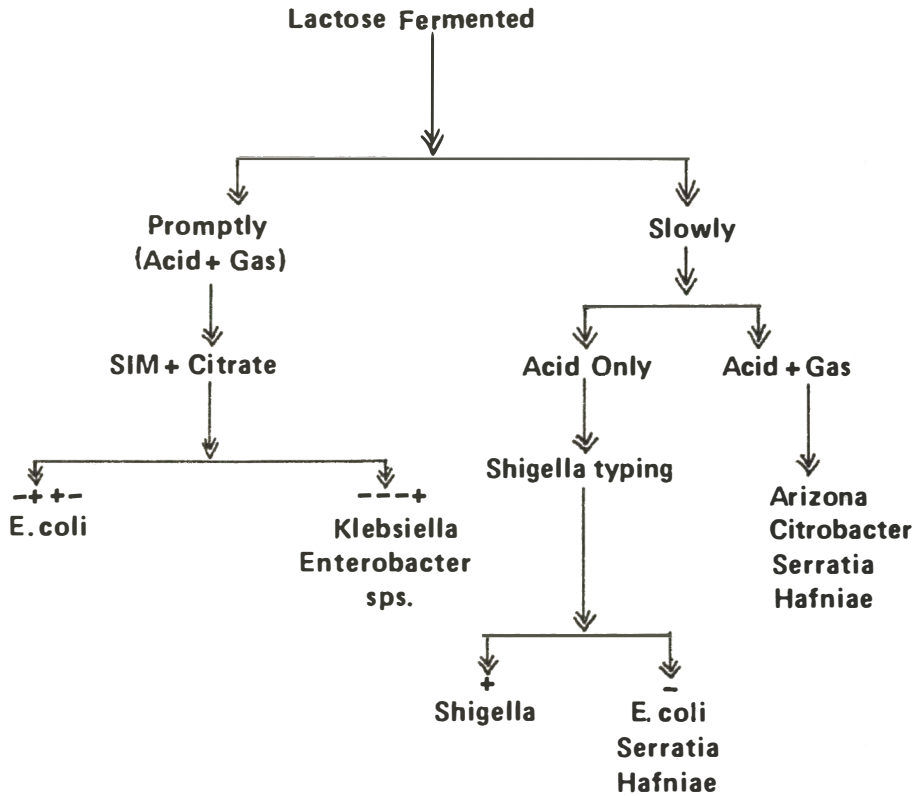
### Collection, Processing and Identification of Bacteria in Stool Specimens and Rectal Swab

Unless the specimen can be taken immediately to the laboratory and properly handled, all swabs may be added to a screw capped vial container of Cary and Blair and transported to the laboratory for culture.

Fresh stool specimens are directly plated to the medium as illustrated.



ANNEX $I_b$


Lactose Fermented
Promptly
(Acid + Gas)
Slowly
SIM + Citrate
Acid Only
Acid + Gas
-+ +-
E. coli
---+
Klebsiella
Enterobacter
sps.
Shigella typing
Arizona
Citrobacter
Serratia
Hafniae
+
Shigella
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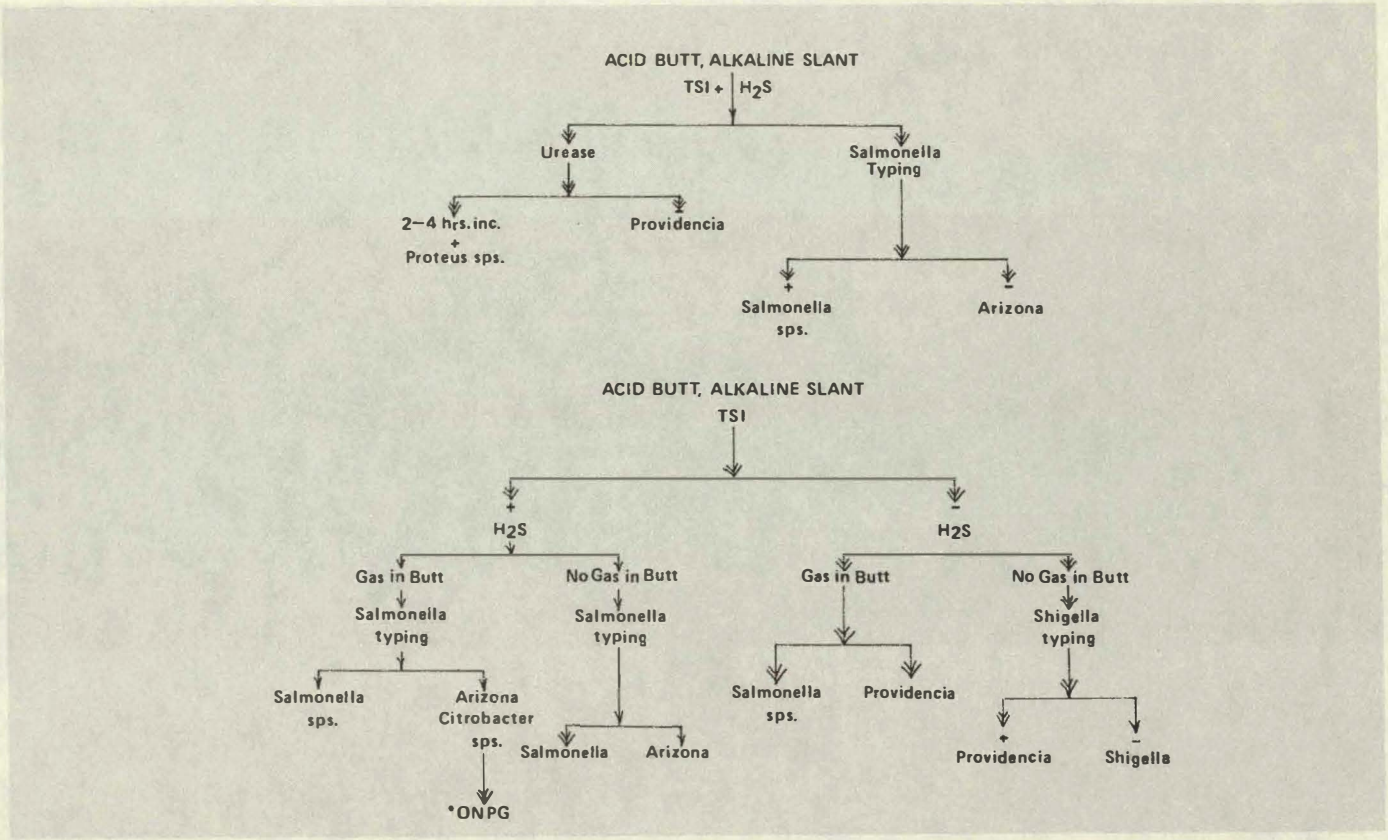
E. coli
Serratia
Hafniae

## ANNEX $I_c$




*Orto-nitrophenyl Galactosidase

**Virginia Basaca-Sevilla, Discussant**

I have always hoped that researchers on the subject of etiologies of diarrheas do not encounter difficulties ordinary laboratories like the Bureau of Research and Laboratories are faced with, everyday. But the statement of the authors at the end of the paper like "expectation of questions, complications, suggestions, disregarding of procedures and omission" make me surmise that most probably they did have problems similar to us. We have to admit that each agency or institution doing this kind of work, has a different work environment and clientele and their own work limitations which may be funding for supplies and reagents, technical personnel, interested clinicians and enough time without sacrificing time for service work load. To solve part of this, we have learned to collaborate with other institutions so that objectives of a particular study can effectively be divided and accomplished efficiently. I hope my guess is also right that there was not much funding problem since the researchers proceeded to sensitivity testing (which is expensive) of all isolates, without having to go through proving that the isolate surely caused the diarrhea or they were also very fortunate in having pure cultures on isolation.

Allow me to add a little bit of information gathered from the data of others working on the etiologies of diarrhea whether as a routine effort or for research.

Using accepted standard technics and the minimum of standard media and reagents like Cary and Blair, MacConkey, S.S. Agar, Nutrient Agar Bile Salts Medium, Selenite broth and Peptone water, the BRL cultures rectal swabs samples from diarrheal patients to isolate Salmonellas, Shigellas, Vibrio choleras, V. parahemolyticus, Edwardsiella tardas and E. coli.

For 1982, there were 407 or 21.40% isolations for bacterial enteric pathogens, from 1901 rectal swab samples from diarrheic children confined at San Lazaro Hospital. Of the isolates, 51.38% were Salmonellas, 36.57% were Vibrio choleras, 4.62% were Shigellas, 0.46% V. parahemolyticus, 3.93% non-agglutinable vibrios, and 3% biochemically Salmonellas. In 1983, of 1,691 samples from diarrheic children confined in the said hospital there were positive isolations in 363 or 21.52%. Of the bacterial enteric pathogens, 59.35% were Salmonellas, 33.42% Vibrio choleras, 5.88% were Shigellas, 1.07% V. parahemolyticus, 0.26% biochemically Shigellas. From the no more than 2 years of age, there were 65 or 3.41% isolates for E. coli in 1982 and 119 or 7.3% in 1983.

Of the 1,266 samples from the regional and provincial surveillance system from children with diarrhea in 1982, the BRL found that 190 or 15% had positive bacterial enteropathogenic isolation. Of these isolates 73.71% were Salmonellas, 24.22% were V. choleras and 1.03% each of V. parahemolyticus and biochemically Salmonellas. In 1983 there were 226 or 11.23% isolations from 2,011 samples. Of the isolates 59.91% were Salmonellas, 37.65% were V. choleras, 1.21% were Shigellas, 0.8% E. tardas and 0.4% Arizona Hinshawa. There were 104 or 8.21% E. coli isolated in 1982 and 108 or 5.37% in 1983.

Of 74 strains of E. coli isolated by the BRL from children below 2 years of age, 33 or 44.59% were found to be toxigenic by Dr. Antonio Jacalne of the Institute of Public Health, U.P. utilizing the intestinal loop test in rats. The most common salmonella serotypes isolated are S. typhimurium, S. senftenberg, S. worthington, S. anatum, S. stanley, S. newport and S. derby. It is mostly V. cholera ogawa that's isolated and Shigella Group B. The youngest child from whom isolation was made for cholera, shigella or salmonella is one month old.

The report of the preliminary evaluation of a study on the etiologies of pediatric diarrheas in the National Children's Hospital done by the Research Institute of Tropical Medicine showed that the prevalence of rotavirus in 620 patients studied was 16%, for Salmonella 57%, for enterogenic coli 24%, for the shigellas 10%, for the choleras 4.81%, for the campylobacter 0.6% and for the E. histolyticas 4.19%, and for the Giardia lamblia 0.32%. This is research data.

The research paper under discussion gives the prevalence of isolation for Salmonellas in the Children's Medical Center as 10.8% for 1980, 9.3% for 1981 and 15.9% for 1982. Shigella prevalence varied from 1.4% to 3.8%. From the Lungsod ng Kabataan the Salmonella prevalence was 5.3% for 1981 and 10.45% for 1982. The prevalence of isolations for Salmonellas, Shigellas and Vibrio choleras are certainly much lower in these institutions. In both hospitals the E. coli isolation predominate varying from 31% to 45%.

It is hoped that the information and experience of others will add to the data obtained by the researchers of this study. It is further hoped that more funds will be provided to adequately serve each patient suffering from diarrheas as the determination of the etiology of diarrheas is not done only when there is a research going on but an everyday activity of each laboratory, whether it is a public health laboratory or a hospital laboratory.

To save money and time it is suggested that sensitivity tests be not a routine procedure in enteric bacteriology except in certain situations where surveillance of the response to antibiotics is regularly being done for certain microorganisms like S. typhi or S. para A. This regular monitoring is done by the Central Laboratory. Sensitivity tests also should be done on specific strains of organisms not grouped together. It is further hoped that as we know more and more of the etiologies of our diarrhea, the control and eradication should not be far off.