LITERATURE SURVEY

Enteropathogenic Organisms. The important enteropathogens are the Salmonella, Shigella, Enteropathogenic Escherichia coli and the Vibrio. The Salmonella causes typhoid, paratyphoid fever and food poisoning. Bacillary dysentery, in both acute and chronic stages, is caused by the genus Shigella, Escherichia coli is the common cause of acute severe diarrhea in newborns and infants or even in adults. Cholera vibrios are present in enormous numbers in the characteristic "rice water" stool produced by profuse diarrhea in the early state of cholera.

The definition of the Family Enterobacteriaceae:

1. Organisms belonging to the family Enterobacteriaceae do not produce cytochrome oxidase. This addition to the definition provides a simple biochemical method of eliminating from the group a number of polar flagellated organisms including Aeromonas, cultures of which often are mistaken for Enterobacteriaceae and particularly for Aerobacter types. Also excluded by means of the cytochrome oxidase tests are members of such groups as Pseudomonas, Alcaligenes, and Vibrio.

2. Members of the Enterobacteriaceae do not liquefy Sodium pectinate medium. This emendation of the definition permits exclusion from the family of a number of organism producing soft rots of plants, the so-called Pectobacterium group generally classified in the

genus Erwinia. The Erwinia group, as it usually is defined includes at least two distinct entities.

a. The microorganisms which generally cause galls and blights of plants and which might be called the "true Erwinia group". These bacteria fail to reduce nitrate to nitrite and bear little resemblance to enteric bacteria in their cultural characteristics and biochemical properties.

b. The bacteria which produce soft rots of plants and which generally reduce nitrate to nitrite and resemble the Aerobacter group in their habit of growth and physiological properties. These organisms generally possess the property of liquefying sodium pectinate gel whereas Enterobacteriaceae, as defined here,do not. To the writers it seems advisable to exclude such organisms from Enterobacteriaceae, at least for the present. Later, when the status of the Erwinia group as a whole is clarified, if it is decided to include the soft rot organisms as a special Pectobacterium group, this could be done.

The criteria for recognition of the principle divisions is in Table I. It is based upon the IMViC (Indol, Methyl-red, Voges-Proskauer and Citrate) reactions, hydrogen sulfide production, urease production, ability to grow in Moeller's KCN medium, and production of phenylalanine diaminase.

Table 1

Differentiation of the principal divisions within the Enterobacteriaceae by biochemical methods

(Reactions of typical cultures)

Substrate or Test	Shigella–Escherichia Division	Salmonella–Arizona–Citrobacter Division	Klebsiella–Aerobacter–Serratia Division	Proteus–Providence Division
Indol	d	–	–	d
Methyl–red	+	+	–	+
Voges–Proskauer	–	–	+	–
Simmons' citrate	–	+	+	d
Hydrogen sulfide (TSI)	–	+	– or (+)	d
Urease	–	–	– or (+)	d
KCN	–	d	+	+
Phenylalanine	–	–	–	+

N.B. Salmonella typhi, Salmonella paratyphi A and some of the rare types are citrate negative. Salmonella paratyphi A and other rare types are H_2S negative. Proteus vulgaris and Proteus mirabilis may be V-P + when incubated at 26°C

\+ positive in one or two days. (90 % or more of cultures tested)

\- negative. (90 % or more of cultures tested)

(+) delayed positive

d different biochemical types.

(Edwards, P.R., and Ewing, W.H. 1955. Identification of Entero - bacteriaceae, p. 3).

The Genus Salmonella. The genus Salmonella is defined by Edwards and Ewing (1955) as "Usually motile but non-motile forms occur. Produce acid and gas from glucose, maltose, mannitol and sorbitol (except that in Salmonella typhosa and Salmonella gallinarum no gas is produced). Lactose, sucrose and salicin are not attacked. Do not clot milk, form indol or liquefy gelatin. Reduce trimethylamine oxide to trimethylamine. All of the known species are pathogenic for warm blooded animals including man, causing food infection and enteric fevers. A few are found in reptile. Some or all may also live in decomposing foods".

For all practical purposes it may be said that Salmonellae possess the following properties:

Glucose	=	Fermented, usually with gas (a)
Mannitol	=	Fermented
Sorbitol	=	Fermented
Adonitol	=	Not fermented
Dulcitol	=	Usually fermented
Lactose	=	Not fermented
Sucrose	=	Not fermented
Indol	=	Not produced
Methyl-red	=	Not produced
Voges-Proskauer	=	Negative

Simmon's citrate	=	Usually utilized[b]
H_2S	=	Usually positive[c]
Urea	=	Negative
Motility	=	Positive[d]
Decarboxylases		
Lysine	=	Positive[e]
Arginine	=	Positive, usually delayed
Ornithine	=	Positive[f]
Phenylalanine diaminase	=	Negative
Gelatin	=	Rarely liquefied

There are some exceptions:

(a) Salmonella typhi and Salmonella gallinarum are anaerogenic. Occasionally anaerogenic forms of other types appear.

(b) Salmonella paratyphi A, Salmonella cholerae - suis (diphasic), Salmonella typhi, Salmonella sendai, Salmonella pullorum, Salmonella gallinarum, and some other types fail to utilize ammonium salt as a sole source of nitrogen.

(c) Salmonella paratyphi A, Salmonella cholerae - suis (diphasic), Salmonella typhi, Salmonella sendai, Salmonella berta, some cultures of Salmonella typhi, and a few other types which occur very rarely fail to produce H_2S (Triple Sugar Iron agar).

(d) Salmonella pallorum and Salmonella gallinarum are non-motile. Non motile variants of other forms occur rarely.

(e) Salmonella paratyphi A gives a negative or delayed positive result in lysine tests.

(f) Salmonella typhi and Salmonella gallinarum are negative in ornithine tests.

The biochemical identification of the species of Salmonella is still widely used along with commercial antisera agglutination. Suspected colonies of Salmonellae are transplanted on TSI agar slants. If they show acid, gas and H_2S in butt, with an alkaline slant, and in addition prove to be urease-negative, they may be tested with Salmonella polyvalent antiserum.

Serologic reactions. The Kauffman-White antigenic scheme presently tests several hundred Salmonella serotypes that are arranged in subgroups on the basis of the O antigens, while the H or flagella antigens, along with the somatic complex, represent the type examplifies a substantially reduced. There are a far greater number of types in which the identification of Enterobacteriaceae by Edwards and Ewing (12) should be consulted.

The great majority of Salmonella cultures isolated from man, including the types mentioned above are numbers of the first five somatic groups of Kauffman-White classification (A,B,C,D,E). It is necessary for the average laboratory to have O serums only for group A through E of the genus. A polyvalent serum which at least contains agglutinins for antigens 1,2,3,4,5,6,7,8,9,10,11,12,15 and 19 should be available. The group identification is determined by the use of O

grouping sera, and type identification is determined by H antisera.

Procedure usually employed in serological examination is the slide agglutination test, using a concentrated suspension of the cell in saline. The important views should be considered at this point are:

1. The culture to be tested must be smooth colonies and thus not autoagglutinable in saline. A priliminary test with a 0.2% solution of acriflavine (in 0.85% saline) is an excellent indicator of smoothness. If a loopful of the test suspension is mixed gradually on a slide with a loopful acriflavine and the cells remain in homologous suspension, the culture may be considered smooth.

2. If the culture fails to agglutinate in the diagnostic antisera aviable, it should be sent to a reference center (e.g. enteric Bacteriology Unit, Communicable Disease Center, Atlanta, U.S.A.) for identification. Few laboratories are equipped to carry out a complete serological analysis.

3. Due to the fact that some Salmonella possess K antigen (Vi antigen in Salmonella typhi and Salmonella paratyphi C and other envelope antigens in certain serotypes) which may block O agglutination, live cells may be in agglutinatable in O antisera and should be heated at 100°C for 10 to 30 minutes, cooled, and retested. Live cell for Salmonella typhi and Salmonella paratyphi C, however, will agglutinate in Vi antiserum.

4. The majority of Salmonellae are diphasic, that is the motile types may exhibit two antigenic forms referred to as phases. These phases have the same O antigen but normally have different H antigens, and in order to identify the type, it is necessary to identify the H antigen in both phases. Both phases are not always in evidence, and phase suppression procedures may be necessary in order to bring out the latent phases. Such procedures can be carried out only by laboratories equipped with the proper facilities.

5. The complete identification of O antigen and H antigens complexes, the use of absorbed single factor sera is required. These antisera are prepared by adding a concentrated suspension of cells, containing the appropriated antigens, to a suitable dilution of the multifactor serum, incubating in a water bath for 2 hours at 50°C and followed by over night refrigeration such a procedure is referred to as agglutinin absorption.

The Genus Shigella. The Shigella commission of the International Enterobacteriaceae Subcommittee recommended the following definition in 1950 "The genus Shigella consists of gram-negative aerobic, non-motile, non-sporulating rods, corresponding to Shigella dysenteriae (Shiga's bacillus) in staining properties and morphology. All numbers ferment glucose, some ferment mannitol and with a few exceptions, all are non-production of gas from fermentable substances. They do not acidify salicin and adonitol not grow in Simmon's citrate agar, not

hydrolyse urea, not liquefy gelatin, not form acetylmethyl carbinol. Lactose is fermented by Shigella sonnei but only after prolonged incubation all members of the genus have an antigenic structure by which they can be recognized.

The biochemical characteristics are as follows:

Gas from Glucose	-
Lactose	-
Sucrose	-
Mannitol	d
Dulcitol	d
Salicin	-
Adonitol	-
Inositol	-
Indol	d
Methyl red	+
Voges-Proskauer	-
Ammonium citrate	-
Hydrogen sulfide	-
Urease	-

\+ = Fermented
\- = Not fermented
d = Delayed

Gelatin Liquefaction	-
Growth in Potassium cyanide medium	-
Phenylalanine deaminase	-
Sodium malonate	-

A guide in biochemical identification are shown in the Diagram 1.

Serological identification

The Shigella scheme was proposed by Ewing and then modified and extended by the Shigella commission of Enterbacteriaceae subcommittee. The scheme is based in part on biochemical characteristics of O antigenic relationships, and on tradition (Names of Shiga, Boyd). There are presented four subgroups as follows:

Sub group A - Shigella dysenteriae, types 1 to 10

Sub group B - Shigella flexneri, types 1 to 6

also X and Y variants

Sub group C - Shigella boydii, type 1 to 15

Sub group D - Shigella sonnei

In sub-group A all types are mannitol - non fermenting and each type exhibits a type specific antigen not related to other Shigella.

In sub-group B the types are usually mannitol fermenting (mannitol - negative variants of Shigella flexneri 6 do exist) and in

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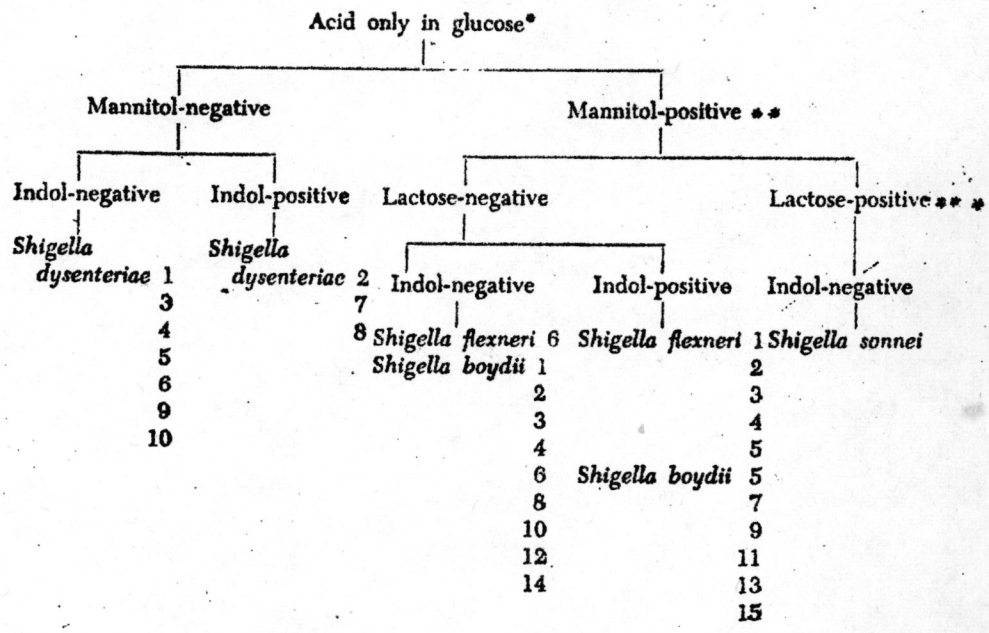
\+ = Fermented
\- = Not fermented
d = Delayed

Diagram 1

Biochemical identification of the genus Shigella.



* Shigella flexneri 6 varieties may be aerogenic (Newcastle and Manchester).

** Certain cultures of Shigella flexneri 4, Shigella flexneri 6, and Shigella boydii 6 may not produce acid from mannitol.

*** Lactose fermentation is delayed with Shigella sonnei (usually 4 to 7 days). Closure of the fermentation with a tightly fitting stopper will hasten the reaction.

(Bailey, W.R., and Scott, E.G. 1970. Diagnostic Microbiology, p.152).

addition are interrelated through group or subsidiary antigen. Each type, however, possesses a type-specific antigen that differentiates it from other Shigellae. The X and Y variants are forms that have lost the type-specific antigen.

In sub-group C the types are mannitol - fermenting and possess individual type specific antigens not related significantly to other Shigellae.

In sub-group D this is only one type, Shigella sonnei, and this possess a type-specific antigen which is not related significantly to other Shigella.

The slide agglutination is adequate serological identification, the technique is similar to that used for the Salmonella O antigens. Since K antigens are found in some Shigellae, living cells may fail to agglutinate. In that case the test suspension should be heated at 100°C for 30 to 60 minutes and retest in the polyvalent antisera.

The Genus Escherichia. The Escherichia group consists of motile or non-motile bacteria that conform to the definition of the family Enterobacteriaceae, and have the following biochemical characteristics.

Gas from Glucose	+
Lactose	+ or X

+ = Fermented
- = Not fermented
d = Delayed
X = Varied

Sucrose	d
Mannitol	+
Dulcitol	d
Salicin	d
Adonitol	-
Inositol	-
Indol	+
Methyl red	+
Voges-Proskauer	-
Ammonium citrate	-
Hydrogen sulfide	-
Urease	-
Gelatin liquefaction	-
Growth in Potassium cyanide medium	-
Phenylalanine deaminase	-
Sodium malonate	-

anaerogenic variants are not uncommon

Escherichia coli are common bacterial flora of gastrointestinal tract. They may be associated with various disease syndromes. Besides gastrointestinal tract, they may be isolate from the urinary tract

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\+ = Fermented
\- = Not fermented
d = Delayed
X = Varied

infection, septicemia, endocarditis. The Enteropathogenic Escherichia coli are isolated from many cases of infantile diarrhea (5,6,11,15,19, 21,33,34,43).

The Antigens of Escherichia coli. There are three classes of antigens that are important in Escherichia coli serology:

1. The O antigens which are somatic antigens not inactivated by heat at 100°C or 121°C

2. The K antigens which are somatic antigens that occur as sheaths envelopes or as capsules and which act as masking antigens that inhibit O agglutination. The K antigens are inactivated by heat at 100°C or 121°C

3. The H antigens or flagellar antigens inactivated by heat at 100°C

Typing of unknown Escherichia coli serotype is dependent upon the determination of its O, K and H antigens. One hundred forty-five O antigen groups have been characterized, 86 K antigen have been recognized and given numbers, and 48 H (flagella) antigens are known.

The K antigens are divisible into at least three varieties, L, A, and B based on physical behavior are given in Table 2.

The most striking difference between L and B antigens is the fact that the binding power of the former is inactivated by heat at 100°C for 1 hour. Thus, one may prepare L antiserum by absorption of an OL antiserum with a heated suspension of hemologous strain which

Table 2

THE K ANTIGENS OF *ESCHERICHIA COLI*

Variety of K Antigen	Characteristics
L	1. Agglutinability of L antigen in L antiserum inactivated by heat at 100°C, 1 hr. 2. Suspensions rendered agglutinable in O antiserum by heat at 100°C, 1 hr. 3. Antibody binding power inactivated by heat at 100°C, 1 hr. 4. Antigenicity inactivated by heat at 100°C, 1 hr. 5. Occur as envelope or sheath, occasionally as a capsule.
A	1. Agglutinability of A antigen in A antiserum inactivated by heat at 120°C, 2 ½ hrs. 2. Suspensions rendered agglutinable in O antiserum by heat at 120°C, 2 ½ hrs. 3. Antibody binding power not inactivated by heat at 100°C, 2 ½ hrs. or at 121°C, 2 hrs. 4. Antigenicity inactivated by heat at 120°C, 2 ½ hrs. 5. Occur as capsules.
B	1. Agglutinability of B antigen in B antiserum inactivated by heat at 100°C, 1 hr. 2. Suspensions rendered agglutinable in O antiserum by heat at 100°C, 1 hr. 3. Antibody binding power not inactivated by heat at 100°C, 2 ½ hrs. or at 121°C, 2 hrs. 4. Antigenicity inactivated by heat at 100°C, 1 hr. 5. Occur as envelopes or sheaths or as capsules.

(Edwards, P.R., and Ewing, W.H. 1955. Identification of Enterobacteriaceae, p.65).

removes the O agglutinin but leaves L agglutinin. This cannot be alone with B antiserum since the binding power of B antigen is not inactivated by heat at 100°C and if one absorbs an OB antiserum which a heated suspension of the homologous strain, agglutinins for both O and B antigen are absorbed from the antiserum.

Enteropathogenic Escherichia coli strain is Enteropathogenic Escherichia coli

The serotypes of the Enteropathogenic Escherichia coli groups are biochemically similar to the flora Escherichia coli strains. Therefore they must be identified by serological procedures.

Serological identification. The Enteropathogenic Escherichia coli types associated with infantile diarrhea are inagglutinable in O antisera and must be heated at 100°C for 1 hour and retests. Live cells will agglutinate readily in OB antiserum. Each of the serotypes is associated with this syndrome possesses a B type of K antigen that is serologically distinct from the other B antigens recognized in the other types. The Enteropathogenic Escherichia coli serotypes are listed below:

O 26 : B 6

O 55 : B 5

O 86 : B 7

O 111 : B 14

O 112 : B 11

O 119 : B 14

O 124 : B 17

O 125 : B 15

O 126 : B 16

O 127 : B 8

O 128 : B 12

Enteropathogenic Escherichia coli O 124 is probably a definite pathogen for adult also (18).

The Genus Proteus. They are lactose-negative and usually usease positive. The species are actively motile at 27°C but weakly motile or non-motile at 37°C. On moist agar or agar of 1.5% concentration, some Proteus tend to swarm, producing a luish grey confluent growth. Swarming may be inhibited on MacConkey and on Eosin Methylene Blue agar plate also if the agar concentration is increased to 5%.

Because of their lactose-negative characteristics, Proteus colonies are often selected from isolation plates as suspected Salmonellae, Shigellae, or other gram negative organisms that do not readily show up as lactose positive forms on such media. Since the rapid urease-positive of Proteus, they can be easily differentiated from the pathogenic group (Salmonella, Arizona, and Shigella). They are frequently found in large numbers in stool of individuals undergoing oral antibiotics therapy. They are also incriminated in cases of gastroenteritis both sporadic and of epidemic nature (45).

The Genus Arizona. The Arizona group is composed of lactose fermenting bacteria which are closely related to the Salmonella group.

The biochemical properties of the group are as follow:

Glucose	-	Fermented with gas
Mannitol	-	Fermented
Sorbitol	-	Fermented
Adonitol	-	Not fermented
Dulcitol	-	Not fermented
Inositol	-	Not fermented
Lactose	-	Fermented[(a)]
Salicin	-	Not fermented
Sucrose	-	Not fermented[(b)]
Indol	-	Not produce[(c)]
Potassium cyanide	-	Negative[(d)]
Methyl Red	-	Positive
Voges-Proskauer	-	Negative
Simmons' Citrate	-	Positive
H_2S	-	Positive
Urea	-	Negative
Nitrates	-	Reduced
Motility	-	Positive
Decarboxylases		
Lysine	-	Positive
Arginine	-	Positive, usually delayed
Ornithine	-	Positive

Phenylalanine Deaminase - Negative

Gelatin - Liquefied slowly

Remark:

(a) The majority of cultures ferment lactose promptly but some produce acid from lactose only after 7 to 10 days incubation. Very rarely lactose fermentation cannot be demonstrated.

(b) Sucrose is fermented rapidly by occasional strains.

(c) Occasional strains produce indol.

(d) Almost all cultures of O antigen group 21 are positive, very rarely cultures of other O antigen groups are positive.

Since Arizona strains are related biochemically and serologically to the Salmonella group, they are often mistaken of Salmonellae. Such mistake are not serious since the organisms produce clinically similar disease and the same precautions should be taken to prevent transmission of the infections. The two groups are so closely related that borderline strains are found however, the great majority of Arizona strains ferment lactose and all fail to ferment dulcitol, whereas most of the human pathogens of Salmonella strains fail to ferment lactose.

An antigenic scheme has been established for the group, and it lists over 140 serotypes in 32 different O antigen groups. The relationships between the O antigens and H antigens of Arizona and the O antigen and H antigens of Salmonellae are numerously reported (12).

The identification of other enterobacteria was based on the second edition of Identification of Enterobacteriaceae by Edwards and Ewing (12).

The Genus Vibrio. A genus belongs to the family Spirillaceae which is causative agent of cholera found in intestinal content of cholera patients and convalescents, and in nature as the result of fecal contamination from cholera cases.

Slightly curved rods 0.3 to 0.6 x 1 to 5 μm occur single or joined end to end gram negative, active motile, possesses a single polar flagellum, noncapsulated, no spores. They are aerobic and facultative anaerobic grows best on alkaline media non-pigmented.

Biochemically, the organism fermented glucose, sucrose and mannitol, producing acid but no gas. Lactose fermentation is delayed or negative, while arabinose, xylose, adonitol, dulcitol, salicin and sorbitol are not fermented. Indol is produced and gelatin is liquefied. Nitrate are reduced to nitrite. The organism gives the cholera-red reaction-a reaction which is obtained by cultivating the organism in nitrate-peptone broth and then adding a small amount of sulfuric acid. A red color develops.

Of the more than 30 species of Vibrio described in Bergey's Manual (7), only a few species are pathogenic and of medical importance.

These are Vibrio comma, and Vibrio El Tor. The causitive agents of cholera, Vibrio parahemolyticus encounted in gastroenteritis in Japan. The microaerophelic Vibrio fetus primarily and animal pathogen but isolated occasionally from a few clinical condition in man. Cholera is important in endemic areas. In Thailand Vibrio El Tor are occasionally found as endemic disease and in carrier.

The normal inhibitat of the cholera Vibrio is the human intestinal tract. The acute stage of cholera is characterized by frequent, waterly opalescent stools containing flakes of intestinal mucosa, often described as "rice water" stools. The organisms are eliminated in feces during the disease and about 10 days during convalescence.

Vibrio comma

Culture examination. Although Vibrio comma grows readily on ordinary media (47). It is very sensitive to low pH. On the usual laboratory media, it is difficult to differentiate the Vibrio colonies from the prevalent enteric organisms Vibrio comma tolerates of a relatively high pH, as well as the presence of tellurite in the culture medium.

Isolation and Identification

1. Streak the stool specimens on an enrichment medium such as Monsur agar (26), Bismuth Sulfide medium (pH 9.2) and incubate at 37^{o}C for 24 to 48 hours. At the same time inoculate the stool specimen into a tube of alkaline peptone-water incubate for 6 to 20 hours

at 37°C. The Vibrio grow rapidly and form a film on the surface of the medium; this may be streaked on a nutrient agar plate, pH 8.4 to obtain isolated colonies. Do biochemical tests and serological tests. The Vibrios are separated into several groups on the basis of their O antigens (47). All pathogenic species belongs to O group 1. The certain O antigens present are A, B and C. There are three types Inaba, Ogawa, and Hikojima.

Inaba - containing O antigens A and C.

Ogawa - containing O antigens A and B.

Hikojima - containing O antigens AB (C)

For practical purpose only a single O - group I antiserum containing the Inaba, and Ogawa factors is required to identify the pathogenic Vibrios

Both Vibrio comma and Vibrio El Tor show agglutination with O group I antiserum. About 1% of strains of Vibrio comma isolated from typical cases of cholera are non agglutinable.

Bacteriophage typing Mukerjee (28) isolated four distinct cholera phages, which he used to subdivide strain of Vibrio comma into five types according to their patterns of lysis by the phages. The phagetypes identified are shown in Table 3 less than 1% of strains of Vibrio comma are non typable by the phages used. There is no correlation between the phage types and serological types.

Table 3

Bacteriophage types of Vibrio comma*

Phage	Bacteriophage type				
	1	2	3	4	5
I	+	O	+	O	+
II	+	+	O	O	+
III	+	+	+	+	O
IV	+	+	+	+	+

* Modified from Mukerjee (1963)

\+ indicates lysis

O indicates absence of lysis

(Blair, J.F., Lennett, E.H., and Truant, J.P. 1967. Manual of Clinical Microbiology, p. 234).

Vibrio El Tor

Pathogenic strains of Vibrio El Tor produce a syndrome of gastroenteritis identical to that caused by Vibrio comma. The organisms have been isolated from clinical cases of cholera in Tor and in the Celebes (42), and nonpathogenic strains have been isolated from water.

The morphological straining and cultural characteristic of Vibrio El Tor are identical with those of Vibrio comma, and pathogenic strains of Vibrio El Tor share the same major O - group I antigens with Vibrio comma, however Vibrio El Tor differs from Vibrio comma in its ability to produce hemolysin, and it can be differentiated further by its resistance to polymyxin B and to cholera phage IV.

The stool cultures

The collection of the specimens

In the selection of methods for isolation of pathogenic bacteria from feces it should be born in mind that certain form, e.g. Shigella, may decrease rather rapidly in numbers after the specimen is voided. Therefore it is desirable to plant specimens on isolation mediums as soon as possible. When fresh specimens are available it is advantageous to select bits of epithelium in a greater number of isolations than the indiscriminate streaking of feces on plates.

The rectal swab from hospitalized patients plate immediately yield a slightly higher number of isolations of Shigella than

did the examination of fecal specimens passed by the same individuals (17).

The period of stool collection is important. In the acute stage of the diarrheal disease usually the bacillary incidence are present in large number and often are the predominant organisms in the stool. The numbers of the causative agent rapidly decrease so that in cultures taken after acute stage of the disease, the organisms may be isolated with difficulty or may not be found.

When fecal specimens must be held some time before they are plated, they should be place in a preservation. Buffered glyceral saline solution suggested by is the most widely used as preservative medium Sachs (35) and Coleman. The semisolid thioglycolate medium (40) for transportation of specimens obtained with charcoal treated swabs, and disodium ethylenediamine tetraacetate (37) were recommended with good results.

The culture media

A great variety of culture media have been devised for isolation of enteropathogen. It is evident that no single medium can be used for all purposes. The combination of the media is very useful.

1. Differential media such as Eosin Methylene Blue agar, MacConkey agar, or Leifson Desoxycholate agar, contain certain carbohydrates, indicators and chemicals that are inhibitory to many gram-positive bacteria. Differentiation of enteric bacteria is achieved

through the incorporation of lactose (and sucrose in Eosin Methylene Blue agar). Since the organisms which attack lactose will form colored colonies, white those that do not ferment lactose will appears as colorless colonies. Salmonella and Shigella are in the latter group.

2. Differentially selective media such as Salmonella-Shigella agar, Wilson and Blair Bismuth Sulfite agar, Desoxycholate Citrate agar,and Kauffmann Brilliant Green agar, are complex combinations of nutrients and chemicals that serve to inhibit many coliform bacilli, especially strains of Escherichia and Proteus. They permitted the growth of most Salmonella and many Shigella organisms. These organisms along with slow or lactose - non fermenting organisms, generally form colorless. Some may appear as black or greenish colonies on Bismuth Sulfite agar. The lactose fermenting organisms which are not inhibited grow as pink or red colonies on media other than Bismuth Sulfite agar.

3. Enrichment media.

It is always advisable to use enrichment procedures. This is particularly necessary in examination of feces where the organisms are present in small number. Two satisfactory enrichment media for Salmonella are Selenite - F broth of Leifson (22) and Tetrathionate broth of Mueller (27). The chemicals are tetrathionate (formed through the oxidation of thiosulfate by addition of iodine just prior to inoculation) and selenite salts. They temporaly (12 to 18 hour) inhibit the growth of coliforms while permitting any Salmonella or Shigella present

to multiply both of these liquid media must be subcultured to differential or selective plating media within 24 hours.

Blood agar plate may also be inoculate to obtain the growth of enteropathogenic Escherichia coli (EEC). Since Enteropathogenic Escherichia coli are inhibited by the selective media used for isolation of Salmonella and Shigella (Desoxycholate citrate agar). It is necessary to use the less inhibitory media such as Eosin Methylene blue or Mac Conkey agar to recover them. However, Escherichia coli serotypes are associated with infantile diarrhea has been observed that they did not grow on media such as Mac Conkey agar but the grow well on Blood agar plate (3).

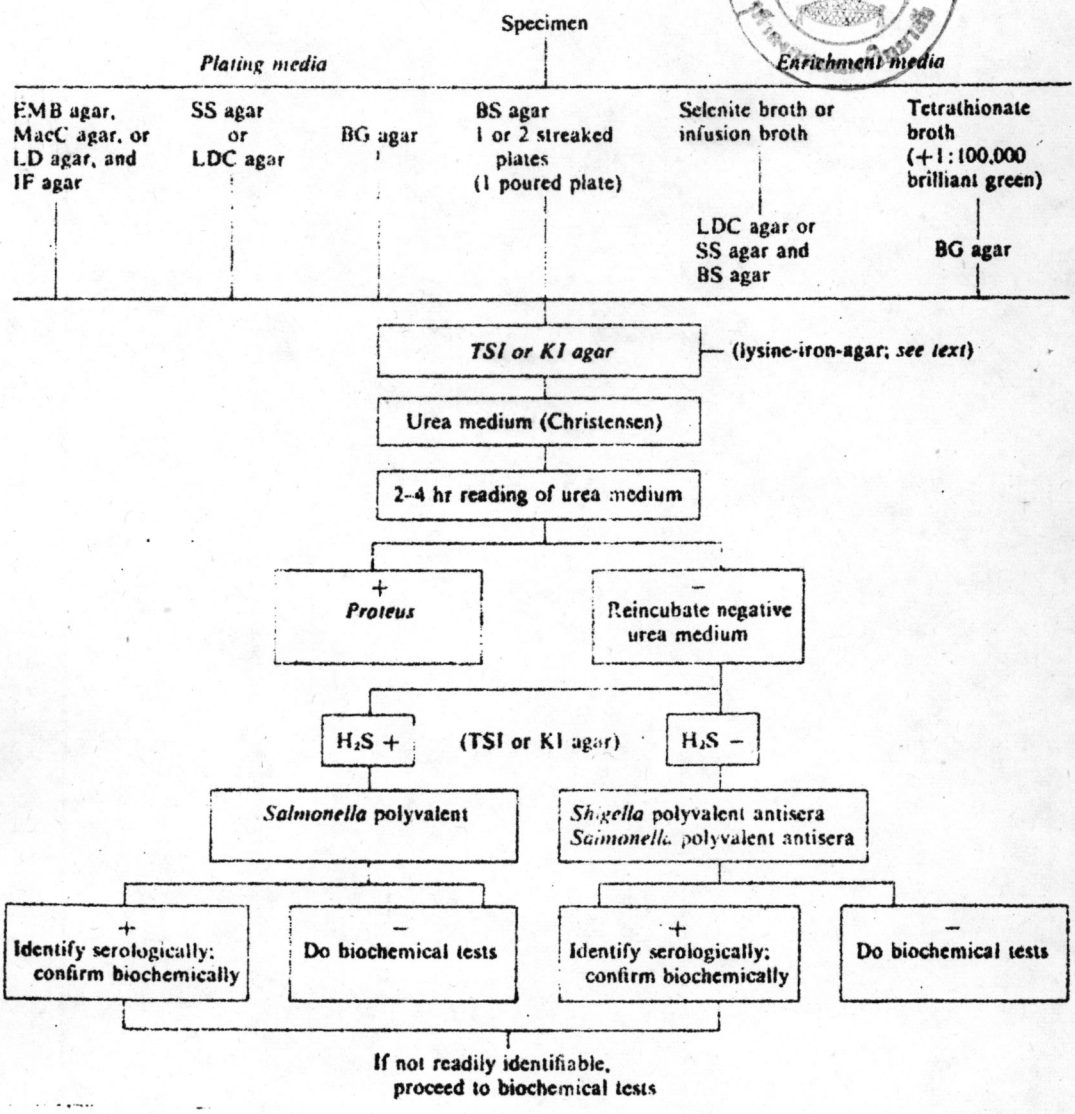
In case of expected Vibrio, an Alkaline Peptone broth (pH 8.4) is used effectively as an enrichment medium. Sodium Desoxycholate Citrate medium (at pH 8.4), Nutrient agar (pH 8) containing 0.5% Sodium taurocholate, and the modified Bismuth Sulfite medium (adjusted to pH 9.2) are very effective plating media. The high pH tends to inhibit other enteric bacteria.

The Isolation

The stool culture procedures which were recommended by American Society for Microbiology (3) are shown in Diagram 2.

The differential selective and enrichment media are used in combination. No single medium can be used for all purposes. The following procedures are obtained for culturing stool for Salmonella,

Diagram 2



Isolation of Enterobacteriaceae from stool specimens. EMB, Eosine Methylene Blue agar; MacC, MacConkey agar; LD agar, Leifson's Desoxycholate agar; LDC agar, Leifson's Desoxycholate Citrate agar; SS agar, Salmonella-Shigella agar; IF agar, Infusion agar; BS, Bismuth Sulfite agar; BG agar, Brilliant Green agar; TSI, Triple Sugar Iron agar; KI, Kligler's Iron agar.

(Blair, J.E., Lennett, E.H., and Truant, J.P. 1967. Manual of Clinical Microbiology, p.153).

Shigella and Enteropathogenic Escherichia coli:

1. Inoculate heavily on one of selective medium for example Salmonella Shigella agar.

2. At the same time slightly inoculate on differential medium e.g. Eosin MethyleneBlue, MacConkey, and a Blood agar plate.

3. Since Bismuth Sulfite is the best medium for Salmonella typhi. It is recommended whenever typhoid fever is suspected. Heavily streak a Bismuth Sulfite agar plate is useful.

4. Also inoculate heavily a tube of Selenite enrichment with fecal specimen. After over night incubation, transfer several loopful of this to an Salmonella Shigella agar or Brilliant Green agar plate and Eosin Methylene Blue agar or Mac Conkey agar plate.

5. Incubation all media at 37°C for 18 to 24 hour and again in 48 hour if indicated (Bismuth Sulfite plates usually require a longer period).

Although Salmonella and Shigella produce typical colorless colonies on these media (except Salmonella typhi, which forms black, opaque colonies on Bismuth Sulfite agar). Transfer from the suspected colonies to Triple Sugar Iron agar slants by making a deep stab to the bottom of the tube and follow by streaking the slant. The Triple Sugar Iron slants are incubated over night and read. The interpretation reaction on Triple Sugar Iron agar slant in shown in Table 4.

Table 4

Interpretation of reactions on Triple Sugar Iron agar

Reaction	Carbohydrates fermented	Possible organisms
Acid butt Acid slant Gas in butt No H_2S	Glucose with acid and gas Lactose and/or sucrose with acid and gas	Escherichia Klebsiella or Enterobacter** Proteus or Providence Intermediate coliforms
Acid butt Alkaline slant Gas in butt H_2S produced	Glucose with acid and gas Lactose and sucrose not fermented	Salmonella Proteus Arizona (certain types) Citrobacter*(certain types)
Acid butt Alkaline slant No gas in butt No H_2S	Glucose with acid only Lactose and sucrose not fermented	Salmonella*** Shigella Proteus Providence
Acid butt Acid slant Gas in butt H_2S produced	Glucose with acid and gas Lactose and/or sucrose with acid and gas	Arizona Citrobacter*
Alkaline or neutral butt Alkaline slant No H_2S	None	Alcaligenes**** Pseudomonas**** Herellea

* Formerly Escherichia freundii.

** Formerly Aerobacter.

*** Salmonella typhi produces a small amount of H_2S but seldom gas.

**** Included here because colonies of these may be confused frequently with lactose-negative members of Enterobacteriaceae and may be selected from isolation plates.

(Bailey, W.R., and Scott, E.G. 1970. Diagnostic Microbiology, p. 142).

Antimicrobial susceptibility tests

The value of the clinical laboratory can be measured only by the significance of susceptibility test which is useful the practicing physician in the treatment of his patient. Since microorganisms differ markedly in resistance and sensitivity to the various antibiotics, not only between genera and species but also between strains of the same species. In vitro susceptibility should be informed along with the pathogen isolated.

In vitro susceptibility of an organism may be accomplished in several ways. The test tube serial dilution technique is a qualitative method which gives a fairly precise determination of susceptibility to a measured amount of the test antimicrobial. The paper disk technique estimates of susceptibility by the measurement of zones of inhibition produced one an agar streak plate. The agar plate dilution method in which graded amounts of antibiotics are incooporated in agar plates and inoculated in sections with the organisms under study. Inhibition of growth at the minimum concentration of antibiotic is taken as the end point. This is a place for all of these methods in routine diagnostic laboratory.

Antimicrobial Agents

Antimicrobial agents must have a selective action against microorganisms as compared with mammalian tissues, i.e. they must possess a

high therapeutic index. This specifity may depend upon the target mechanism being to the microbes than to the mammalian tissues. The basis for such activity is probably often the specific inhibition of particular enzymes.