Enterobacteriaceae

-Classification:

These family classify into many genera there are:

1-Escherichia 2-Enterobacter 3-Klebsiella 4-Proteus 5-Salmonella 6-Shigella ...etc

-General characteristics

1-gram negative ,short rods may form chain or single.

2-motile by peritrichous flagella [1,2,4,5] or non motile [3,6].

3-capsule is large and regular [3],less in [2] and un common in other species.

4-natural habitat in intestinal tract of humans and animal, some of them normal flora such as[1],pathogenic [5,6] and some opportunistic.

5-aerobic and facultative an aerobic.

6-catalase positive* , oxidase negative** , reduce nitrate to nitrite***.

7-ferment a wide range of carbohydrates with gas production [1-5] or without[6].

8-posses a complex antigenic structures.

9-produce a variety of toxins [end toxins and exo toxins].

–specimens : urine , blood , pus , sputum , spinal fluid , stool.

–smear: Gram stain , capsule swelling test.

–culture: samples can cultured on blood agar and macconky agar or EMB agar and incubation at 37c for 24-48h in aerobic or an aerobic condition.

Most genera of these family are grow on routine culture media like blood , mac-conkey , EMB and chocolate agar also on peptone water nutrient broth ....etc.

* this test demon strates the presence of catalase , an enzyme that catalyses the release of oxygen from hydrogen peroxide .

Method : take amount of bacterial growth on slid and add 3 % from H_2O the production of bubbles (gas) is positive reaction

$$2H_2O_2 \xrightarrow{\text{catalase}} O_2 + 2H_2O$$

** This test detection presence cytochrome C . (respiratory enzyme) that will catalyse the transport of electrons between electron donors in the bacteria and a redox dye tetramethyle – p-phenylen diamine (reagent) to purple color

Method : put some drops of reagent on filter paper and put amount of bacterial growth after cultured for 24h at 37c then mixed together , purple color is positive results .

*** inculate organism in broth containing nitrate then incubate at 37c after 4h record the results . if nitrate reducing to nitrite , which reacts to form red coloer with sulfanilic acid and alpha naphthyl amine is positive test .

–culture characteristics

E.coli and most enteric bacteria form circular , convex and smooth colonies with distinct edges

-on mac-conkey agar:

-*E.coli* form large , metallic sheen and pink colonies because it is lactose fermented

-*Enterobacter* colonies are similar but more mucoid , lactose fermented

-*klebsiella* colonies are large and very mucoid and tend to coalesce with prolong incubation appear pale pink because it ferment lactose but does not greatly reduce PH.

-Proteus spp. non lactose fermented appear pale.

–*salmonella* colonies appear creamy white , does not ferment lactose.

–*Shigella* colonies are rough , flat and have irregular edge, which in places is effuse and spreading, non lactose fermented.

On blood and chocolate agar:

All *Enterobacteriaceae* produce similar growth on blood and chocolate agar, the colonies are large , gray and smooth.

–some strains of *E.coli* produce hemolytic (beta hemolytic)

-some species of *klebsiella* form rasied and viscid colonies and smaller than on mac-conkey .

–*Proteus spp*. resulting swarming on blood and chocolate agar by production of thin film of growth on the agar surface.

Biochemical tests

1-Kligler test:

use triple sugar iron agar media (TSI) to help differentiate Salmonella and Shigella from other enteric bacteria in stool culture.

these media contains 1% glucose, 1%sucrose, 1%lactose, ferrous sulfate(for detection of H2S production), tissue extracts(protein growth substrate) and phenol red (PH indicator).

Method: inoculate media and incubate for 24h at 37c then record the results.

If the organism ferment G the slant and butt initially turn yellow ,the small amount of acid produce ,as fermentation products are subsequently oxidized to CO2 and H2S and released from the slant and oxidative decarboxylation of proteins (amino acid) continues with formation of amines ,the slant turns red (alkaline),if the S or L are fermented so much acid is produced that the slant and butt remain yellow (acid).

2-Indol production test:

use tryptone broth media to demonstrates the ability of bacteria to decompose the amino acid tryptophan to indol by tryptophanase enzyme ,indol which accumulates in media then tested by color metric reaction with Kovac's reagent (p-dimethyl amino-benzaldehyde.

Method: inoculate media and incubate for 24h at 37c then add o.5ml from reagent.

Red color ring is positive reaction.

Tryptophane $\xrightarrow{\text{Tryptophanase}}$ indol

3-Citrate utilization test :

use Siummon's citrate agar to demonstrates ability of bacteria to utilize citrate as the sole of carbon and energy source , an ammonium salt as the sole of nitrogen and PH indicator bromo thymal blue is green at PH=6.8 and blue at PH=7.6 .

Method : inoculate media and inculate for 24h at 37c blue color is positive reaction and original green color is negative.

Sodium citrate $\xrightarrow{\text{citrate aldolase}}$ oxalo acetate $\longrightarrow$ pyrovate + CO_2

$CO_2 + Na \longrightarrow NaCO_2$

$Na + H_2O \longrightarrow NaoH$

$NaCO_2$ + $NaoH$ > ALK.

4-Urease test:

use agar base with 5ml from 40% urea solution, to differentiate the bacteria can decompose urea by urease enzyme ,PH indicator is phenol red .

Method: inoculate media and incubate for 24h at 37c,purple-pink color is positive and yellow color is negative reaction.

Urea +2 H_2O $\xrightarrow{\text{Urease}}$ 2 NH_3 + CO_2 ALK.

TSI	*E.coli*	*Enterobacter*	*Klebsiella*	*proteus*	*Salmonella*	*Shigella*
Slant	Acid (Y)	Acid (Y)	Acid (Y)	Alk . (R)	Alk . (R)	Alk . (R)
Butt	Acid (Y)	Acid (Y)	Acid (Y)	Acid (Y)	Acid (Y)	Acid (Y)
Gas	+	+	+	+	+	-
H_2S	-	-	-	+	+ /-	-
Indol	+	-	-	+/-	-	+/-
Citrate	-	+	+	+/-	+/-	-
Urease	-	-	+	+	-	-

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Widal test

is serelogical diagnosis test measures the patient's antibodies against of the somatic antigens (O Ag)and flagellar antigens (H Ag) for the causative agent for typhoid fever Salmonella typhi and Salmonella paratyphi A,B,C....etc.

Method: 1-tube dilution agglutination test ,2-rapid slide titration.

1-preparation serial dilution of the patient's serum by using normal saline.

2-addone drop of each antigen (O,H)to each serum titration.

3-mix well using stick then record the results after 2minute .

4- agglutination of O Ag is granular , blue stained and agglutination of H Ag floccular red stained .

_ recording where the agglutination in (O or H Ag) for *S. typhi* or *S. pharatyphi* and the titration .