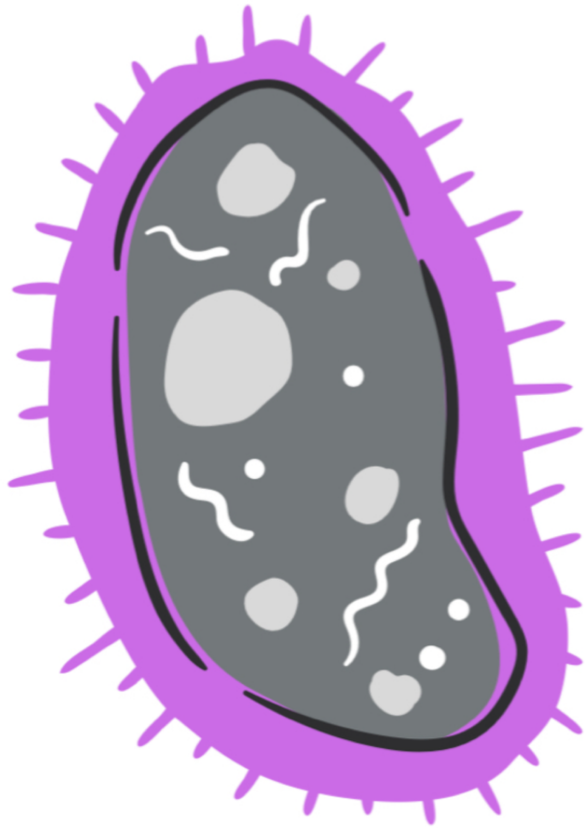
GIS

Sheet no. 3

Microbiology



Done by: Salsabeel Al-Jawabrah
Correction: Saif Al-Tayar
Doctor: Nader Alaridah

Pay attention!

-I will write the slides content that the doctors mentioned, with bold black.

-What was mentioned in the slides while the doctor skipped them, with small letters.

-<u>What doctor focused on and said اللي بهمني تعرفه, with underlined shape .</u>

-The rest of Dr. Nader speech will be written in a normal shape.

……

This topic will be relatively long, will be divided into 2 lectures:

Enteric Gram-Negative Rods (Enterobacteriaceae)

Here we go

The family is too large, we will focus on those related to gastrointestinal tracts infections**

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A. Overview

1. NAMES:

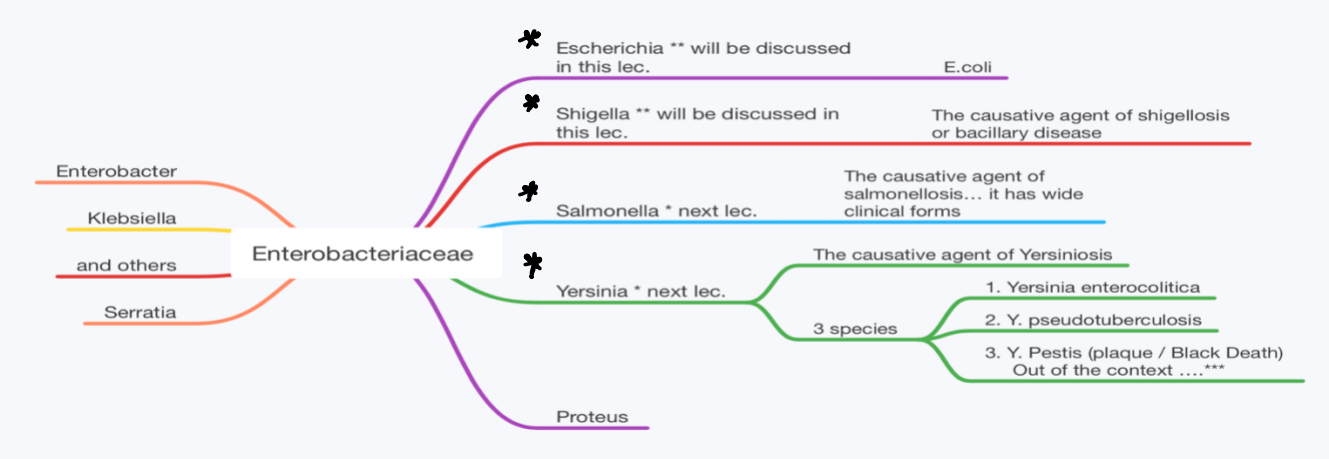
   Enterobacteriaceae **has 2 other names: enteric bacteria & may also be called coliforms** (because they can be part of colonic microbiota or microbiome).

2. SOME FEATURES:

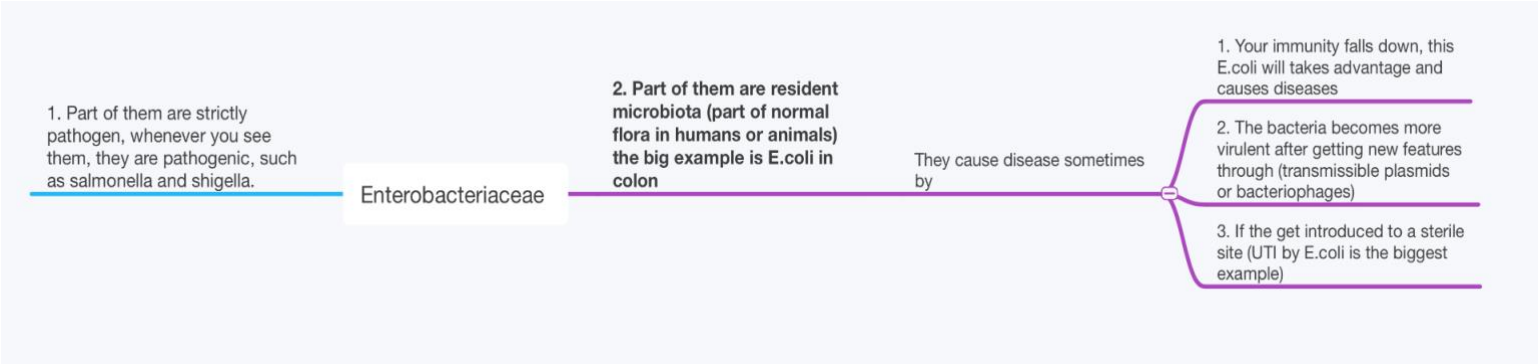
   This family is a large, heterogeneous group (If you will be accepted in microbiology/immunity residency program after fighting these amazing years, you will face a lot of Enterobacteriaceae + streptococcus + staphylococcus…. You will see them a lot!!!) they cause a variety of infections, all types of infections you could imagine!!!

3. GRAM STAIN: They <u>are all gram-negative rods (bacilli).</u>

4. Four genera that we will talk about:



5. Enterobacteriaceae….. Pathogen Vs Microbiota!!!!!!



UTI: urinary tract infection.

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UTI

Out of the context, but Dr. Nader did mention it to clarify how bacteria gets access to sterile sites, so enjoy these information ✍

Some information about UTI that was mentioned in the 3ed mechanism of causing diseases by normal flora:
UTI will cause either:
1.*cystitis* (inflammation of urinary bladder) or 2. *Pyelonephritis* (inflammation in the pelvis of the kidneys).

Don't miss the meaning of sterile site: sites in the body where we don't find any microbe in healthy people (no pathogens, no microbiota).
Sterile sites: blood, CNS and urinary tract! Yeah, urinary tract!

Doctor Nader said (خلي في بالك) that E.coli falls under 3 categories:
1.E.cloi as a part of normal flora inside the intestines and colon, no diseases, no problems, everyone is happy 😃

2.E.coli (uropathogenic E.coli) is the most common cause of urinary tract infection (UTI), how do they do that?
More than 90% of UTI cases, the patient was infected by his own flora, E.coli from the intestines of this patient gets access to the urinary tract, often the perineal flora (something related to personal hygiene), especially in females due to short urethra.

3.What we will talk about (enteropathogenic E.coli) the E.coli that cause gastroenteritis, this type gets virulence factors and starts to attack the GIT (gastrointestinal tract).

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6. MOTILITY:

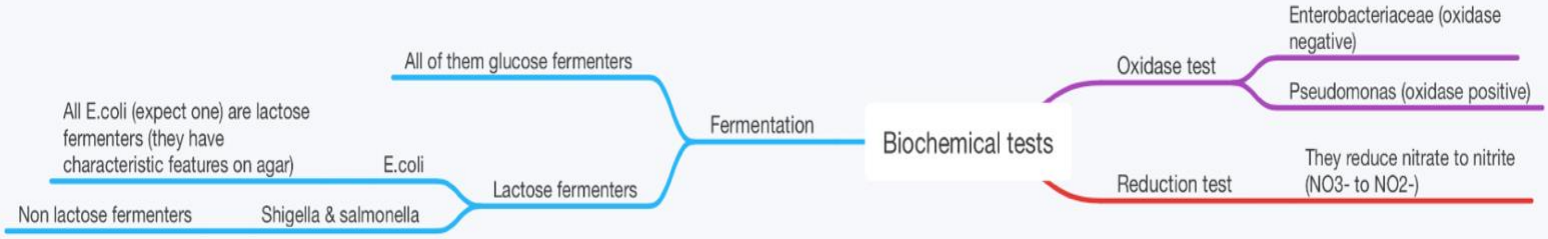
They are either motile (most of them) with peritrichous flagella or nonmotile (just some of them).

Non motile: 1) shigella. 2) klepsiella. 3)yersinia.

7. WHICH ENVIRONMENT THEY PREFER?

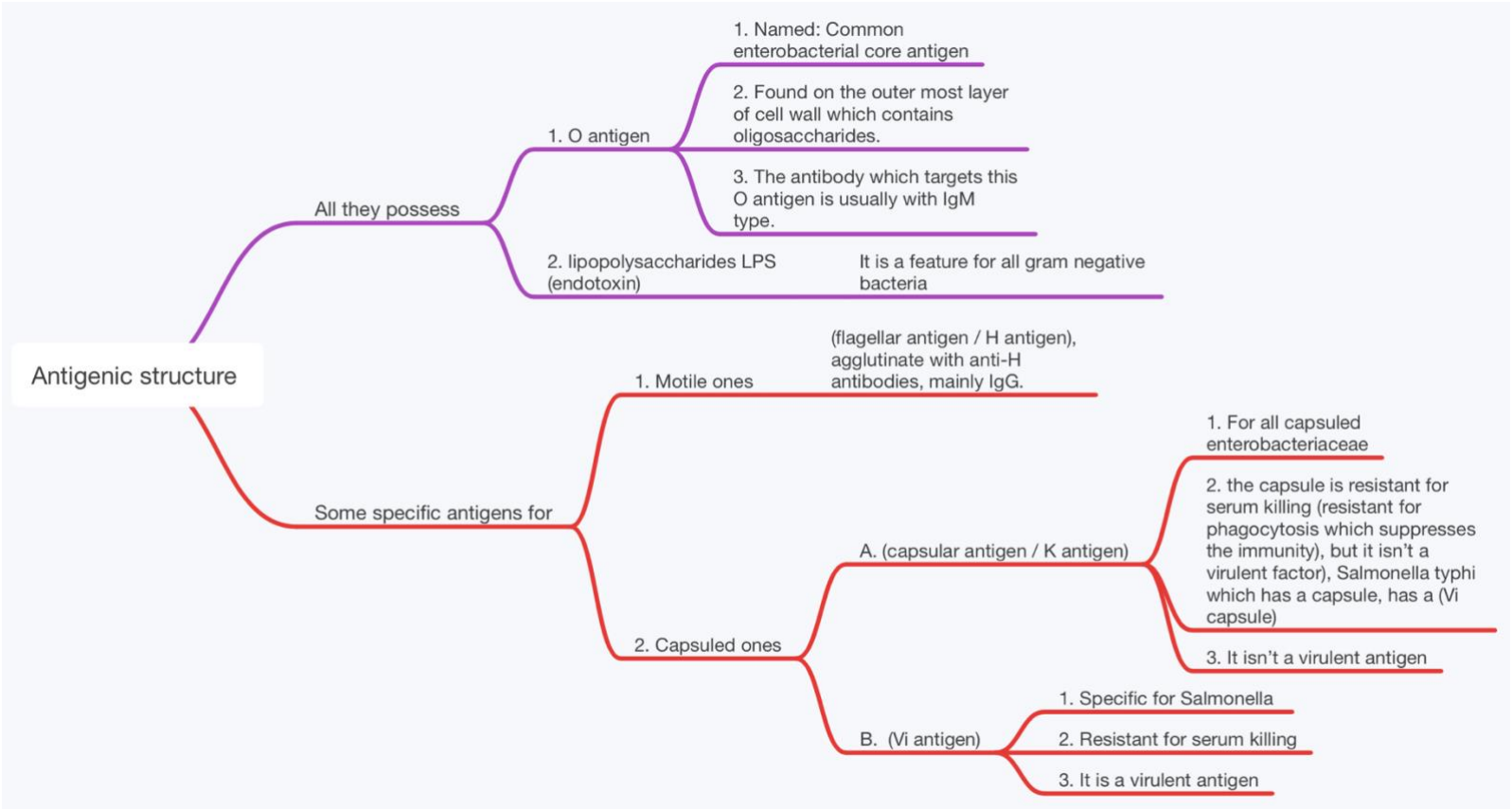
They grow **aerobically** and anaerobically (**are facultative anaerobes**).

8. SOME BIOCHEMICAL TESTS: important



9. ANTIGENIC STRUCTURE:

Why are all of these genera and species are put under one family (enterobacteriaceae / coliform)?! They all have some antigens common between them:



10. Many gram-negative organisms produce Colicins (bacteriocins).

These are peptide compounds produced by the bacteria to reduce the competition by other bacteria, which are produced from the E.coli are specifically called colicins, colicins are used to derive antibiotics from them, maybe it has a bacteria-static effect and that helps it to get more nutrients and achieve more area for growth.

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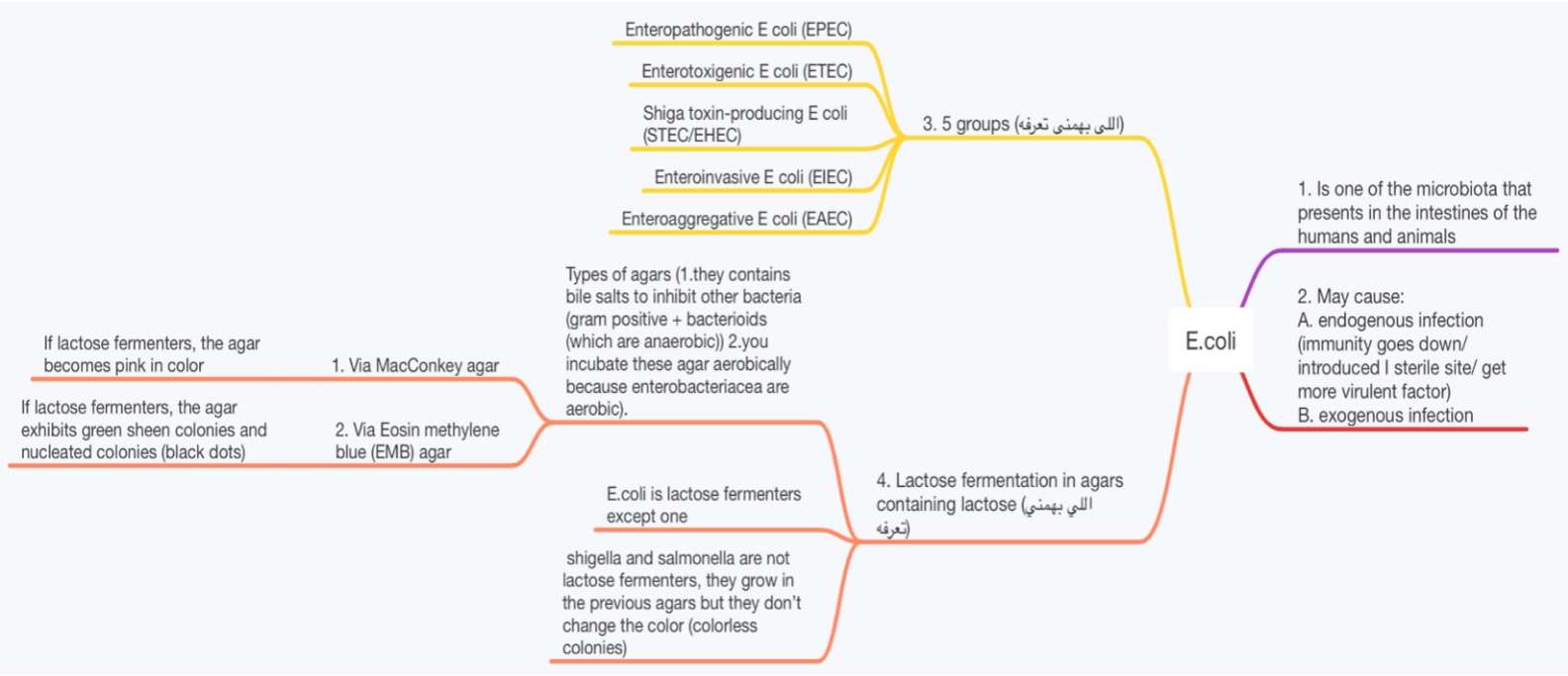
Now, we will talk specifically about E.coli:

B. overview of E.coli

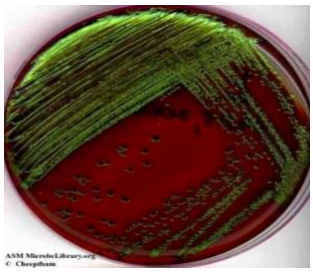
1. General overview of E.coli including biochemical testing:



Typical lab question …..

MacConkey Agar

Notice the pink color that represents lactose fermenters…



EMB Agar

Notice the green sheen color and the nucleated colonies (black dots)

If you get a feces sample, you will find thousands of micro-organisms, so there is a protocol that we should follow to differentiate between the pathogenic and non-pathogenic microbes, we will dig deeper into that protocol in salmonella , in general this protocol includes (in order):

1) enrichment agar plate.
2) Differential agar plate.
3) Selective agar plate.

Mainly for differentiation of E.coli from other microbes we use the feature of the lactose fermentation اللي بهمني تعرفه by using one of two types of agars (MacConkey or EMB) these two agars are the major agars for testing lactose fermenters.

يعني متى ما شكيت ب E.coli او أي بكتيريا بتشتغل على تخمير اللاكتوز راح استخدم هدول الوسطين.

……

After discussing: A. an overview of enterobateriaceae B. An overview of E.coli ,Now C. let's dig deep in the five groups of E.coli (back to the map where I wrote the five groups to take an overview about their names, this will help you in organizing your knowledge):

C. Five groups of E.coli

These five groups cause gastroenteritis (they cause diarrhea)and they don't cause long lived immunity (pay attention to some details in ETEC),:

1- Enteropathogenic E.coli (EPEC)

a. The main cause of **infantile diarrhea**,
   - Nurseries were explosive diarrhea outbreaks take place.
   - Bottle-feed infants (their ages is less than 2 years).

b. The pathogenesis (Two words: **Attachment and Effacement)**
   - This bacteria has fimbriae and villi (not that importance)
   - what is important is to know that there is something called:

.**plasmid encoded genes:**

Bundles forming pilus for attachment to colonic mucosa.

.chromosomal encoded genes:

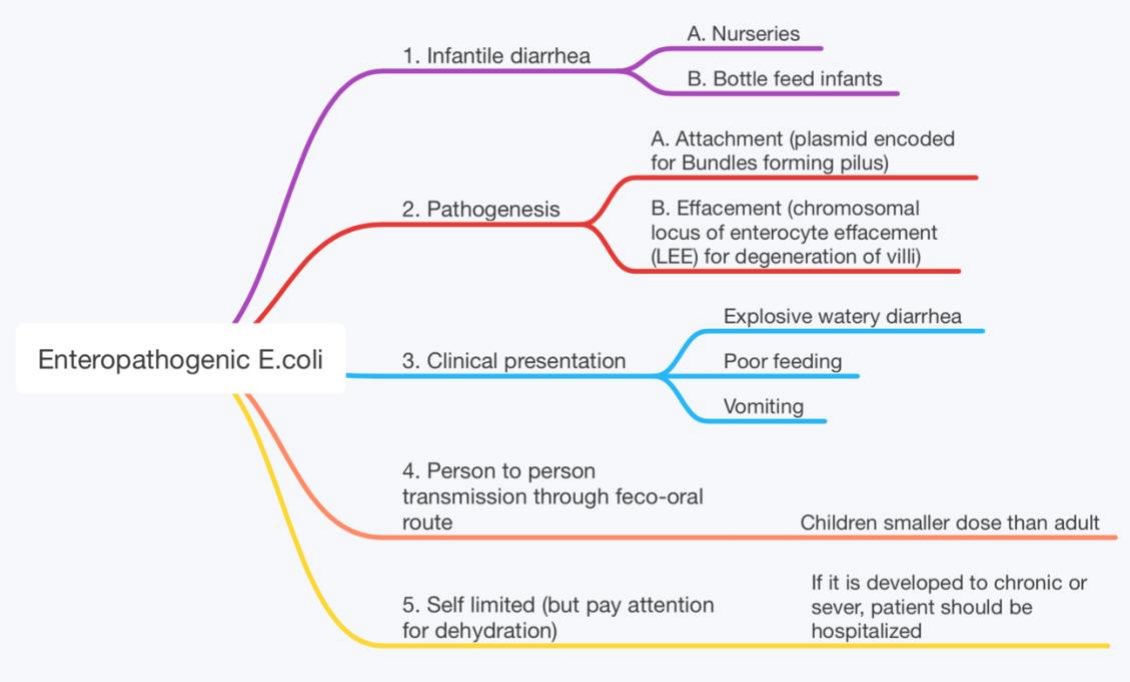
chromosomal locus of enterocyte effacement (LEE) pathogenicity island that promote the tight adherence characteristic of EPEC, but the main mechanism of it is effacement (**degeneration of microvilli or the brush borders of the enterocytes epithelial cells**).

c. The clinical presentation
   - The result of the previous attachment and effacement: explosive **Watery diarrhea (secretory diarrhea)**.
   - The result of EPEC infection in infants is severe, watery diarrhea; vomiting; and fever. Diarrheal stool often contains **mucus but not blood**.

- Person-to-person transmission is common in this one in children population, adults need very high dose children need about 10^3 CFU (colony forming unit). If an infected child with a specific strain was in a nursery, he will transmit the infection with same strain to all children because the fomites and surfaces are contaminated and children put their hands in their mouth (personal hygiene issues), so feco-oral transmission takes place.
- Often self-limited, within (48-72) hours the child will recover, but can be prolonged or chronic, people with sever or chronic infection or any sign of dehydration should be hospitalized .
- Doctors and mothers should take care of dehydration (because of diarrhea they lose a lot of fluids).
- The cornerstone (حجر الأساس) in the treatment regardless of the type of infection in the gastrointestinal tract is the fluid restoration then the antimicrobial or antitoxins.
- The duration of the EPEC diarrhea can be shortened and the chronic diarrhea cured by antibiotic treatment.

d. EPEC diarrhea has been associated with multiple specific serotypes of E coli; strains are identified by O antigen and occasionally by H antigen typing.

e. It doesn't cause immunity.



2- Enterotoxigenic E.coli (ETEC)

a. From its name, there is a toxin to be produced.
b. Traveler diarrhea, travelers who come from the developed countries to developing countries, face gastroenteritis (traveler diarrhea, water diarrhea) due to (hygiene issues).
c. Pathogenesis: mainly toxin but before producing the toxin they should attach.

- ETEC colonization factors (known as colonization factor antigens [CFAs]) specific for humans promote adherence (attachment) of ETEC to epithelial cells (mucosa) of the small bowel. (Attachment)
- The main pathogenesis (toxins), there are 2 toxins cause hyper secretion of fluids and electrolytes and poor absorption of sodium:
  1. Heat stable toxin (ST):
     -cGMP (Guanylyl cyclase)
  2. Heat labile toxin (LT):
     -one A active subunit + 5 active B subunits.
     -cAMP (Adenylyl cyclase).
- Immunogenicity: the ability of body to produce antibodies against the antigen.
  -LT and the colonization factor are immunogenic (but with short lived antibodies for specific strain), the patients will suffer again from the **watery diarrhea (secretory diarrhea).**

3- Shiga Toxin-producing E.coli (STEC) / Enterohemorrhegic E.coli (EHCE) / Verocytotoxin-producing E. coli (VTEC)

I will summarize all information after finishing this type maybe it is useful to read the summary before digging in deep of the information, don't panic about the big amount of information.

a. From the names:
   - Vero cells are monkey kidney cells which are killed by the toxin.
   - Named for the cytotoxic toxins they produce (**the main pathogenesis**).
   - Hemorrhage, **Bloody diarrhea, there are RBCs, mucus and WBCs (leukocytes) in patients' stool.**

b. They start with watery diarrhea then they develop **bloody diarrhea** (there are 2 explanations)has to do with the production of Shiga toxin :
   1. There are at least two antigenic forms of the toxin referred to as Shiga-like toxin 1 and toxin 2 that affect 60S ribosomal subunit (so it inhibits protein synthesis).
   2. it causes occlusion for the capillaries in intestinal mucosa, so once the RBCs are coming and want to transverse through the occluded capillaries so they squeeze ,and as a result they are distributed (hemolysis) without any invasion for the intestinal mucosa so the pathogen stays in the lumen (attached and multiplying), this is the most acceptable explanation.

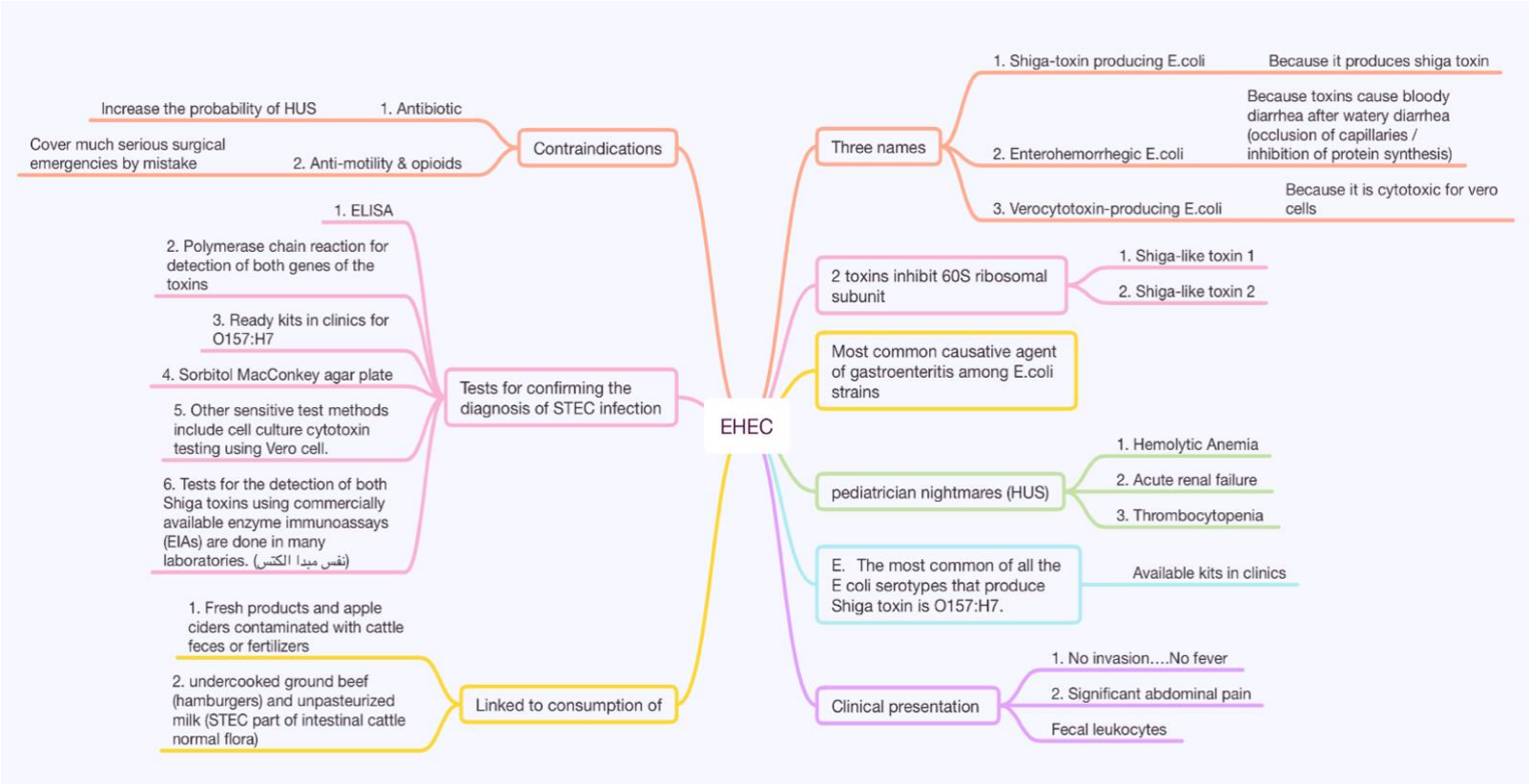
C. It is the most common E.coli strain causative agent of gastroenteritis.
D. Why should we take care of patients with STEC?
   - 10% of patients infected with STEC develop **HUS (hemolytic-uremic syndrome)**. HUS is a disease resulting in microangiopathic *hemolytic anemia (hemolytic)*, *thrombocytopenia* (low platelet count) and *acute renal failure (uremic)*; it occurs frequently in children (pediatrician nightmares).
E. The most common of all the E coli serotypes that produce Shiga toxin is O157:H7. It is the one that can be identified most readily in clinical specimens (kits), you can diagnose it quickly in your clinic.
F. No invasion….No fever, Significant abdominal pain and fecal leukocytes are common (70% of cases).
G. If you doubt that the patient may be infected with STEC (**antibiotics are contraindication**), if you give them anti microbial agents the probability of developing HUS goes up ↑ **anti-motility drugs and opioids are contraindicated as well.**

If your patient has gastroenteritis (bloody diarrhea), avoid giving him opioids (morphine, methadone) the strong analgesics, because you might do a mistake (bloody diarrhea has a wide differential diagnosis from infections and surgical emergencies where you should do a surgical intervention immediately) so you can say: "yeah! It's STEC while in fact it is intussusception (ileum enters the cecum)" and you actually mask acute condition (Note that absence of fever can incorrectly lead to consideration of noninfectious conditions (e.g., intussusception or ischemic bowel disease)), the patient will sleep without any pain and at the morning you will see that he has a septic shock in his abdomen that will cause perforation, so avoid anti-motility agents and opioids in case of gastroenteritis.

H. We should take care about confirming the infection with STEC not just E.coli without knowing which type, and for that purpose you can use:
   1. The options that deal with enzymes, such as :ELISA (enzyme-linked immunosorbent assay, Polymerase chain reaction (search for genes that code for shiga toxin).
   2. Sorbitol MacConkey agar plate is used for diagnosis because EHEC is the only E. coli that doesn't ferment sorbitol→so it will not grow on the plate and thus we can differentiate it from other E. coli strains which are sorbitol fermenters .
      Actually the doctor said the opposite, but after reviewing 018/019 sheet, some scientific papers as well, we know that, I left a part from a research in the end of this sheet.
   3. Other sensitive test methods include cell culture cytotoxin testing using Vero cell.
   4. Tests for the detection of both Shiga toxins using commercially available enzyme immunoassays (EIAs) are done in many laboratories + readily used kits in clinics .
I. Linked to consumption of fresh products (e.g., lettuce, spinach, sprouts), undercooked ground beef (hamburgers), apple cider, and unpasteurized milk.

➢ Why? Because STEC can be found in the intestinal tract of cattle. The vegetables and dropped apples can be contaminated by cattle feces and fertilizers.

A summary for all information …. Summary not new knowledge to learn !!!!!!



4- Enteroinvasive E.coli (EIEC)

a. Name (<u>invasive</u>), so it invades the intestinal mucosa and causes <u>bloody diarrhea</u>. Remember the map of diarrhea 2 Pages before…..
b. The big exception of lactose fermenter rule, <u>this one can not ferment lactose. EIEC strains are **non-lactose or late lactose fermenters**</u>.
c. Unlike shigella, EIEC require large inoculum (10^8–10^10 CFU) to establish infection, shigella needs (100-1000) CFUs.
d. The main pathogenesis is <u>invasion</u>.
e. Produces a disease very similar to shigellosis. The disease occurs most commonly in children in developing countries and in travelers to these countries.

5- Enteroaggregative E.coli (EAEC)

a. There is no big knowledge about it.
b. The confirmation of the presence of this bacteria is expensive and laborious work.
c. Types of diarrhea associated with EAEC:
   1. **<u>Chronic diarrhea</u>**.
   2. Prolonged diarrhea (gastroenteritis) that lasts more than 14 days.
   3. Diarrhea that happens to HIV patients.

d. They are characterized by their specific patterns of adherence to human cells. The organisms exhibit a diffuse or "stacked-brick" pattern of adherence to small intestine epithelial cells (that's why we call them **enteroaggregative**).
e. There is no single pathogenesis. There are a lot of pathogenesis like:
    1. They produce shiga toxin like toxin.
    2. They have adherence.
    3. They have enterotoxin.

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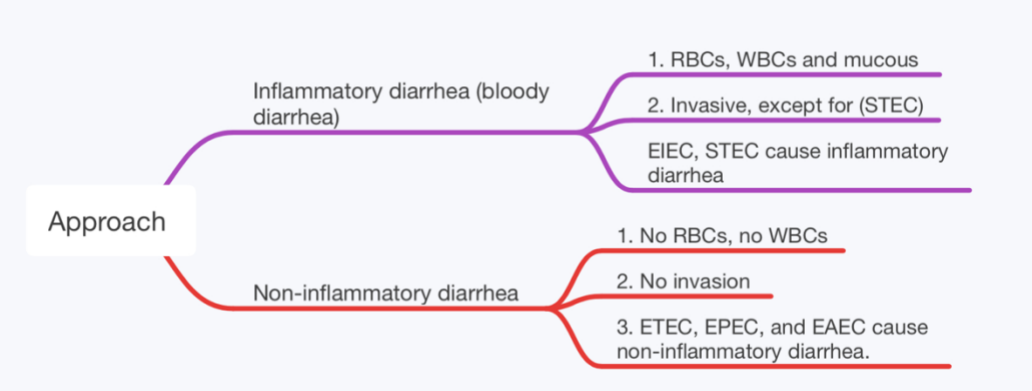
We have finished the 5 groups, now we will talk about:

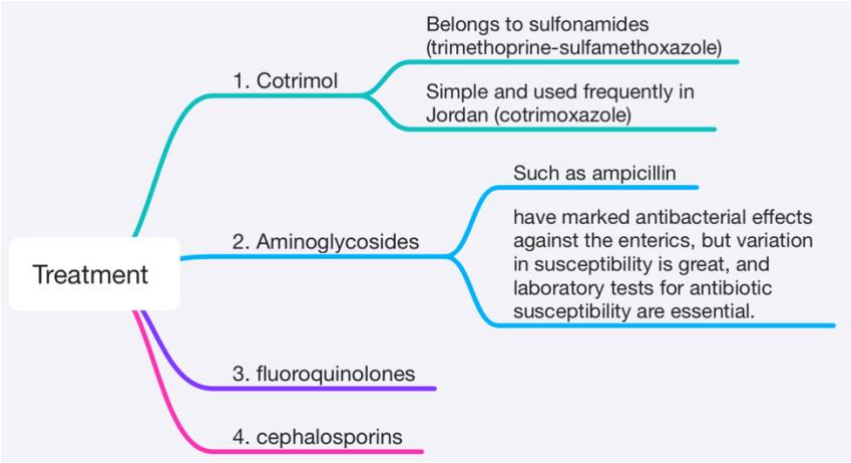
D. The approach

We are interested in the clinical approach because each presentation has different differential diagnosis:



E. Treatment

a. The first line of treating diarrhea is fluid and electrolytes restoration.
b. Some anti-microbial drugs, which are grams negative anti-microbial:



c. The resistance of these drugs are high, you should pay attention to drug sensitivity test.
d. <u>Antibiotic are contraindicated in STEC.</u>

G. Prevention

a. Should pay attention to sanitation, disinfection, sterilization, washing and avoiding the eating of undercooked meat especially (EHEC).

b. Travelers can avoid travelers diarrhea (ETEC) by chemicals as a preventive drugs

- Daily ingestion of (bismuth subsalicylate) suspension, bismuth subsalicylate can inactivate E coli enterotoxin in vitro, firstly this drug was approved as anti acid drug, but also it has a bactericidal effect, has a good efficiency as a preventive drug but not 100%.
- Regular doses of tetracyclines or other antimicrobial drugs for limited periods.

C. Because none of these methods are entirely successful or lacking in adverse effects, caution to be observed in regard to food and drink in areas where environmental sanitation is poor and that early and brief treatment (eg, with ciprofloxacin or trimethoprim–sulfamethoxazole) be substituted for prophylaxis.

H. Control

a. You know that there is **endogenous infection** (difficult to control, has to do with personal hygiene) remember UTI and **exogenous infection.**

b. The doctor didn't comment in this slide:

Control

- The enteric bacteria establish themselves in the normal intestinal tract within a few days after birth and from then on constitute a main portion of the normal aerobic (facultative anaerobic) microbial flora.
- E coli is the prototype. Enterics found in water or milk are accepted as proof of fecal contamination from sewage or other sources. Control measures are not feasible as far as the normal endogenous flora is concerned.
- Enteropathogenic E coli serotypes should be controlled like salmonellae. Some of the enterics constitute a major problem in hospital infection. It is particularly important to recognize that many enteric bacteria are "opportunists" that cause illness when they are introduced into debilitated patients. Within hospitals or other institutions, these bacteria commonly are transmitted by personnel, instruments, or parenteral medications.
- Their control depends on handwashing, rigorous asepsis, sterilization of equipment, disinfection, restraint in intravenous therapy, and strict precautions in keeping the urinary tract sterile (ie, closed drainage).

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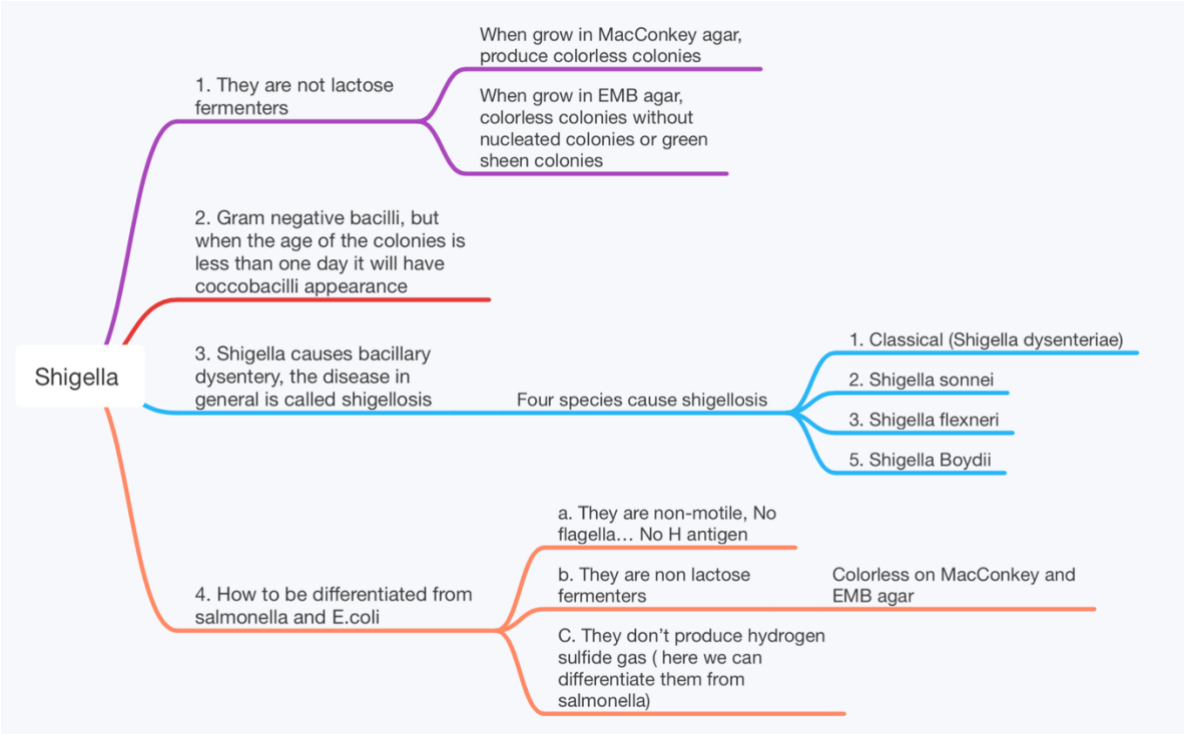
I. Shigella (another genera of enterobacteriaceae)

a. Overview



b. Shigellosis:

❖ **Clinical Findings:**

• After a short incubation period (1–2 days), there is a sudden onset of abdominal pain, fever, and watery diarrhea. The diarrhea has been attributed to an exotoxin acting in the small intestine. A day or so later, as the infection involves the ileum and colon, the number of stools increases; they are less liquid but often contain mucus and blood.

• {Each bowel movement is accompanied by straining and tenesmus (rectal spasms), with resulting lower abdominal pain.}

• In more than half of adult cases, fever and diarrhea subside spontaneously in 2–5 days. However, in children and elderly adults, loss of water and electrolytes may lead to dehydration, acidosis, and even death. The illness caused by S. dysenteriae may be particularly severe.

• On recovery, most persons shed dysentery bacilli for only a short period, but a few remain chronic intestinal carriers and may have recurrent bouts of the disease. Upon recovery from the infection, most persons develop circulating antibodies to shigellae, but these do not protect against reinfection.

- Any age but commonly under 5 or 10 y/o (it's mainly disease of children).
- Many cases are **self-limited**
- Temperate zones and rain seasons.
- In industrialized/ developed countries, the most common serotype is S. sonnei with S. flexneri second.
- In developing countries (like Jordan), the most common one is S.flexneri.

- S.dysentriae (the classical one) is the most serious one with the highest mortality rate.
- The main pathogenesis for all species is <u>invasion</u>, even if they produce shiga toxin, shiga toxin is like a synergistic effect.
- The transmission is eco-oral route, we abbreviated the transmission in four Fs
  1. Food (contaminated food)
  2. Fingers
  3. Feces that contaminate food
  4. Flys
- Shigella needs a low dose to establish the infection (100-1000) CFU or even less.
- Restrictedly, humans pathogen, don't affect animals, so the source is carriers, those are treated but still shedding shigella, **<u>the human is the only reservoir.</u>**
- Shigella is one of enterobacteriaceae family, so it has O antigen, and according to it, shigella has four types:

  - Group A, Shigella dysenteriae (the classical one), the most serious and the less common, it produces shiga toxin while other may not produce shiga toxin and might produce it if they acquire this features (if they are infected by a phage with the gene).
  - Group B Shigella flexneri: {8 serotypes and mild disease}. (most common in developing countries).
  - Group C Shigella boydii: {18 serotypes}.
  - Group D Shigella sonnei single , intermediately sever disease, most common in developed countries.
- it's highly **communicable, small inoculum is needed.**

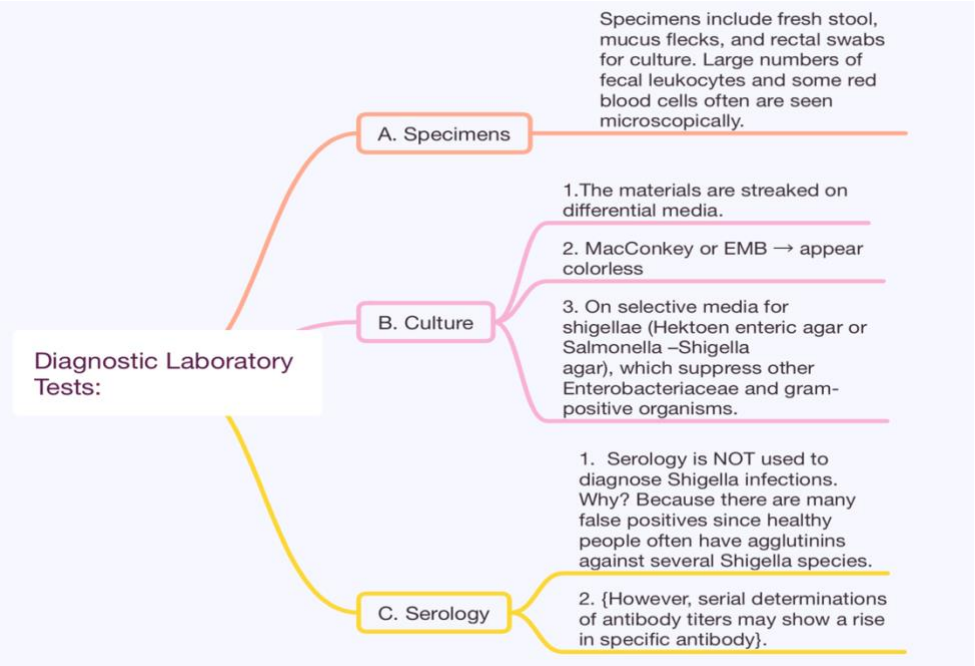
C. Toxins:

- Enterotoxin (lipopolysaccarides) including O antigen, because it is gram negative bacteria, contributes to the irritation of the bowel wall.
- Shigella dysenteriae (the classical one), it produces **exotoxin** (shiga toxin) which inhibits protein synthesis through ribosomal subunit 60S, shiga toxin is:
  1. Neurotoxin. 2. Cytotoxin 3. Enterotoxin

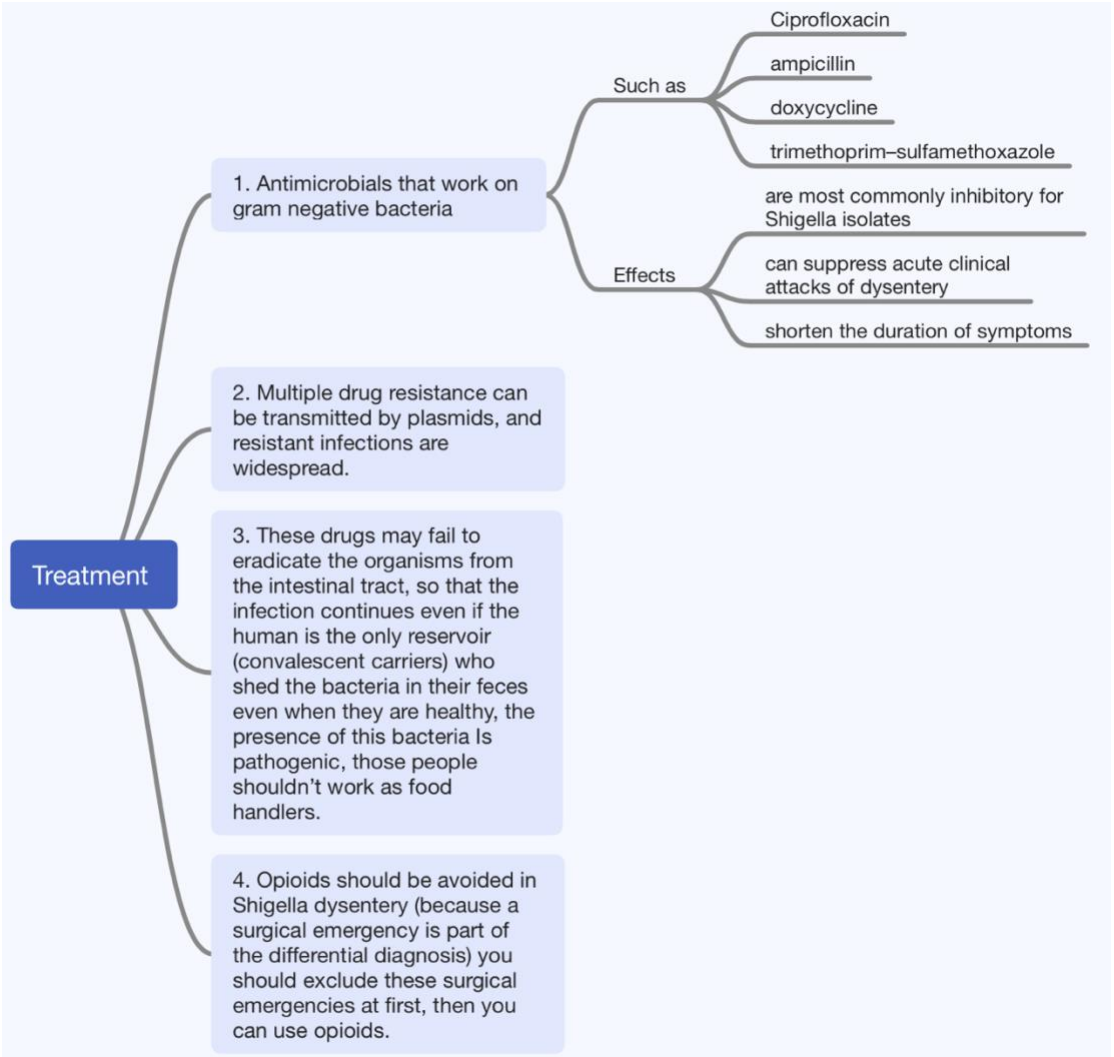
  And that's why s.dysenteriae has a high mortality rate.

  Neurotoxin: when the children are infected they face (bloody diarrhea (invasion), fever, febrile convulsion) one of the differential diagnosis that can result in seizures. Shiga toxins that are produced either E.coli and shigella are not 100% similar, shiga toxin of s.dysenteriae causes febrile convulsion, shiga toxin in E.coli causes HUS.

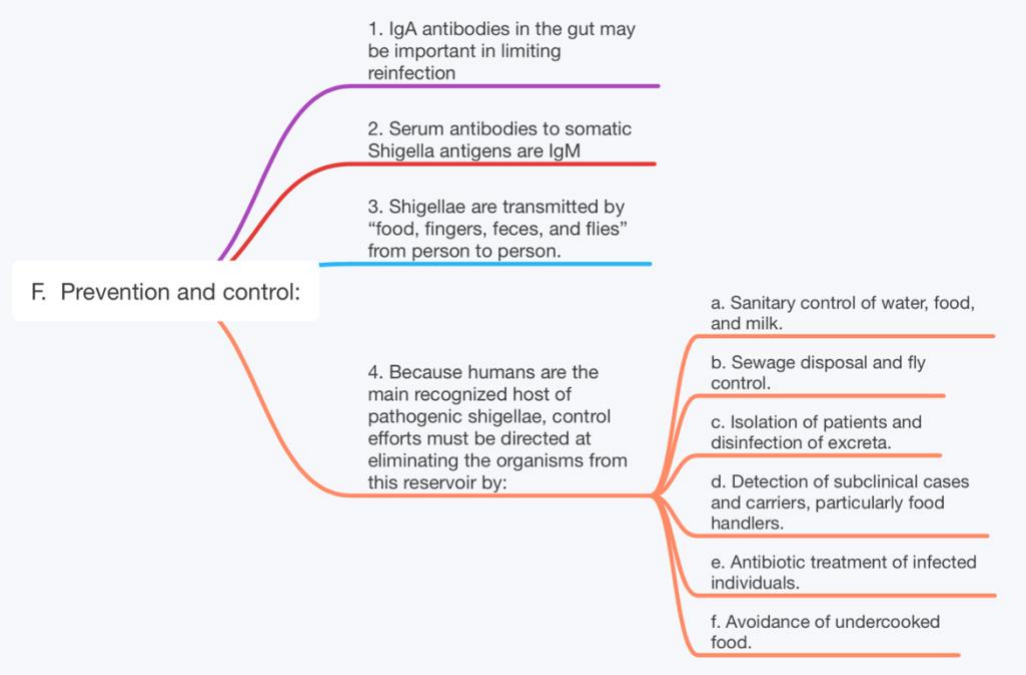
D. Diagnosis:



E. Treatment:



F. Prevention and control:



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Sorbitol-MacConkey medium for detection of Escherichia coli O157:H7 associated with hemorrhagic colitis

S B March, S Ratnam

PMID: 3519658 PMCID: PMC268739 DOI: 10.1128/jcm.23.5.869-872.1986

Free PMC article

Abstract

Escherichia coli serotype O157:H7 is a recently recognized human pathogen associated with hemorrhagic colitis. Unlike most E. coli strains, E. coli O157:H7 does not ferment sorbitol.

The End

Qalsabeel