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Lecture title: Enterobacteriaceae

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Summary: *Enterobacteriaceae*

Escherichia coli

Escherichia coli is usually motile with peritrichous flagella and is often fimbriate. This lactose fermenter produces pink colonies on MacConkey agar and has characteristic biochemical reactions in IMVIC TESTS (Table.1).

Some strains produce colonies with a metallic sheen when grown on Eosin–methylene blue agar. Haemolytic activity on blood agar is a characteristic of certain strains of E. coli.

Somatic (O), flagellar (H) and sometimes capsular (K) antigens are used for serotyping E. coli.

Colonization of the mammalian intestinal tract by *E. coli* from environmental sources occurs shortly after birth. These organisms persist as important members of the normal flora of the intestine throughout life. Most strains of *E.coli* can be regarded as commensal organ and are of low virulence but may cause opportunistic infections in extra intestinal locations such as the mammary gland and urinary tract.

Strains of *E. coli* which produce extra intestinal disease frequently colonize the intestinal tract of normal animals also. In recent years, *E. coli* O157:H7 and other entero- haemorrhagic serotypes have emerged as major food-borne, zoonotic pathogens in humans, responsible for the haemorrhagic colitis–haemolytic uraemic syndrome.

Pathogenesis and pathogenicity

The virulence factors of pathogenic strains of E. coli include capsules, endotoxin, structures responsible for adherence and colonization, enterotoxins and other secreted substances

1. **Capsular polysaccharides**, which are produced by some E. coli strains, interfere with the phagocytic uptake of these organisms. Capsular material, which is weakly antigenic, also interferes with the antibacterial effectiveness of the complement system.
2. **Endotoxin, a lipopolysaccharide**(LPS) component of the cell wall of Gram-negative organisms, is released on death of the bacteria. It is composed of a lipid A moiety, core polysaccharide and specific side chains.

   The role of LPS in disease production includes pyrogenic activity, endothelial damage leading to disseminated intravascular coagulation, and endotoxic shock. These effects are of greatest significance in septicaemic disease.

3• **Fimbrial adhesins** which are present on many strains of *E. coli* allow attachment to mucosal surfaces in the small intestine and in the lower urinary tract. The most significant adhesins in strains of *E. coli* producing disease in domestic animals are K88 (F4), K99 (F5), 987P (F6), F18 and F41.

4• **An adhesin termed intimin**

is associated with attaching and effacing E. coli (AEEC). This adhesin is one of the products of genes encoded in a pathogenicity island termed the locus of enterocyte effacement (LEE).

5• The pathological effects of infection with pathogenic E. coli, other than those attributed to endotoxin, derive mainly from the production of enterotoxins, shigatoxins or verotoxins or cytotoxic necrotizing factors.

6. Two types of enterotoxins, heat-labile (LT) and heat-stable (ST), have been identified. Each type of enterotoxin has two subgroups.

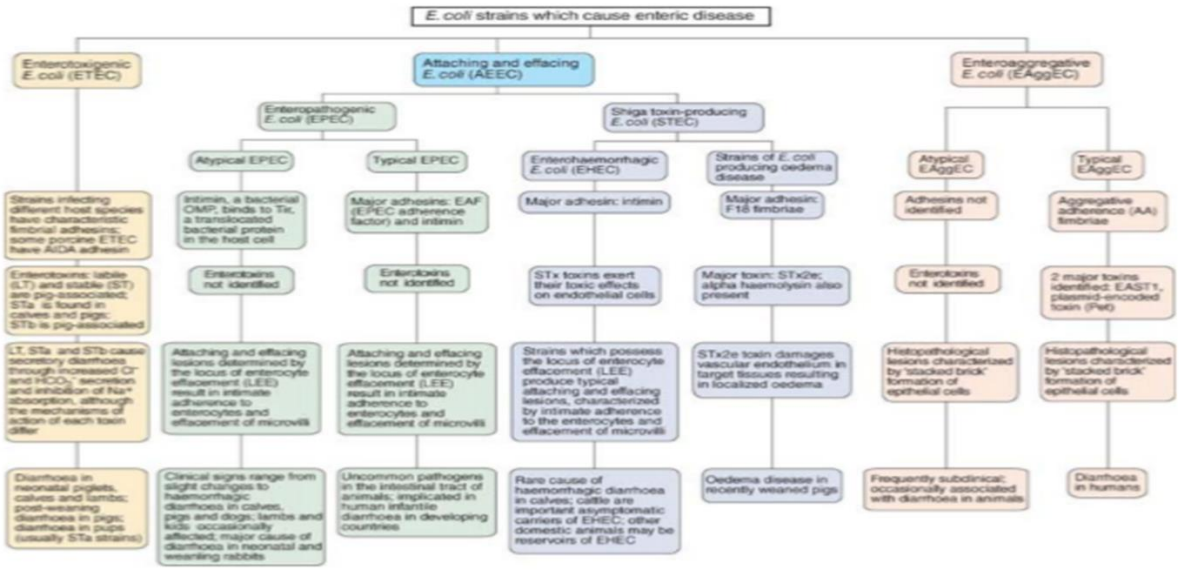
- Enteroaggregative heat stable toxin 1 (EAST1) is found in some Enterotoxigenic *E. coli* (ETEC) and Enteropathogenic *E. coli* (EPEC) strains, and is found in all Enterohaemorrhagic *E. coli* (EHEC) strains.Also Enteroinvasive *Ecoli* (EIEC) IS PRESENT
- Verotoxins (VT), also known as shigatoxins (ST), are similar structurally, functionally and antigenically to the shigatoxin of Shigella dysenteriae. These toxins are heat-labile and lethal for cultured Vero cells.

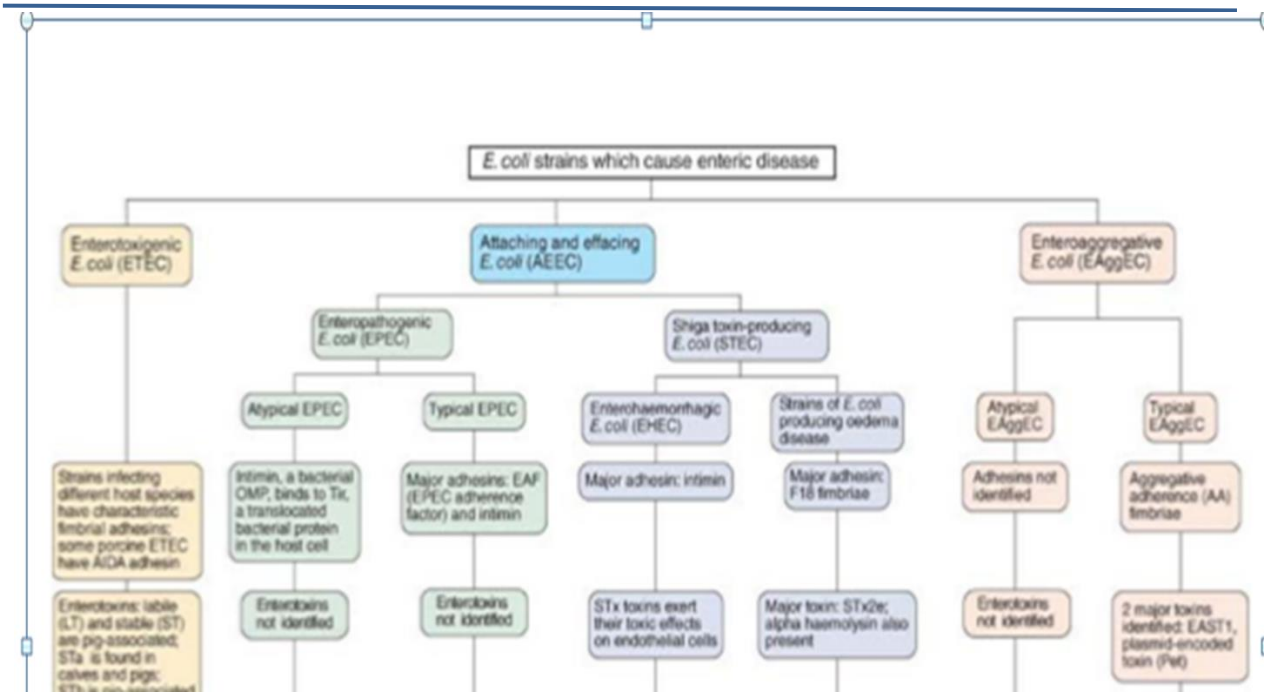
7. Cytotoxic necrotizing factors, CNF1 and CNF2, and recently CNF3), have been demonstrated in extracts of strains of E. coli isolated from extraintestinal E. coli infections in animals and humans.

.8. Alpha-haemolysin, although often a useful marker for virulence in certain strains of *E. coli*, It has been suggested that the action of alpha-haemolysin may increase the availability of iron for invading organisms.

9 • Siderophores, iron-binding molecules such as aerobactin and enterobactin, are synthesized by certain pathogenic strains of *E. coli.* When available iron levels in the tissues are low, these iron-binding molecules may contribute to bacterial survival.

Figure 2 Pathotypes of *E. coli* which produce enteric conditions in animals and humans





Clinical infections

Clinical infections in young animals may be limited to the intestines (enteric colibacillosis, neonatal diarrhoea), or may manifest as septicaemia (colisepticaemia, systemic colibacillosis) or toxaemia (colibacillary toxaemia).

Enteric colibacillosis

Enteric colibacillosis primarily affects newborn calves, lambs and piglets.
Oral infection with a pathogenic strain of E. coli, colonization of the intestine and toxin production are
The incidence and severity of the disease increases under intensive systems of management.
This may reflect heavy exposure of young animals to pathogenic strains of E. coli as a result of build-up of infection in the environment.

Factors which may predispose young farm animals to infection with pathogenic Escherichia coli strains

- Insufficient or no colostral immunity
- Build-up of pathogenicE.colistrains
- Overcrowding and poor hygiene, facilitating increased transmission of organisms
- Normal flora of neonates not fully established
- Naive immune system in neonates
- Receptors for ETEC adhesins are present only during first week of life in calves
- Pigs retain receptors for some adhesins past weaning age (post-weaning diarrhoea)
- Digestive tract of young pigs equipped only for easily digested foods. Accumulation of undigested and unabsorbed nutrients encourages replication of *E. coli*

• Stress factors such as cold ambient temperatures and frequent mixing of animals.

Colisepticaemia

Systemic infections with E. coli are relatively frequent in calves, lambs and poultry. Septicaemic strains of E. coli have special attributes for resisting host defence mechanisms. They invade the bloodstream following infection of the intestines, lungs or umbilical tissues (navel ill) Septicaemic spread throughout the body commonly occurs in calves with low levels of maternally-derived antibodies and the severity of the disease corresponds to the degree of hypo gamma globulinaemia. Colisepticaemia often presents as an acute fatal disease with many of the clinical signs attributable to the action of endotoxin. Pyrexia, depression, weakness and tachycardia, with or without diarrhoea, are early signs of the disease. Hypothermia and prostration precede death which may occur within 24 hours.

Meningitis and pneumonia

are commonly encountered in affected calves and lambs. Postsepticaemic localization in the joints of calves and lambs results in arthritis with swelling, pain, lameness and stiff gait .Watery mouth occurs in lambs up to 3 days of age and has been associated with systemic invasion by *E. coli*. It is characterized by severe depression, loss of appetite, profuse salivation and abdominal distension.
Morbidity rates may exceed 20%.
Mortality in affected lambs is high, many dying within 24 hours of clinical onset. Death DUE TOto endotoxic shock.

In poultry, airsacculitis and pericarditis,

develop following septicaemia. Coligranuloma (Hjärre's disease) is characterized by chronic inflammatory changes resembling tuberculous lesions which are encountered at post-mortem examination in laying hens.

Coliform mastitis

Infection of the mammary glands of cows and sows by members of the Enterobacteriaceae, including *E. coli*, occurs opportunistically. In dairy cows, the source of infection is faecal contamination of the skin of the mammary gland, and relaxation of the teat sphincter following milking increases vulner ability to infection. Peracute disease may be fatal within 24 to 48 hours. Affected animals are severely depressed with drooping ears and sunken eyes. Mammary secretions are watery and contain white flecks.

Urogenital tract infections

Opportunistic ascending infections of the urinary tract by certain uro pathogenic strains of *E. coli* result in cystitis, especially in bitches. These strains possess virulence factors such as fimbriae which facilitate mucosal colonization.

Diagnostic procedures

1 . The age and species of the affected animal, the clinical signs and the duration of illness may suggest the type of infection and the category of disease

2. Suitable specimens include faecal samples from animals with enteric disease, tissue specimens from cases of septicaemia, mastitic milk, samples of midstream urine and cervical swabs from suspected cases of pyometra or metritis.

3 Specimens cultured on blood and MacConkey agar are incubated aerobically at 37°C for 24 to 48 hours.

4• Identification criteria for isolates:

– On blood agar, colonies are greyish, round and shiny with a characteristic smell. Colonies may be haemolytic or non-haemolytic.

–On MacConkey agar, colonies are bright pink.

– IMVIC tests can be used for confirmation (Table.1)

–The colonies of some E. coli strains have a metallic sheen on EMB agar.

–A full biochemical profile may be necessary to identify isolates from coliform mastitis or cystitis.

–Some serotypes are found in association with certain disease conditions. Slide agglutination tests for O and H antigens are employed for serotype identification.

In suspected cases of colisepticaemia, isolation of the organism in pure culture from the blood or from parenchymatous organs is considered confirmatory.

5. When enterotoxigenic strains of E. coli are suspected, the presence of either enterotoxins or fimbrial antigens can be confirmed by immunological methods or molecular techniques such as the polymerase chain reaction.

– Enterotoxins in the small intestine can be detected, using methods employing monoclonal antibodies (Carroll et al., 1990). Some of these reagents are available commercially.

– For expression of fimbrial antigens, isolates should be sub cultured on Minca medium. Fimbrial antigens can be identified using ELISA or latex agglutination (Thorns et al., 1989)

– PCR techniques using primers specific for genes encoding heat-labile and heat-stable enterotoxins may be used to identify enterotoxigenic strains of E. coli.

Treatment

Therapeutic measures are determined by the severity and duration of disease process.

•In calves with neonatal diarrhoea, milk supplemented with fluids containing electrolytes. Severely dehydrated calves require parenteral fluid replacement.

• Calves with hypo gamma globulinaemia can be given bovine gammaglobulin intravenously.

• In most domestic species, enteric diseases may be treated, if required, by oral administration of antimicrobial compounds which are active in the gastrointestinal tract. Systemic and localized infections require parenteral administration of therapeutic agents. **Treatment should be based on susceptibility testing of isolates.**

• Antimicrobial resistance is a major problem with respect to E. coli organisms . Multiple resistance to three or more classes of antimicrobial agents is common in isolates of *E.coli* from pigs and poultry at time of slaughter and in clinical isolates from all animals, both farm animals and pets. Increasing resistance is of importance because resistant *E. coli* may be transferred from animals to humans, either through food or by direction tact.

• Because of the extensive local tissue damage, intramammary treatment of coliform mastitis is often of limited value. Therapy is aimed at counteracting shock and eliminating toxic material from the mammary gland by frequent stripping of affected quarters.

Control

•Newborn animals should receive ample amounts of colostrum shortly after birth.

Colostral antibodies can prevent colonization of the intestine by pathogenic *E. coli*. Absorption of gammaglobulin from the intestine declines progressively after birth and is negligible by 36 hours.

- A clean, warm environment should be provided for newborn animals.
- Dietary regimes may contribute to the development of oedema disease and other post-weaning conditions.

To avoid factors that may contribute to the occurrence of disease, new feed should be introduced gradually

- Vaccination is of value for a limited number of the diseases caused by *E. coli*. Vaccination methods used for prevention of enteric disease in piglets and calves include:

– Vaccination of pregnant cows with purified *E. coli* K99 fimbrial or whole-cell preparations, often combined with rotavirus antigen, can be used to enhance colostral protection.

–Commercially available killed vaccines containing prevalent pathogenic E. coli serotypes can be given to pregnant sows..

–Acommercial vaccine is available for protection against *E. coli* mastitis in cow.