Salmonella

- Salmonellae are usually motile.
- Do not ferment lactose. Rarely, lactose-fermenting strains are encountered.
- The genus *Salmonella* contains more than 2,400 serotypes. Serotyping is based on the Kaufmann and White scheme in which somatic (0) and flagellar (H) antigens are identified.
- Occasionally, capsular (Vi) antigens may be detected.
- In a modification of this scheme**,** two species are proposed, *S. enterica* and *S. bongori. Salmonella enterica* has been divided into six sub-species .
- The majority of salmonellae of veterinary importance belong to *S. enterica subspecies enterica***.** The subspecies are further qualified by the serotype to give a final designation such as *S. enterica subspecies enteric serotype Typhimurium.*

Salmonella serotypes occur worldwide and infect many mammals, birds and reptiles and are mainly excreted in faeces. Ingestion is the main route of infection in salmonellosis although it can also occur through the mucosae of the upper respiratory tract and conjunctiva. Organisms may be present in water, soil, animal feeds, raw meat, and in vegetable material. The source of environmental contamination is invariably faeces. In poultry, some serotypes such as *Salmonella enteritidis* infect the ovaries, and the organisms can be isolated from eggs. Salmonellae can survive in damp shaded soil for up to 9 months.

Pathogenesis and pathogenicity:-

The virulence of salmonellae relates to their ability to invade host cells, replicate in them and resist both digestions by phagocytes and destruction by the complement components of the plasma.
Following adherence, probably through fimbrial attachment, to the surface of intestinal mucosal cells, the bacteria induce ruffling of cell .The ruffles facilitate uptake of the bacteria in membrane-bound vesicles, which often coalesce. The organisms replicate in these vesicles and are eventually released from the cells, which sustain only mild or transient damage. The complex invasion process is mediated by the products of a number of chromosomal genes, whereas growth within host cells depends on the presence of virulence plasmids.

Resistance to digestion by phagocytes and to the lethal action of complement components facilitates the spread of organisms within the host. The toxic oxidative effects of free radicals produced by phagocytes are minimized by bacterial catalase and superoxide dismutase activities.

Resistance to killing by complement is partially dependent on the length of O antigen chains of lipopolysaccharide. Long chains of LPS prevent the complement components of the membrane attack complex from interacting with and damaging the bacterial cell membrane. The LPS is also responsible for the endotoxic effects of infection with salmonellae. It may contribute to the local inflammatory response which damages intestinal epithelial cells and results in the development of diarrhoea. Bacterial cell wall LPS also mediates the endotoxic shock which may accompany septicaemic salmonellosis.

Salmonellosis is of common occurrence in domestic animals and the consequences of infection range from subclinical carrier status to acute fatal septicaemia.

- Some *Salmonella* serotypes such as *Salmonella pullorum* and *Salmonella gallinarum* in poultry, *Salmonella choleraesuis* in pigs and *Salmonella dublin* in cattle are relatively host-specific.
- In contrast, *Salmonella typhimurium* has a comparatively wide host range.
- It is recognized that healthy adult carnivores are innately resistant to salmonellosis.

❖ *Salmonellae* often localize in the mucosae of the ileum, caecum and colon, and in the mesenteric lymph nodes of infected animals.

- ❖ Although most organisms are cleared from the tissues by host defense mechanisms, subclinical infection may persist with shedding of small numbers of salmonellae in the faeces.
- ❖ Latent infections, in which salmonellae are present in the gall bladder but are not excreted, also occur.
- ❖ Clinical disease may develop from subclinical and latent infections if affected animals are stressed. Some of these factors such as transportation and overcrowding have proved to be significant in outbreaks of the disease in young animals and in adult sheep and horses.
- ❖ Salmonellosis in adult cattle is usually sporadic and is also often associated with stress.

Other factors which determine the clinical outcome of infection include:

1- The number of salmonellae ingested
2- The virulence of the infecting serotype or strain
3- The susceptibility of the host. Host susceptibility may be related to immunological status, genetic make-up or age. Young and debilitated or aged animals are particularly susceptible and may develop the septicaemic form of the disease.

In most animal species, both enteric and septicaemic forms of salmonellosis are recorded. A number of serotypes have been associated with abortion in farm animals.
The *Salmonella* serotypes of importance in domestic animals, *Salmonella Dublin* causes a variety of clinical effects in cattle .Terminal dry gangrene and bone lesions are common manifestations in chronic infections with *Salmonella Dublin* in calves.

Clinical infections:-

1-Enteric salmonellosis:-

Enterocolitis caused by salmonella organisms can affect most species of farm animals, irrespective of age.

- ✓ Acute disease is characterized by fever, depression, anorexia and profuse foul-smelling diarrhoea often containing blood, mucus and epithelial casts.
- ✓ Dehydration and weight loss follow and pregnant animals may abort.
- ✓ Severely affected young animals become recumbent and may die within a few days of acquiring infection.
- ✓ On farms with endemic salmonellosis, the milder clinical signs often observed may be attributed to the influence of acquired immunity.
- ✓ Chronic enterocolitis can follow acute salmonellosis in pigs, cattle and horses. Intermittent fever, soft faeces and gradual weight loss, leading to emaciation, are common features of this condition.
- ✓

2-Septicaernic salmonellosis:-

- ✓ The septicaemic form can occur in all age groups but is most common in calves, in neonatal foals and in pigs less than four months of age.
- ✓ Onset of clinical disease is sudden with high fever, depression and recumbency.
- ✓ If treatment is delayed, many young animals with septicaemic salmonellosis die within 48 hours.
- ✓ Surviving animals can develop persistent diarrhoea, arthritis, meningitis or pneumonia.

In pigs with septicaemic *Salmonella choleraesuis* infection, there is a characteristic bluish discoloration of the ears and snout. Intercurrent viral infections often predispose to severe clinical forms of the disease.

3-Salmonellosis in poultry

Salmonella pullorum, Salmonella gallinarum and *Salmonella enteritidis* can infect the ovaries of hens and be transmitted through eggs. The presence of *Salmonella enteritidis* in undercooked egg dishes may result in human food poisoning.

a- Pullorum disease or bacillary white diarrhea ***(****Salmonella pullorum*) infects young chicks and turkey up to 2 to 3 weeks of age. The mortality rate is high and affected birds huddle under a heat source and are anorexic, depressed and have whitish faecal pasting around their vents. Characteristic lesions include whitish nodes throughout the lungs and focal necrosis of liver and spleen**.**

b- Fowl typhoid ***(****Salmonella gallinarum*) can produce lesions in young chicks similar to those of pullorum disease. However, in countries where fowl typhoid is endemic, a septicaemic disease of adult birds occurs, often resulting in sudden deaths. Characteristic findings include an enlarged, friable, bile-stained liver and enlarged spleen.

Salmonella Pullorum *Salmonella Gallinarum*

bacillary white diarrhea Pullorum disease. Multiple grey nodules in the heart.

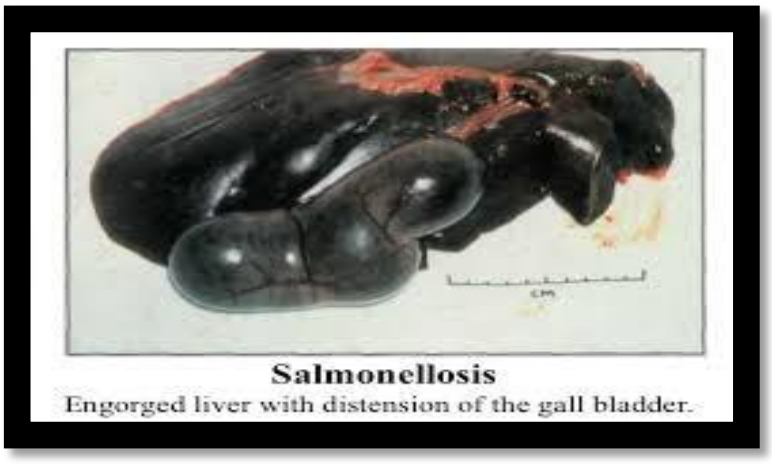


Fig. **Salmonellosis in poultry**

Stress factors which may activate latent or subclinical salmonellosis:-

1- Intercurrent infections.
2- Transportation.

3- Overcrowding.
4- Pregnancy
5- Extreme ambient temperatures.
6- Water deprivation.
7- Oral antimicrobial therapy.
8- Sudden changes in rations altering the intestinal flora.
9- Surgical procedures requiring general anesthesia.

Diagnosis

1- A history of previous outbreaks of the disease on the premises, the age group affected and the clinical picture may suggest salmonellosis.

2- At postmortem, enterocolitis with blood-stained luminal contents and enlarged mesenteric lymph nodes are commonly observed.

3- Laboratory confirmation is required. Specimens for submission should include faeces and blood from live animals. Intestinal contents and samples from tissue lesions should be submitted from dead animals and abomasal contents from aborted fetuses. Isolation of salmonella from blood or parenchymatous organs is deemed to be confirmatory for septicemic salmonellosis.

4- A heavy growth of salmonellae on plates directly inoculated with faeces, intestinal contents or fetal abomasal contents strongly suggests the aetiological involvement of the pathogen. Recovery of small numbers of salmonella from faeces is usually indicative of a carrier state.

5- Specimens should be cultured directly onto BG and XLD agars and also added to selenite F or tetrathionate broth for enrichment and subsequent subculture .The plates and enrichment broth are incubated aerobically at 37^{o}C for up to 48 hours. Subcultures are made from the enrichment broth at 24 and 48 hours.

Identification criteria for isolates:

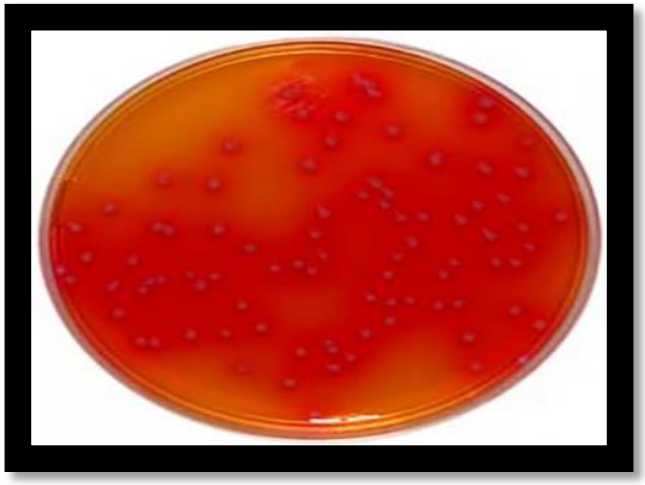
a- On brilliant green agar, colonies and medium are red indicating alkalinity. On XLD agar, colonies are red (alkaline) with a black center, indicating H2S production.
b- Suspicious colonies, subcultured from the selective media into TSI agar and lysine decarboxylase broth, should be examined after incubation for 18 hours at 37^{o}C to establish their biochemical identity as salmonella.

c- If reactions in TSI agar and lysine decarboxylase broth are in conclusive, a biochemical profile using a battery of biochemical tests may allow definitive identification.
d- The isolates from the TSI agar slant are confirmed as salmonellae using commercially available antisera for ***O*** and H antigens in a slide agglutination test. Serotypes with ***O*** antigens in common are assigned to a serogroup.

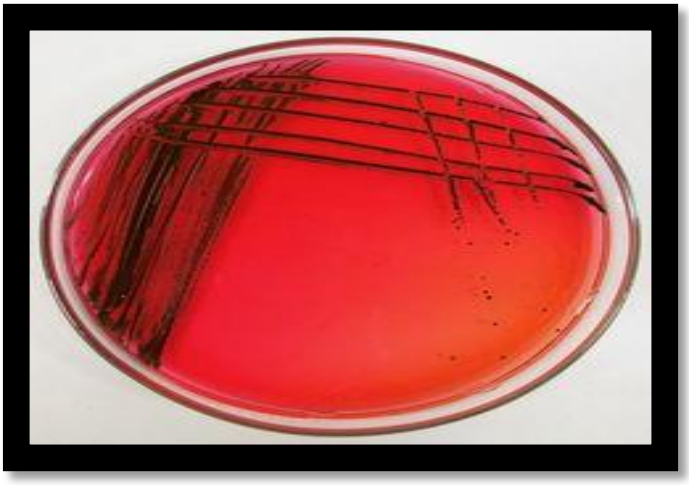
e- Serotypes which have flagellar (H) antigens in two phases, phase 1 (specific) and phase 2 (non-specific) are termed diphasic. The antigens in both phases must be determined. The majority of organisms in these serotypes usually possesses **H** antigens in a single phase and is agglutinated by the appropriate antiserum.
f- Bio-typing is required for serotypes which are antigenically indistinguishable such as *Salmonella pullorum* and *Salmonella gallinarum*.
g- Phage typing is used in epidemiological studies to identify isolates with specific characteristics such as multiple resistances to antibiotics and enhanced virulence.

h- Serological tests such as ELISA and agglutination techniques are of greatest value when used on a herd or flock basis. A rising antibody titer using paired serum samples is indicative of active infection.

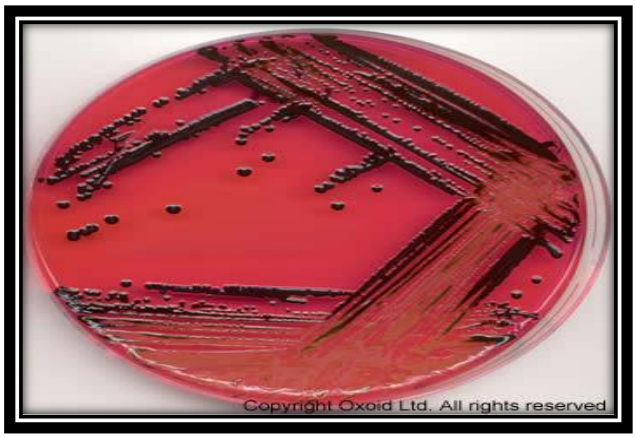
i- DNA probes can be used to screen large numbers of faecal samples for salmonellae.



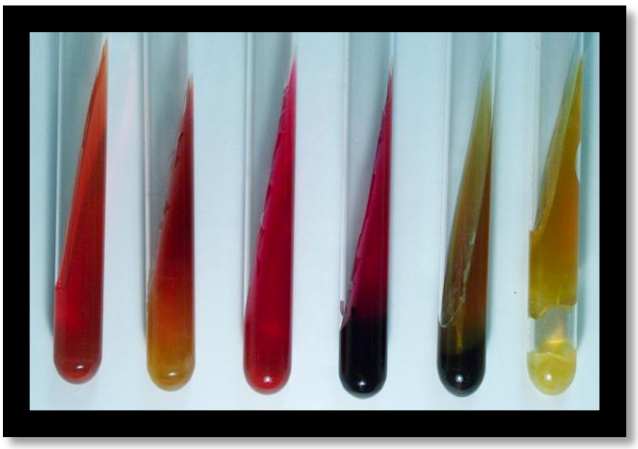
XLD agar



Brilliant Green Agar



S.S agar



TSI

Fig 1.61 cultural characteristic and biochemical test

Yersinia

Yersinia species are non-lactose fermenters
Are motile with the exception of ***Y.*** *pestis*.
Although there are more than 10 *Yersinia* species, only *Y. pestis, Y. enterocolitica* and *Y. pseudotuberculosis* are pathogenic for animals and man.
Yersinia ruckeri causes perioral hemorrhagic inflammation in some species of fish.
Growth of yersinia tends to be less rapid than other members of the *Enterobacteriaceae***.**
They characteristically demonstrate bipolar staining in Giemsa-stained smears from animal tissues.
Of the ten serotypes of *Y. pseudotuberculosis***,** serotypes **I,** II and I11 contain the majority of pathogenic isolates.
There are five biotypes and more than 50 serotypes of *Y. enterocolitica*. Serotype O9 is of particular importance because it shares common antigens with *Brucella* species and it may induce false positive reactions in *Brucella* agglutination tests.

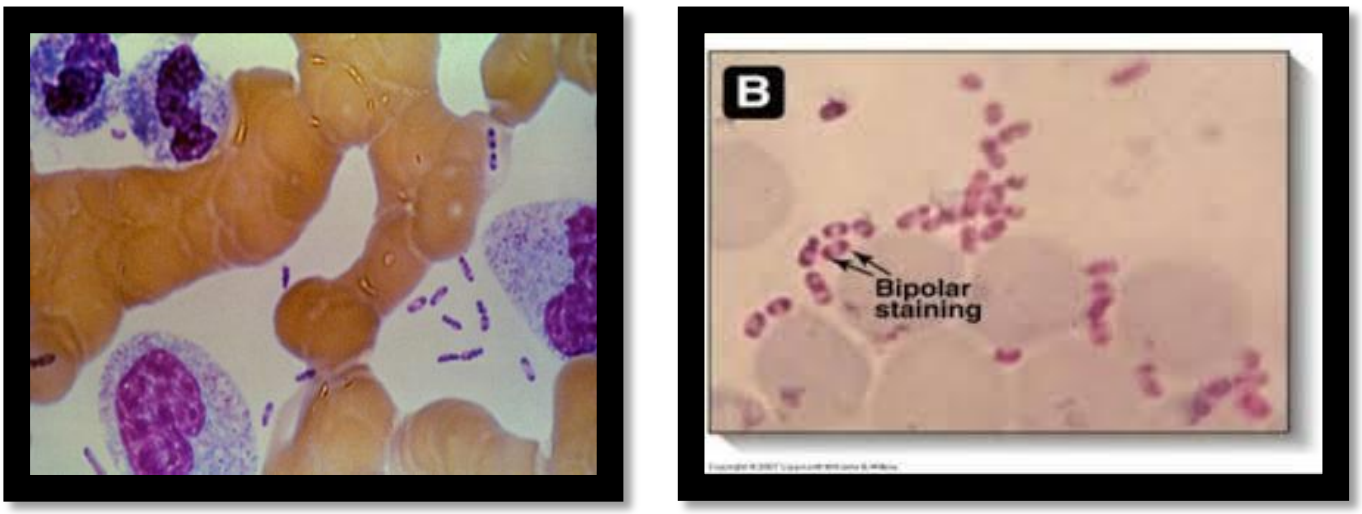


Fig1.62 ***Y.*** *pesti*s Bipolar staining in Giemsa-stained smears

Pathogenesis and pathogenicity:-

Pathogenic *yersinia* are facultative intracellular organisms which possess plasmid and chromosomal encoded virulence factors, many of which are required for survival and multiplication in macrophages. *Yersinia pseudotuberculosis* and *Y. enterocolitica* are less virulent than *Y. pestis* and rarely produce generalized infections. The pathogenesis mechanisms in enteric disease caused by *Y. enterocolitica* and *Y. pseudotuberculosis* are incompletely understood.

It is probable that both organisms gain entry to the mucosa through M cells of Payer's patches. Adhesion to and subsequent invasion through these cells are facilitated by factors such as invasion and adhesion invasion proteins which have an affinity for integrins on cell surfaces. Once in the mucosa, the bacteria are engulfed by macrophages in which they survive and are transported to the mesenteric lymph nodes. Replication in the nodes follows with the development of necrotic lesions and neutrophil infiltration. Survival of *Y. pseudotuberculosis* and *Y. enterocolitica* is enhanced by anti-phagocytic proteins secreted by the organisms which interfere with the normal functioning of neutrophils in the host.

Yersinia pestis is more invasive than *Y. pseudotuberculosis* and *Y. enterocolitica* and possesses additional virulence factors. These include an antiphagocytic protein capsule (Fraction 1) and a plasminogen activator which aids systemic spread. Endotoxin, with properties similar to the endotoxin produced by other members of the *Enterobacteriaceae***,** also contributes to the pathogenesis of disease.

Table 1: The consequences of infection with *Yersinia* species.

Yersinia species	Hosts	Clinical infections:
Y. enterocolitica	Pigs, other domestic animals, wild life Ewes Humans	Subclinical enteric infections, occasionally enteritis. Sporadic abortion Gastroenterocolitis
Y. pseudotuberculosis	Farmed deer, sheep, goats, cattle, buffaloes, pig Cattle, sheep, goats Humans	Enteritis in young animals, subclinical infections common in older animals, mesenteric lymphadenitis. Sporadic abortion Enterocolitis, mesenteric lymphadenitis
Y. pestis	Humans Rodents Cats	Bubonic and pneumonic Plague Sylvatic plague Feline plague

Clinical infections:-

Yersinia pseudotuberculosis causes enteric infections, in a wide variety of wild and domestic animals which are often subclinical. The septicaemic form of disease, known as pseudotuberculosis, can occur in laboratory rodents and aviary birds. Sporadic abortions caused by *Y. pseudotuberculosis* have been reported in cattle**,** sheep and goats.

1-Enteric yersiniosis:-

Enteritis caused by *Y. pseudotuberculosis* is relatively minimized. Common in young farmed deer in New Zealand and Australia. Outbreaks of the disease have been reported also in buffaloes in Brazil. Enteric

disease has been reported in sheep, goats and cattle less than one year of age. Subclinical infection in many species is common and clinical disease may be precipitated in the winter months by stress factors such as poor nutrition, weaning, transportation and cold wet conditions.

Enteritis in young deer and lambs is characterized by profuse watery diarrhoea, sometimes blood-stained, which may be rapidly fatal if untreated. The luminal contents of the small and large intestine are watery at postmortem examination. Severely affected animals may show mucosal ulceration. The mesenteric lymph nodes are often enlarged and oedematous and scattered pale necrotic foci may be present in the liver.
A clinically similar but less severe enterocolitis caused by *Y. enterocolitica* has been described in young ruminants.

2- **Septicaemic yersiniosis:-**

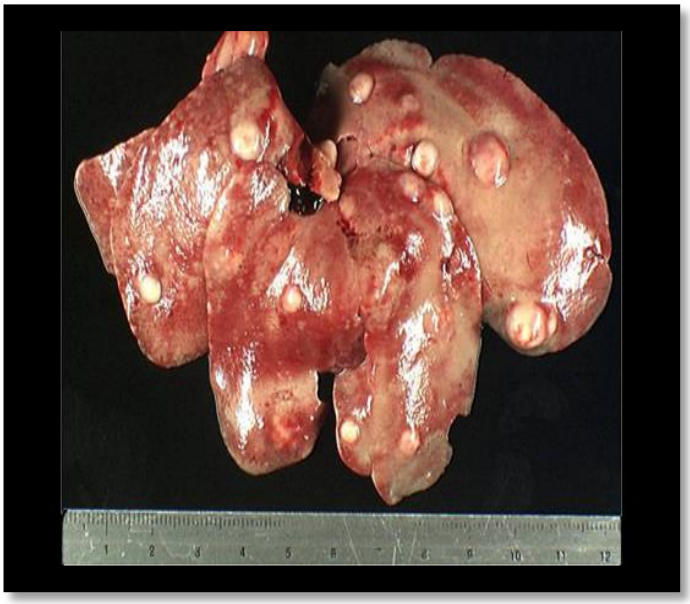
Septicaemia, caused by *Y. pseudotuberculosis* occurs in birds kept in cages or aviaries. It is presumed that infection is acquired through contact with the faeces of wild birds or rodents, or through the feeding of contaminated leafy plants. In aviaries**,** overcrowding may predispose to the development of disease. Infected birds may die suddenly. Pin-point white necrotic foci are present in the liver at postmortem. Confirmation is based on the isolation and identification of Y. *pseudotuberculosis* from the liver and other internal organs. Treatment is seldom feasible due to the acute nature of the disease. Control should be aimed at preventing faecal contamination of food and water by wild birds and rodents.

3- Feline plague

Cats usually acquire infection with *Y. pestis* by ingestion of infected rodents. Three clinical forms of the disease are recognized: bubonic, septicaemic and pneumonic. The most common form of the disease is characterized by enlarged lymph nodes (buboes) associated with lymphatic drainage from the site of infection. Clinical signs include fever, depression and anorexia. Affected superficial lymph nodes may rupture, discharging serosanguineous fluid or pus. Septicaemia may occur without lymphadenopathy and is potentially fatal. Pneumonic lesions may result from haematogenous spread.

Cats with pneumonic lesions are a potential source of human infection through aerosol generation and should be euthanized. Human infection can also be acquired through cat scratches and bites and possibly through the bites of fleas from infected cats. Care should be taken when handling infected animals.

Yersinia Enterocolitica



Y. pseudotuberculosis

Septicaemia *Yersinia pseudotuberculosis*

Feline plague

Fig 1.63 *Yersinia pseudotuberculosis* in domestic animals.

Opportunistic pathogens:-

These groups of enterobacteriaceae, which rarely cause enteric disease in domestic animals, are sometimes involved in localized opportunistic infections in diverse anatomical locations. Faecal contamination of the environment accounts for widespread distribution of the organisms and contributes to the occurrence of opportunistic infection.

Predisposing factors include intercurrent infection, tissue devitalization and the inherent vulnerability of certain organs.

These opportunistic invaders have characteristics which may allow them to circumvent host defense mechanisms and colonize and survive in affected organs.

- *Klebsiella pneumoniae* and *Enterobacter species* produce abundant capsular material which may inhibit phagocytosis and enhance intracellular survival.

- Adhesins are of particular importance in those bacteria which colonize the lower urinary tract.
- Siderophores produced by some opportunistic pathogens, contribute to bacterial survival when the supply of available iron in tissues is limited.
- Some toxic effects of these opportunistic pathogens are attributable to release of endotoxin from dead bacteria. This can induce local and systemic changes which include inflammatory responses, pyrexia, endothelial damage and micro-thrombosis.

Clinical infections

The clinical conditions arising from infections with opportunistic members of the *Enterobacteriaceae* are presented in Table1.28.

Table 1.28 Opportunistic pathogens in the *Enterobacteriaceae* and their associated clinical conditions.

Bacterial species	Clinical Finding
Enterobacter aerogenes	Coliform mastitis in cows and sows
Klebsiella pneumoniae	Coliform mastitis in cows; endometritis mares; pneumonia in calves and foals; urinary tract infections in dogs
Morganella morganii subsp. morganii	Ear and urinary tract infections in dogs
Proteus mirabilis and P.vulgaris	Urinary tract infections in dogs and horses; associated with otitis
Serratia marcescens	Bovine mastitis

Diagnostic procedures:-

1- Clinical signs
2- Specimens for examination should be collected from the infected organ. Blood agar and MacConkey agar inoculated with the specimens are cultured aerobically at 37°C for 24 to 48 hours.
3- Identification criteria for isolates:
   a- Gram-negative rods.
   b- Oxidase-negative, catalase-positive
   c- Growth and appearance on MacConkey agar
   d- Colonial appearance on blood agar
   e- Appropriate biochemical profile for presumptive or definitive identification

P. vulgaris

Serratia marcescens

Klebsiella pneumoniae

Enterobacter aerogenes

Fig. Opportunistic pathogens *Enterobacteriaceae* in MacConkey agar.