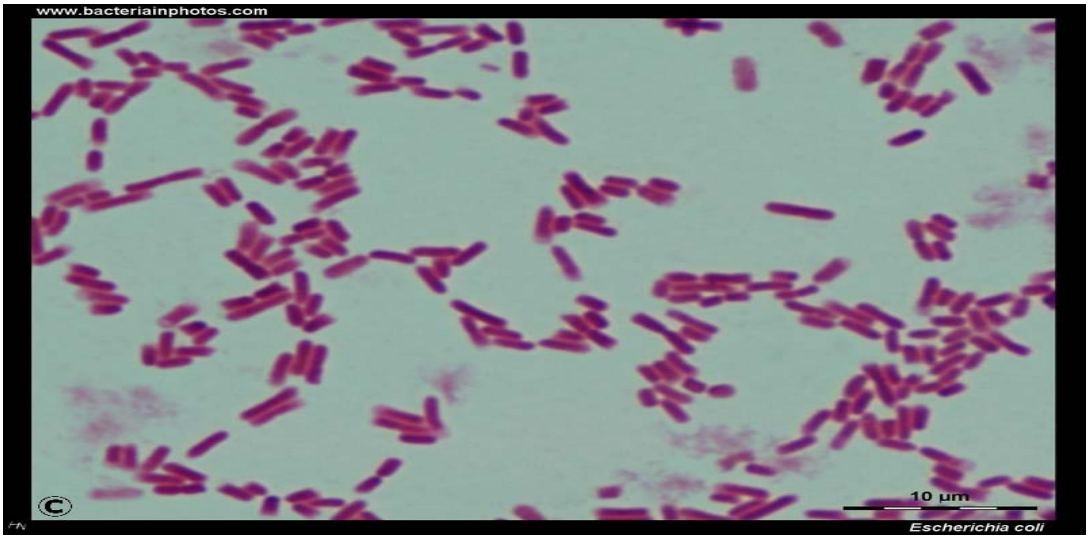
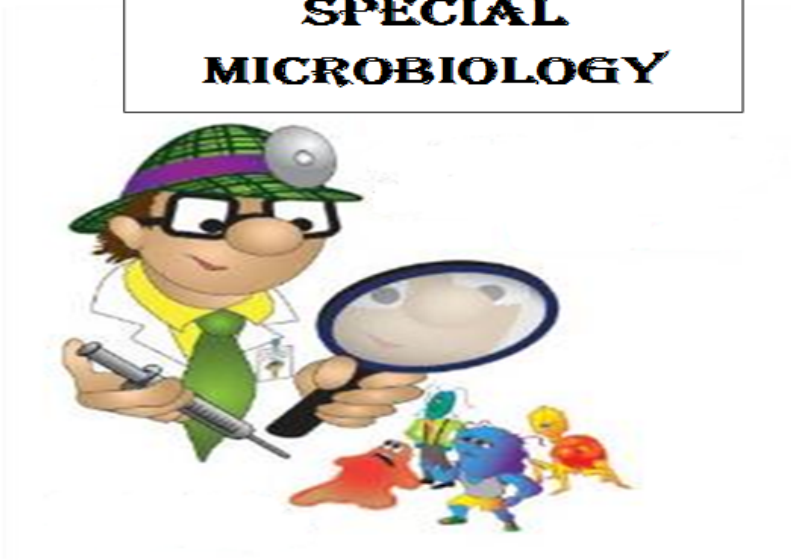
ENTEROBACTERIACEAE

Salmonella & Proteus



SPECIAL MICROBIOLOGY



Instructor

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Salmonella serotypes

- The genus ***Salmonella,*** which belongs to the family Enterobacteriaceae, are gram negative (gram –ve) short rods.
- The genus *Salmonella* contains more than 2,500 serotypes, based on a system devised by **Kaufmann and White** in which **somatic (O)** and **flagellar (H) antigens** are identified. Occasionally, **capsular (Vi)** antigens may be detected. These Ags are responsible of the virulence of *Salmonella* serotypes.
- **Phase Changing**: Serotypes which have flagellar (H) antigens in two phases, phase 1 (specific) and phase 2 (nonspecific), are termed diphasic.
- Salmonellae are typically intestinal pathogens & hence the carriers considered the major cause of shedding of the organisms.
- Recently, two species were proposed:

S. enterica

S. bongori

- *Salmonella enterica* has been divided into six subspecies.
- The majority of salmonellae of veterinary importance belong to ***S.enterica*** **subspecies *enterica***
- A phage typing system has been developed for a small number of serotypes such as Typhimurium and Enteritidis and is used for epidemiological investigation of isolates.
- The source of environmental contamination is invariably faeces.
- Organisms may be present in water, soil, animal feeds, raw meat and offal, and vegetable material.
- In poultry, some serotypes such as *Salmonella* Enteritidis infect the ovaries, and the organisms can be isolated from eggs.

GENERAL CULTURAL CHARACTERISTICS

- Aerobic & facultatively anaerobic.
- Most are Lactose non-fermenter.
- Most of them are motile except avian serotypes (*Salmonella* Pullorum & *Salmonella* Gallinarum*).*
- Are non sporeformer & mucoid strains.
- Grow well on selective media: Salmonella-Shigella (SS) Agar, Brilliant green (BG) Agar, Xylose–Lysine–Deoxycholate (XLD) agar, Selenite Broth or Tetrathionate Broth or Rappaport Broth as well as to on Hektoen enteric agar (HEA).

The most prevalent serotypes in man & animals:

Salmonella serotype	Hosts
***Salmonella* Typhi**	Man
***Salmonella* Paratyphi A**	Man
***Salmonella* T yphimurium**	Many animal species & Humans
***Salmonella* Dublin**	Cattle, sheep, horses & dogs
***Salmonella* Pullorum**	Chicks
***Salmonella* Gallinarum**	Adult birds
***Salmonella* Arizonae**	Turkeys
***Salmonella* Enteritidis**	Poultry, Many other species & Humans.
***Salmonella* Brandenburg**	Sheep

Pathogenicity

- Many of the virulence features of salmonellae are encoded on ***Salmonella* pathogenicity islands (SPI)** and on virulence **plasmids (*spv* gene)**, which is important for intracellular multiplication and other genes, which encode serum resistance play a role in systemic invasion.
- 18 of these 'islands' have been described to date.
- This pathogenicity island is found in all serotypes of *S. enterica* analyzed to date and one of its major effectors is a **Type III secretion system (TTSS)**. The **TTSS** is a complex of proteins which forms a **needle-like structure** for the transfer of virulence factors from the bacterium into the host cell
- ***Colonization of the intestinal tract & enteric disease:***

-Attached to enteric mucosa via **Fimbriae**. Disruption of intestinal normal flora due uptake of antibiotic therapy, diet & water deprivation facilitate such adhesion.

-Some strains producing enteritis & diarrhea appear capable of forming **LT-Like & ST-Like enterotoxins & cytotoxins.**

- ***Invasion & Septicaemia***

-The **LPS** is also responsible for the endotoxic effects of infection with salmonellae.

-Production of exotoxins by *Salmonella* serotypes.

-Siderophores, are secreted by invasive Salmonellae.

Salmonella serotypes of clinical importance and the consequences of infection

Salmonella serotype	Hosts	Consequences of infection
Salmonella Typhimurium	Many animal species	Enterocolitis and septicaemia
	Humans	Food poisoning
Salmonella Dublin	Cattle	Many disease conditions
	Sheep, horses, dogs	Enterocolitis and septicaemia
Salmonella Choleraesuis	Pigs	Enterocolitis and septicaemia
Salmonella Pullorum	Chicks	Pullorum disease (bacillary white diarrhoea)
Salmonella Gallinarum	Adult birds	Fowl typhoid
Salmonella Arizonae	Turkeys	Arizona or paracolon infection
Salmonella Enteritidis	Poultry	Often subclinical in poultry
	Many other species	Clinical disease in mammals
	Humans	Food poisoning
Salmonella Brandenburg	Sheep	Abortion

Diagnostic procedures

- History of previous outbreaks of the disease.
- At post-mortem, enterocolitis with blood-stained luminal contents.
- Specimens:

-Faeces and blood from live animals.

-Intestinal contents, tissue lesions from dead animals

-Abomasal contents from aborted ruminant fetuses.

- Culturing of samples: Preferred first with pre-enrichment medium like Selenite broth & then onto agar media to observe the colonial morphology like: BG (pink to red) , XLD (red with black center), SS (colorless with black center), MAC (colorless to faint yellow) & HEA (blue with black center).

- Biochemical Identification: The above successfully cultured Salmonellae then subjected to:

-Cultivation on TSI & if inconclusive, use biochemical profile identification kits.

- Serological Methods
- Serotyping of *Salmonella* using commercially available antisera for O and H antigens in a **slide agglutination test (Clumping)** or **Tube agglutination test (WIDAL TEST).**
- ELISA : Detection of raised Abs titer in animal serum is an indication of active infection.
- The Craigie tube method for 'phase-changing' salmonella isolates, like the biphasic organism *Salmonella* Typhimurium.

❖ **Phase 1** (flagellar i antigen) & **Phase 2** (flagellar 1,2 antigens)

- Molecular techniques:

- PCR and real-time PCR-based tests can be applied directly to samples.

- Multiplex PCR for the identification and differentiation of serovars Enteritidis, Gallinarum, Typhimurium, Kentucky and Dublin in poultry samples.

Proteus species

The genus *Proteus,* which belongs to the family Enterobacteriaceae, are widely distributed in nature as well as in man & animals intestine.

The most important species of *Proteus* spp. are:

Pr. mirabilis ***Pr. vulgaris***

GENERAL CHARACTERITICS

- Gram negative (gram –ve) short rods.
- Grow under aerobic & facultative anaerobes conditions.
- Are non-sporeformer & some strains are encapsulated.
- Rapidly motile by means of actively peritrichous flagella with a characteristics **Swarming phenomenon** of growth on non-inhibitory culture media like blood agar with bad smell.
- The rapid motility of *Proteus* may contribute to its invasion of the urinary tract.
- *Proteus vulgaris* is important nosocomial pathogens.
- *Proteus* species produce urease, resulting in rapid hydrolysis of urea with liberation of ammonia.
- Strains of *Proteus* vary greatly in antibiotic susceptibility.
- *Pr. mirabilis* is often inhibited by penicillins; the most active antibiotics for other members of the group are aminoglycosides and cephalosporins.
- *Proteus* spp. are reacting positively to **Phenylalanine deaminase test** also known as **phenylpyruvic acid (PPA) test** is used to test the ability of an organism to produce enzyme deaminase which differentiate them from other members of Enterobacteriaceae which reacts negatively to PAA.
- *Proteus mirabilis* and *Pr. vulgaris* are opportunistic pathogens that cause lesions in the urinary tract in dogs, horses & man.

- *Pr. vulgaris* is positive to Indole & ferment maltose whereas *Pr. mirabilis* is negative for both.
- Both can express genes for a number of **adhesins** that bind specifically at different locations within the urinary tract.
- It is believed that the pathogen uses one fimbrial type, ***P. mirabilis* fimbriae (PMF)**, to become established in the bladder.
- Some are differentiated into the heavily flagellated swarming phenotype and proceed up the ureters to the kidneys, where they use another fimbrial type, **mannose-resistant/*Proteus*-like (MR/P) fimbria**, to adhere to epithelial cells of the kidneys.
- These adhesions are responsible for exposure of the renal tissues to bacterial toxins such as **lipopolysaccharide (LPS), haemolysin,** and **Cytotoxin Necrotizing Factor 1 (CNF1).**
- A virulence factor for *Proteus mirabilis* is its **polysaccharide capsule** which facilitates adherence to the luminal surface of the uroepithelial cells. The adherent bacteria divide and form a biofilm.
- **Calcium** and **magnesium ions** may be precipitated within the biofilms, providing the bacteria with protection from host defences and interfering with the efficacy of antimicrobial therapy.
- Phage-typing, bacteriocin-typing and serotyping schemes are used for strain discrimination.
- **Bacteriocins** are proteinaceous toxins produced by bacteria to inhibit the growth of similar or closely related bacterial strain(s).
- Strains of *Proteus* spp. express mannose-resistant haemagglutination and may also produce calcium-dependent and calcium-independent haemolysins.
- *Proteus* spp. express proteases such as an IgAase.

DIAGNOSIS

- Specimens: Mid-stream urine & blood, one half to be stained with Gram's stain (Gram –ve) & the other cultured on blood agar & MAC.
- Biochemical Identification: Urease (+ve), PAA (+ve), Pr. Vulgaris (Indole +ve & Ferment Maltose), Pr. Miarbilis (Indole –ve & Do not ferment Maltose & Citrate +ve). TSI (Red slant/Yellow butt/H2S +ve).
- Rapid kits for biochemical identification.
- Serotyping
- Bacteriocin-typing
- Phage-typing
- Molecular techniques.

DISEASES

- Cystitis
- Pyelonephritis
- Otitis

In Man, Dogs & Horses.

GOOD LUCK