SALMONELLA

- It is a bacterium that causes Salmonellosis.
- It is not a normal GIS flora member of human, lives in the intestinal tracts of warm and cold blooded animals.
- Second most common bacterial food borne illness.
- The most commonly reported cause of enteric disease.
- In humans; the cause of two diseases called **salmonellosis**:
  - **enteric fever (typhoid),** resulting from bacterial invasion of the bloodstream, and
  - **acute gastroenteritis,** resulting from a foodborne infection/intoxication

- A very complex group contains more 2000 ssp.
- Divided into two groups
  - Enteric fever group
  - Food poisoning group- Septicemias

Enteric fever group

- Enteric fever caused by S. Typhi and other groups called as Paratyphi A, B, C
- S. Typhi causes typhoid, S. Paratyphi A, B, C cause paratyphoid fevers.

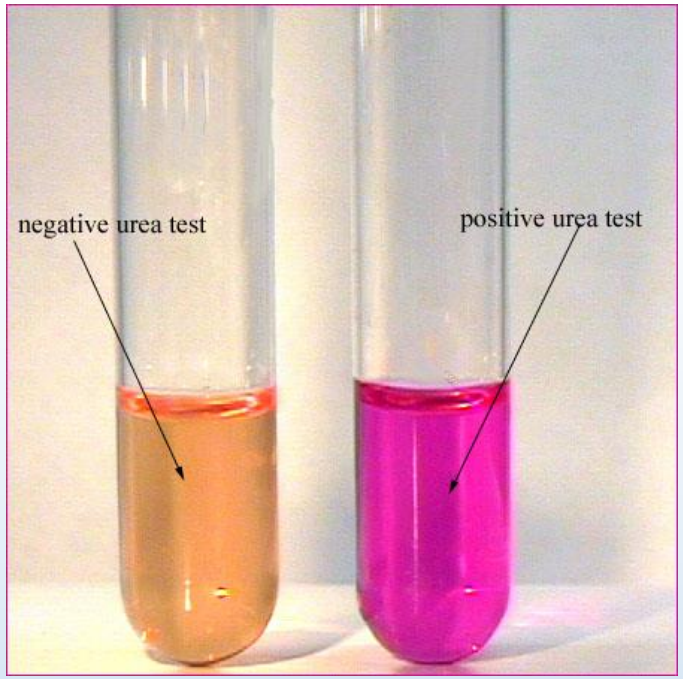
Food poison group

- Spread from animals-humans
- Causes gastroenteritis- Septicemias, localized infection

General Characteristics

- Gram negative, rod-shaped bacilli
- Aerobic/ Facultative anaerobic
- Lactose negative
- Peritrichous flagella (ensure motility)
- Motile (except *S. gallinarum* and *S. pullorum*)
- Uncapsulated (except *S. typhi*)

- H_2S production by S. Typhi
- Grows simple medium
- Temp 15- 41 ºC/ opt 37 ºC
- Colonies appear as large 2-3 mm, circular, low convex
- On MacConkey medium appear colorless (NLF)
- Enrichment medium: Selenite F medium; It is used for isolation of Salmonella from contaminated specimens (stool specimens)
- Urea: negative



- IMVIC: **(-, + , - , +)**
- Resistance of Salmonella• 55º C – 1 hour• 60º C – 15 MT
- Boiling ,Chlorination, Pasteurization Destroy the Bacilli

Selective Broth and Media

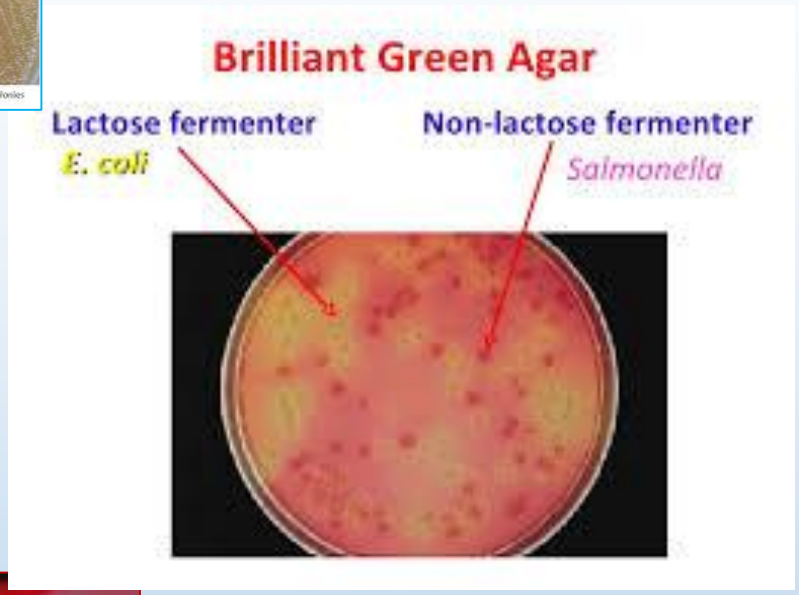
- **Selective Enrichment Broth**
  - Selenite F
  - Tetrathionate enrichment Broth (Tetrathionate reductase enxyme)
- **Selective Media**
  - Brillant green agar
  - MacConkey Agar
  - XLD4

On MacConkey Agar

- Salmonella gives colorless colonies

- On Brillant Greeen Agar

Salmonella colonies are red



- On XLD4 Agar

Colonies are red with a black center due to H_2S production

https://pt.slideshare.net/SandeepPatil31/diff-mediasv/17

Antigenic structure of Salmonella

1. Somatic or O antigens: forms integral part of cell wall (LPS)

Unaffected by boiling, less immunogenic than H antigen

2. Flagellar or H antigens

3. Vi surface antigen:

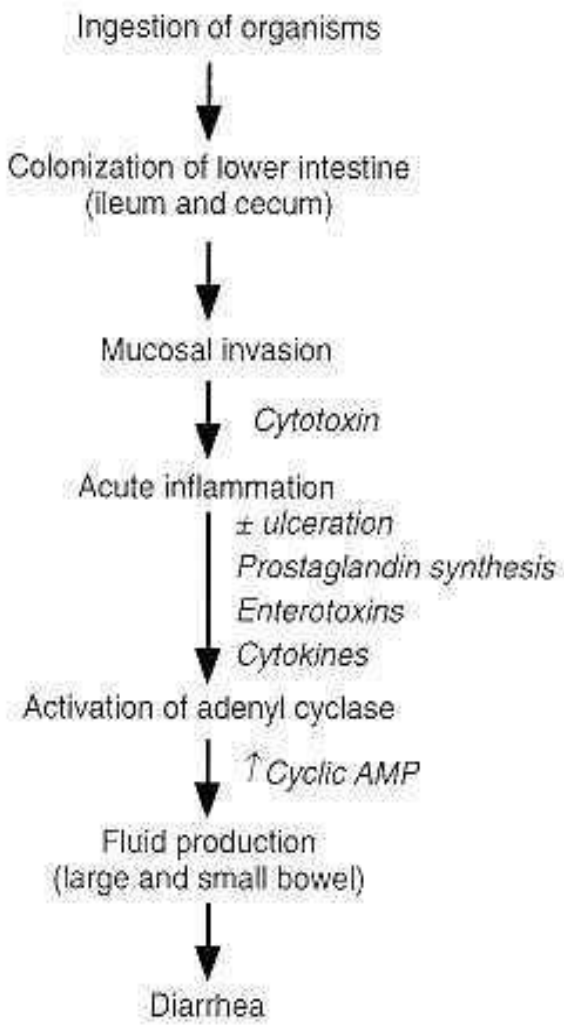
   - Covers O antigen,
   - Acts as virulence factor, protects the bacilli against phagocytosis
   - Poorly immunogenic

Clinical Syndromes

- Although Salmonellae can cause a wide spectrum of clinical illness there are three major syndromes,
- Enteric fever
- Gastroenteritis
- Bacteremia

A) Enteritis

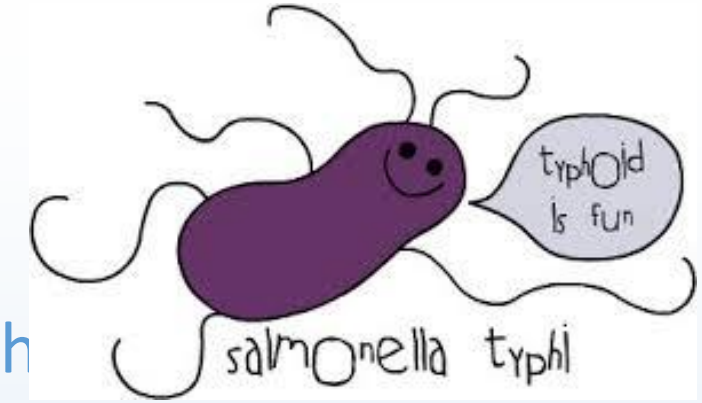
- Most serotypes cause enteritis, an infection that is limited to the terminal ileum. The common agents of enteritis are S. Enteritidis and S. Typhimurium. The salmonellae invade the intestinal wall and produce enterotoxins that cause **nausea, vomiting and diarrhea.** Bacteria rarely spread beyond the gastrointestinal wall.



- In many countries *Salmonella enteritidis* is the third most commonly reported form of “food poisoning”.
- The infection is zoonotic, and the poultry is the source of infection.
- Other sources of infection include milk products, food and water contaminated with animal feces or urine.

- 8-48 hours after the ingestion of food or drink contaminated with Salmonella, enterocolitis begins with nausea, vomiting, abdominal pain, diarrhea which can vary from mild to severe.
- In some cases manifestation include fever, headache and chills.
- Salmonella enteritis last about 5 days, but severe loss of fluids and electrolytes may be life threatening in infants and elderly patients.

B)Enteric Fever



- Two serotypes Typhi and Paratyphi can cause typh
- The salmonella invade the wall of the terminal ileum and than spread to the intestinal lymphatics, where they are phagocytosed by PMNs and macrophages.
- Salmonella phagocytosed by PMNs are killed, but those phagocytosed by macrophages survive and multiply within phagocytic vacuoles.

- Wandering macrophages that contain salmonellae, deliver salmonellae to various reticuloendothelial tissues.
- Infected macrophages are eventually destroyed and salmonellae released from lysed macrophages cause septicemia.

- Some Salmonellae begin to disseminate hematogenously to a variety of ectopic sites, including the bones, lungs, liver, brain where they cause osteomyelitis, pyelonephritis, empyema, hepatic necrosis, meningitis.
- Other salmonella remain in the intestine, where they invade the gut wall and may cause ulceration, perforation and hemorrhage.
- Salmonellae also multiply well in gut associated lymphoid tissue and may ulcerate Payer’s patches

Diagnosis

- The diagnosis of salmonellosis requires bacteriologic isolation of the organisms from appropriate clinical specimens (blood, stool, urine).
- Laboratory identification of the genus Salmonella is done by biochemical tests; the serologic type is confirmed by serologic testing.

(Gruber Vidal test): Tube agglutination test, detect O and H antigens

- Diagnosis of thyphoid and paratyphoid
- First week negative because titers raise in second week , increase of titers is diagnostic
- But nowadays ELISA test and immunoblotting are used for detecting O, H, and Vi antigens.

- But nowadays ELISA test and immunoblotting are used for detecting O, H, and Vi antigens.
- The laboratory diagnosis of bacterial food poisoning depends on isolation of the casual organism from samples of faeces or suscepted foodstuffs.

Prophylaxis

- TAB vaccine: S.Typhi, S Paratyphi A,B)
- TY21a
- The vaccine prepared Vi antigen

Treatment

- Chloramphenicol
- Ampicillin
- Amoxicillin
- Co-tromoxazole

- Fluoroquinolones (Ciprofloxacin)
- Ceftazidime, ceftriaxone, cefotaxime

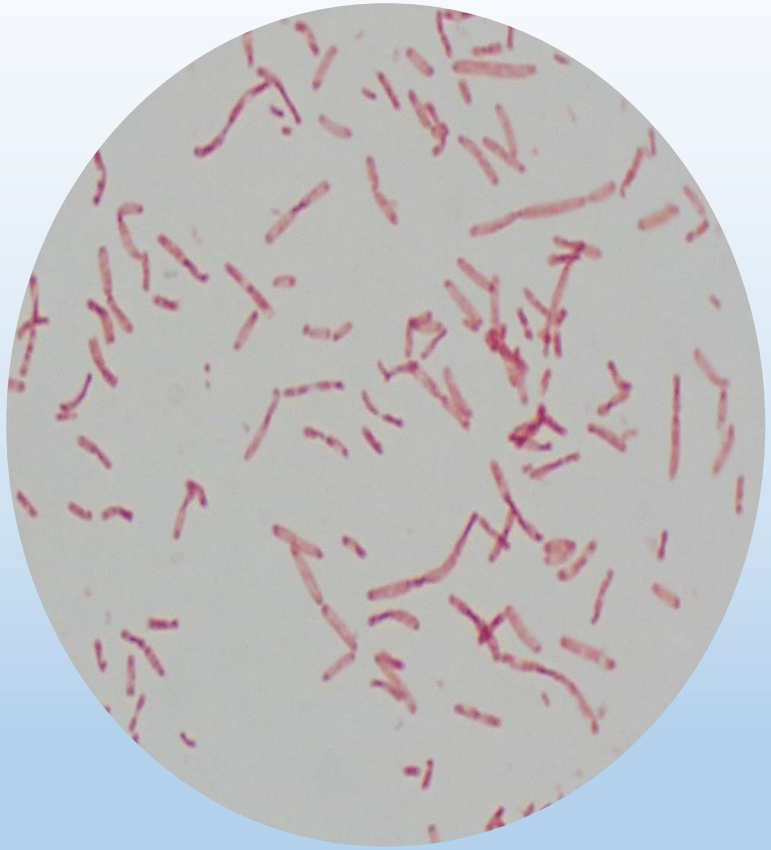
KEY POINTS FOR SALMONELLA

- *There are more than 2000 different antigenic types of Salmonella; those pathogenic to human are serotypes of S. enterica.*
- *Most serotypes of. S. enterica cause food-borne gastro-enteritis and have animal reservoirs.*
- *S. enterica serotypes Typhi and Paratyphi cause typhoid fever.*

- *Typhoid and other systemic salmonella infections are treated with Chloramphenicol, Amoxicillin, Co-tromoxazole*
- *Antibiotics have no place for gastro-enteritis treatments.*
- *Clean water, sanitation, and hygienic handling of foodstuffs are the keys to prevention.*

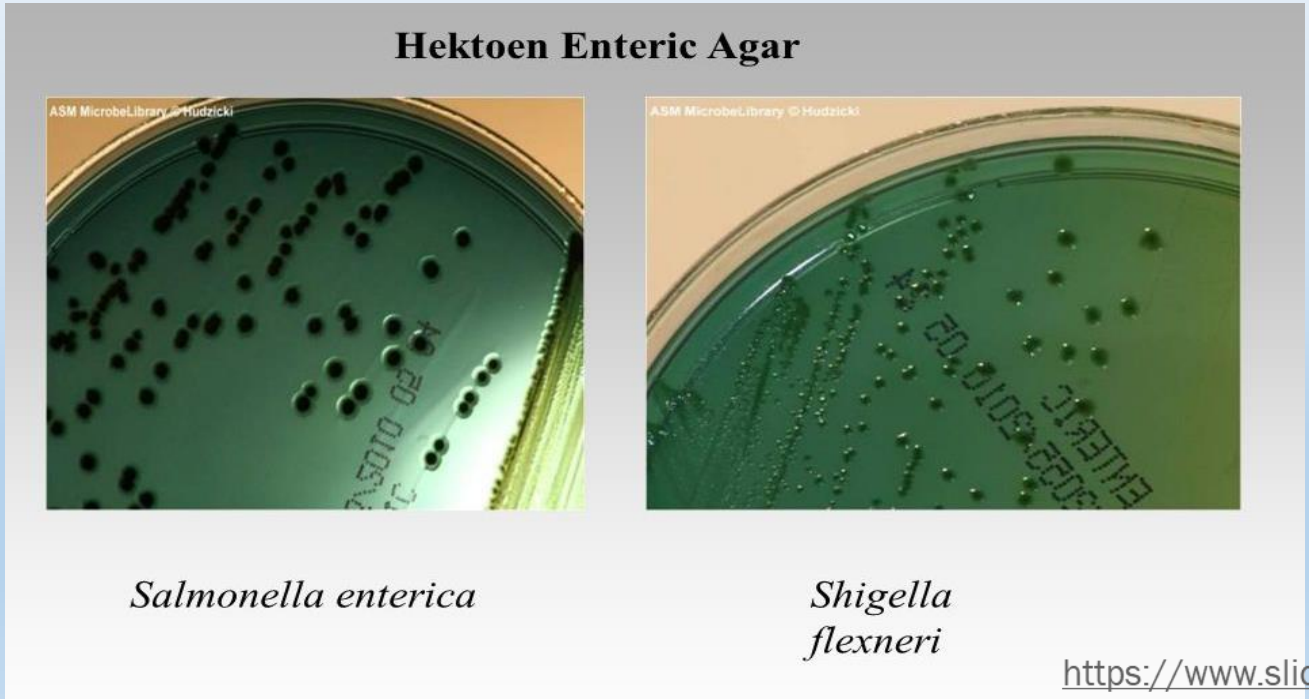
SHIGELLA

- Gram negative,
- Non lactose fermenting, Oxidase negative
- Mostly non-motile (no H antigen), non-sporing
- IMVIC: **(- , +, - , -)**
- Shigella can survive up to 30 days in milk, eggs, cheese



- All members of Shigella are aerobic and facultative anaerobes
- Grow readily in culture media at pH 6.4 to7.8 at 10 ^{o}C - 40 ^{o}C, with optimum of 37 ^{o}C.
- After 24 hours incubation, Shigella colonies reaches a diameter of about 2 mm.
- The colonies are circular, convex, colorless, but moderately translucent with smooth surface, and entire edges.

- In XLD (xylose lysine deoxycholate) they apperar pinkish to reddish



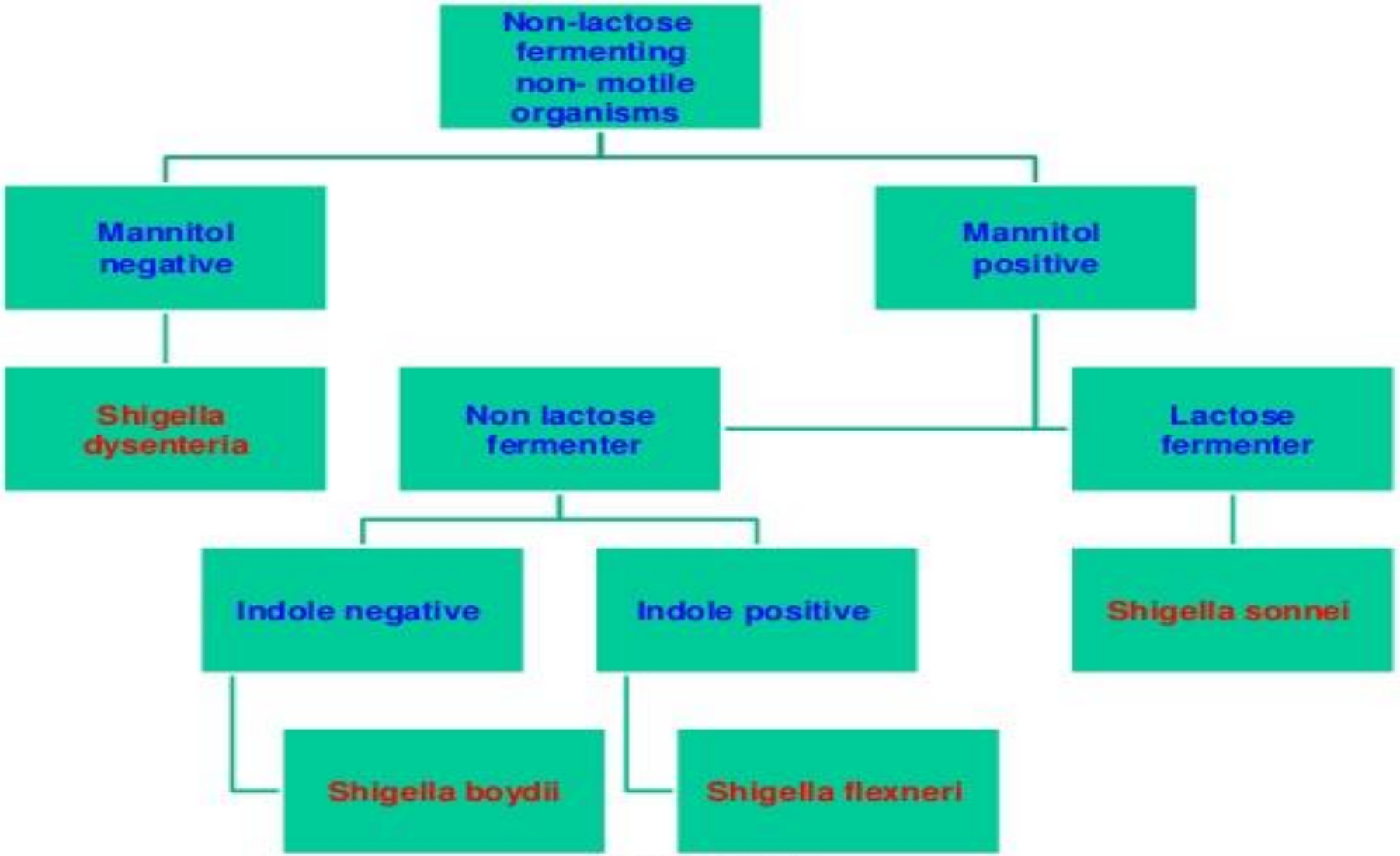
- In Hektoen Agar, green to blue green colonies

https://www.slideshare.net/DanaSinzianaBreharCi/enterobacteriaceae-45934685

CLASSIFICATION

Four Species/Subgroups Based On Biochemical And Serological Characters

- ***Group A: Shigella dysenteriae*** : 12 Serotypes: most serious form of bacillary dysentery
- ***Group B: Shigella flexneri*** : 6 Serotypes, Shigellosis in underdeveloped countries
- ***Group C: Shigella boydii*** **:** 18 serotypes
- ***Group D: Shigella sonnei :*** *Shigellosis in developed country*


Non-lactose fermenting non- motile organisms
Mannitol negative
Mannitol positive
Shigella dysenteria
Non lactose fermenter
Lactose fermenter
Indole negative
Indole positive
Shigella sonnei
Shigella boydii
Shigella flexneri

Dr.T.V.Rao MD

Shigellosis

- Ranges from asymptomatic infection to severe bacillary dysentery.
- Fecal-oral transmission is the main path of Shigella infection.
- Other modes of transmission include ingestion of contaminated food or water, contact with infected objects, or sexual contact.
- Outbreaks of Shigella infection are common in places where sanitation is poor.

- *S. dysenteriae* produce shiga toxin and cause dysentery.
- Two-stage disease: watery diarrhea changing to dysentery with frequent small stools with blood and mucus, tenesmus , cramps, fever

Early stage :

- Watery diarrhea attributed to the enterotoxic activity of Shiga toxin
- Fever attributed to neurotoxic activity of toxin

Clinical Syndromes

- Process involves:

1. Ingestion

2. Non-invasive colonization and cell multiplication

3. Production of the enterotoxin by the pathogenic bacteria in the small intestine;

Second stage

- Adherence to and tissue invasion of large intestine
- Typical symptoms of dysentery
- Cytotoxic activity of Shiga toxin increases severity

Clinical Features

- Fever
- Bloody Diarrhea
- Abdominal Cramps
- Tenesmus
- Mucus
- Pus

- Convulsions
- Mild Infection: Watery Stool
- Bacteremia - Rare
- Reiter’s Syndrome
- Hemolytic – Uremic Syndrome

Diagnosis

- Culture: Cultivate the bacilli from the patient
- Stool-Rectal swabs
  - MacConkey agar, NLF
  - XLD
  - Selenite F Broth

- Microscopy: Leucoctyes, RBC
- Biochemical test
  - TSI, No gas, H_2S, acid
- Non-motile
- Serological test
- Sereny test positive

Treatment

- Fluid and electrolyte replacement therapy
- Antimicrobial therapy: shorten the duration, prevent spread
- TMP-SMX, Ciprofloxacin
- No vaccine

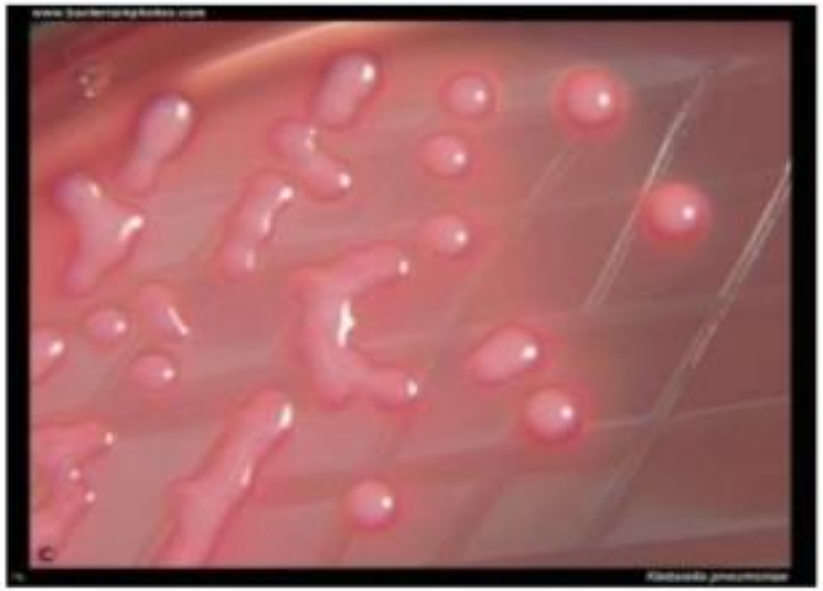
KEY POINTS FOR SHIGELLA

- *Shigella species cause bacillary dysentery*
- *The infective dose is very small*
- *Sh. dysenteriae type 1 produces a toxin that resembles verocytotoxin of certain strains of E. coli and it is responsible for the most serious forms of shigellosis.*

- *Sh. flexneri, Sh. boydii* and *Sh. sonnei* cause enteric disease of varying severity.
- Sonnei dysentery is the most prevalent form of shigellosis in developed countries.
- Most cases of shigellosis o not require antibiotics. Treatment with ciprofloxacin is indicated in severe cases.

KLEBSIELLA

- Klebsiella is a common inhabitant of the human GIS
- Gram negative rods,
- Non-motile
- Lactose fermenting
- Facultative anaerobic
- Capsule formation



Mucous, lactose positive colonies of *Klebsiella pneumoniae* on MacConkey agar. Cultivation 37°C, 24 hours.

- IMVIC: **-, -, +, +**
- Gas positive , H_2S negative

Antigenic structure:

- K (Capsule antigen)
- O Somatic antigen

Four major species;

- *Klebsiella pneumoniae*
- *Klebsiella rhinoscieromatis*
- *Klebsiella ozaenae*
- *Klebsiella oxytoca* (Indole +)

***K. pneumoniae* (Friedlander bacilli)** is mostly commonly isolated species

- Possesses a polysaccharide capsule, which protects against phagocytosis and antibiotics, makes the colonies moist and mucoid
- Frequent cause of nosocomial pneumonia
- Significant biochemical reactions
  - Lactose positive
  - Most are urease positive
  - Non-motile
  - Produce pneumocyin

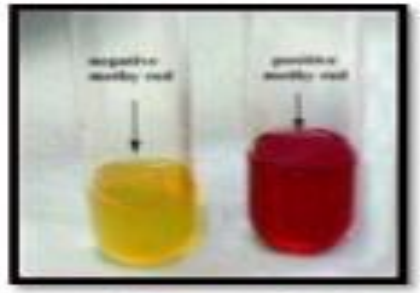
Biochemical Activities of *Klebsiella pneumoniae*

1. Ferments glucose and lactose but no H_2S with gas production

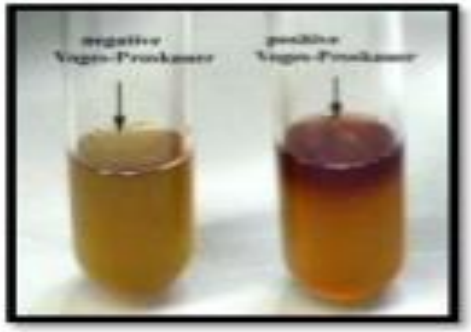
- - + +

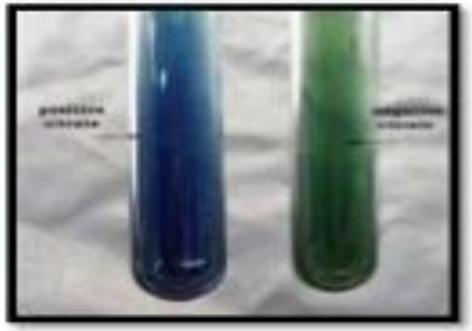
IMViC

2. Indole and methyl red tests are –ve



3. Citrate and Voges proskour tests are +ve





Diseases

- *K. pneumoniae* is the most frequent cause of gram negative bacterial pneumonia. Klebsiella-related pneumonia frequently afflicts debilitated and malnourished persons, particularly chronic alcoholics.

- The most common condition caused by *Klebsiella* bacteria outside the hospital is pneumonia, typically in the form of bronchopneumonia and also bronchitis. These patients have an increased tendency to develop lung abscess, cavitation, empyema, and pleural adhesions. It has a death rate around 50%, even with antimicrobial therapy. The mortality rate can be nearly 100% for people with alcoholism and bacteremia.

- In addition to pneumonia, *Klebsiella* can also cause infections in the urinary tract, lower biliary tract, and surgical wound sites.
- The range of clinical diseases includes; pneumonia,
- UTI’s Nosocomial infections
- Wound infections
- Septicemia
- Meningitis
- Rarely diarrhea

Klebsiella

- ***Klebsiella ozaenae***
  - Ozena (the atrophy of nose with bad smelling)
- ***K. rhinoscleromatis***
  - Rhinoscleroma (A granulomatosis in nose and pharynx)
- ***Klebsiella penomoniea***
  - 5% in upper respiratory and GI systems
  - Nosocomial infection
- ***Klebsiella oxytoca***
  - Nosocomial infection

- Klebsiellae have become important pathogens in hospital-acquired (nosocomial) infections.
- Klebsiella organisms with the ability to produce extended-spectrum b-lactamases (ESBL) are resistant to many antibiotics, including:
- All b-lactams
- Aminoglycosides
- Fluoroquinolones
- Tetracyclines
- Chloramphenicol
- Trimethoprim
- Sulfamethoxazole

- One of many carbapenem-resistant *Enterobacterales* (CRE) is Carbapenem-Resistant Klebsiella pneumoniae (CRKP).
- It was first described in North Carolina in 1996; since then CRKP has been identified in 41 states. It is now the most common CRE species encountered within the United States.Over the past 10 years, a progressive increase in CRKP has been seen worldwide.

Diagnosis

- Cultures on blood agar and MacConkey agar
- Gram staining
- Biochemical reactions

Treatment

- Cephalosporin
- Trimethoprim
- Nitrofurantoin

KEY POINTS FOR KLEBSIELLA

- *Klebsiella spp*. usually cause UTI but may cause bronchopneumonia and septicaemia.
- *Klebsiella spp*. are a major cause of hospital- acquired infections.

PROTEUS

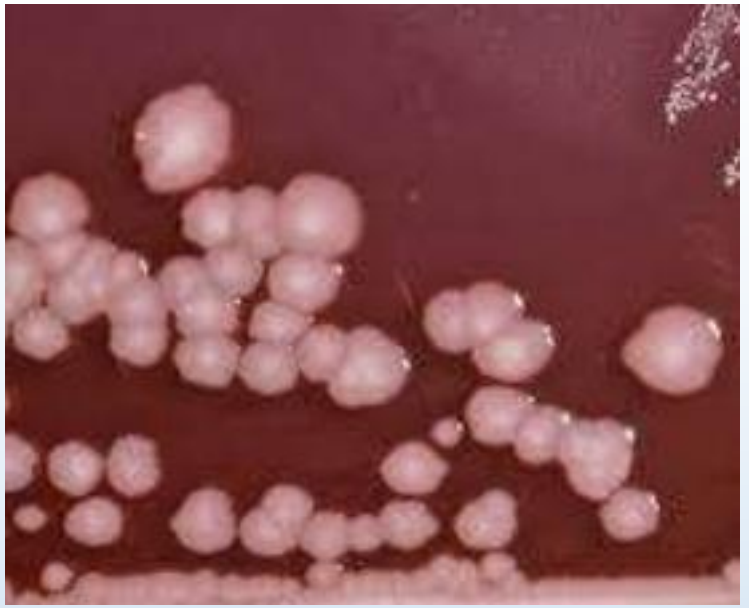
- *Proteus spp*. are most commonly found in the human intestinal tract as part of normal intestinal flora.
- The sub-family include:

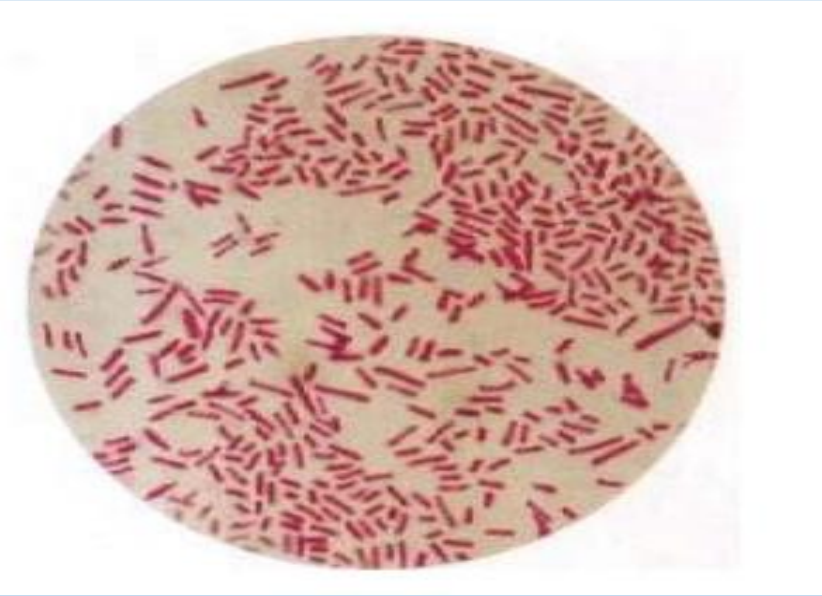
Proteus.
- *P.mirabilis*
- *P.vulgaris*

Morganella.
- *M.morgani*

Providencia.
- *P. Rettgeri*

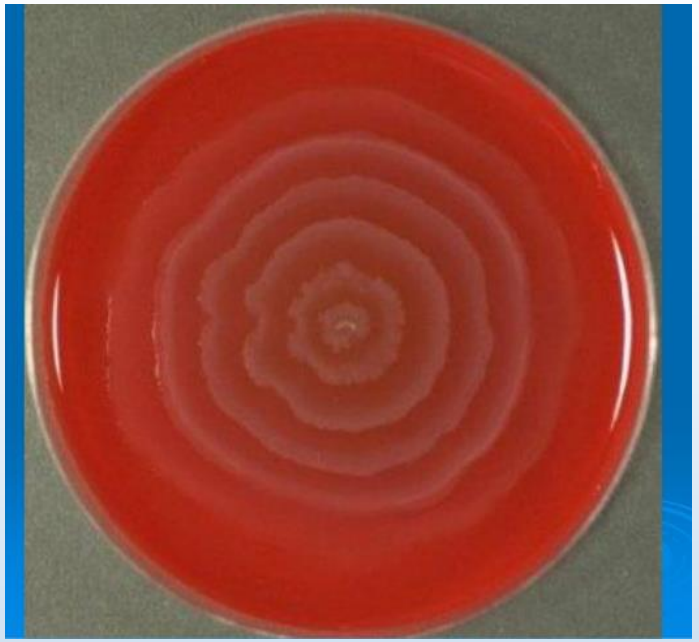
- Large, circular, gray, smooth colonies





Rod shaped gram negative

- Non-capsulated
- Very motile with peritrichous flagella
- Urease positive
- Lactose negative
- H_2S positive
- MR: +, VP: -
- **Indole** and **Citrate** variable
- Fenil alanine deaminase test positive (different from Enterobacterales)



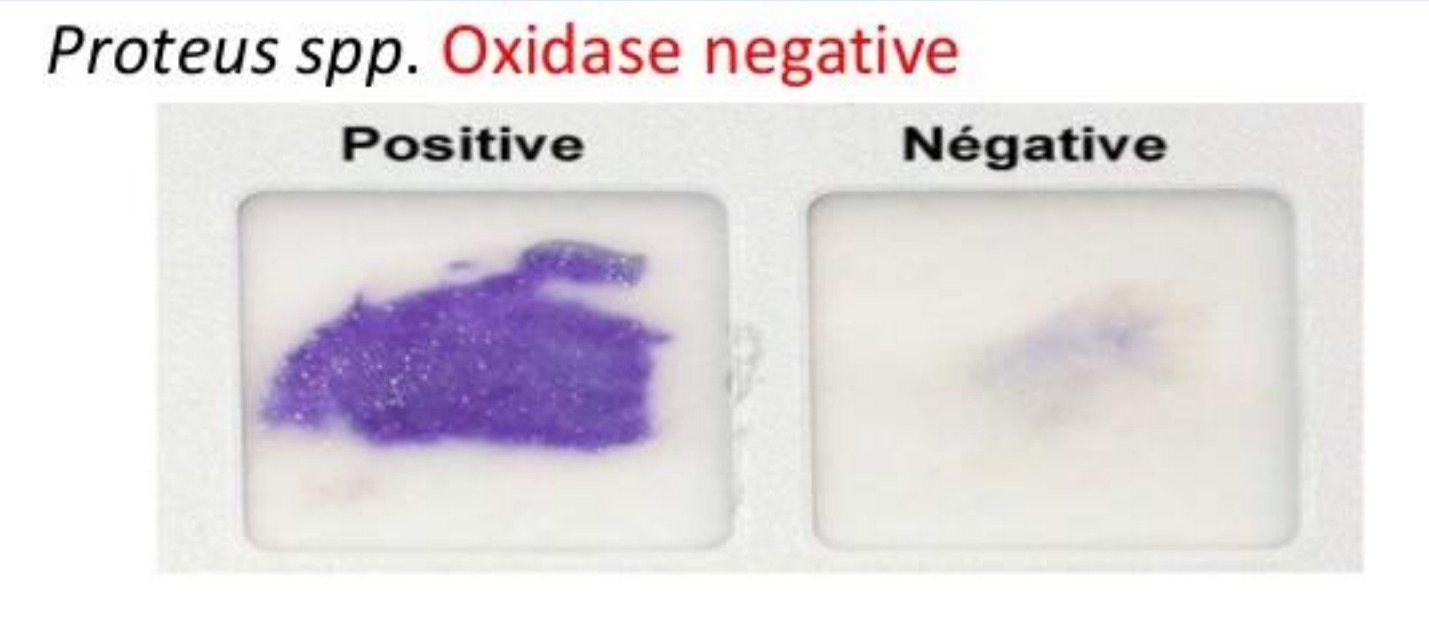
P. vulgaris and *P. mirabilis* have ability to swarm on solid media

*The main species of medical importance are *P. mirabilis* and *P. vulgaris. They are opportunistic pathogens and may cause many types of infections.*

	P. mirabilis	*P. vulgaris*	*Morganella morganii*	*Providenica rettgeri*
Indole	-	+	+	+
Methyl Red	+	+	+	+
VP	+ (50%)	-	-	-
Citrate	+ 65%)	-	-	+
Motility	+	+	+	+
Urea	+	+	+	+
TSI	Alk/Acid	Acid/Acid Ferment sucrose	Alk/Acid	Alk/Acid
KIA	Alk/Acid	Alk/Acid	Alk/Acid	Alk/Acid
H_2S	+	+	-	-

Biochemical reactions

- **Differences between *P. vulgaris* and *P. mirabilis***
- **Oxidase test:** It is used for determining if a bacterium produces certain cytochrome c oxidase.

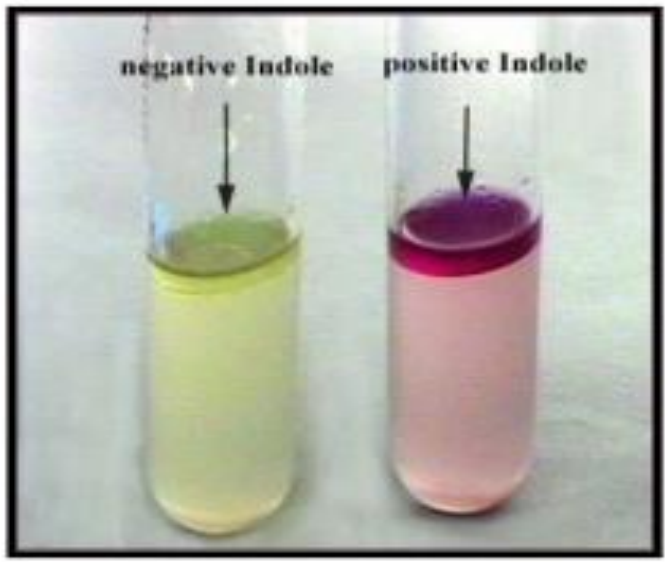


Indole test: It is used to determine the ability of bacteria to convert tryptophan into indole.

P. mirabilis can be differentiated from *p.vulgaris* by indole test.

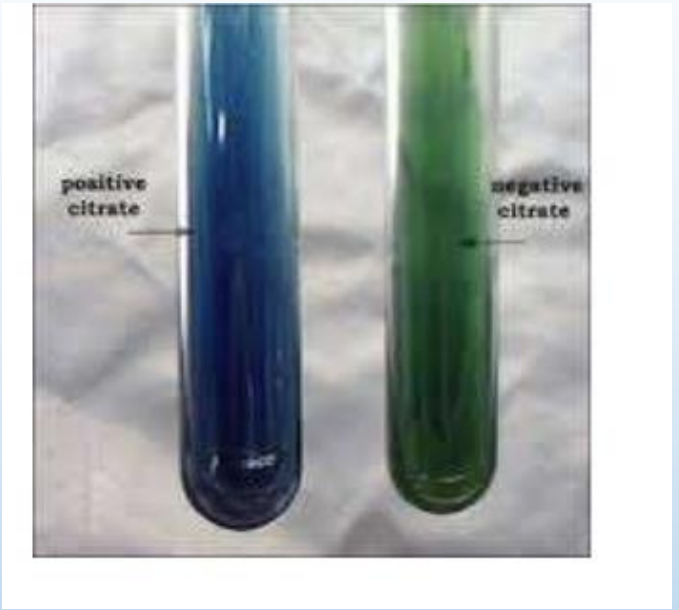
P. mirabilis → **negative**

P. vulgaris → **positive**



Citrate test

- *P. vulgaris*: negative
- *P. mirabilis*: positive



Pathogenicity

- ***P. mirabilis*** causes 90% of all Proteus infections in human.
- UTI
- Wound infection.
- Otitis media.
- Septicemia (complication of UTI & wound infection).

Antigenic structure

- O antigen
- H antigen

- ***P. vulgaris*** important nosocomial pathogen, and isolated in wound infection and UTI
- Proteus In UTI infection, cause forming of calculus: It breaks down urea, makes the urine alkaline, Ca and Mg salts precipitate and calculus is formed.

Weil-felix reaction

- There are cross reaction between certain species of Proteus and Rickettsia. Reckettsial infection (typhus fever) will cause heterolphilic antibodies that agglutinate some strains of Proteus.

Weil-felix test

- Heterophile agglutination test
- Using non motile *Proteus vulgaris* strains (OX 19, OX 2, OX K) to find rickettsial antibodies in patient's serum.
- Procedure:
  - ✓ Serum is diluted in three separate series of tubes followed by the addition of equal amount of OX 19, OX 2, OX K in 3 separate series of tubes.
  - ✓ Incubation at 37^{0}C for overnight.
  - ✓ Observe for agglutination.
- Interpretation:
  - ✓ Strong Agglutination with OX 19 => epidemic & endemic typhus.
  - ✓ Strong agglutination with OX 19 & OX 2 => Spotted fever
  - ✓ Strong agglutination with OX K => Scrub typhus (Scrub typhus by *Orientia tsutsugamushi*)

Diagnosis

- Culture (specimen: according to the side of infection; pus, swabs, urine, blood….etc.)
- Gram stain
- Colonial morphology
- Biochemical reaction

Treatment

- Ampicillin
- Cephalosporins
- Aminoglycosides

- Proteus species are resistant to polymyxin and nitrofurantoin

KEY POINTS FOR PROTEUS

- Strains of *Proteus*, *Providentia*, and *Morganella* are closely related.

  They are regularly isolated from UTI

- Indole test is important to determine differences between *P. mirabilis* and *P. vulgaris*.
- Cause forming of calculus in UTI infection.

ENTEROBACTER

- ***Enterobacter aerogenes***
- ***E. cloacae***
- *E. sakazakii*
- *E. agglomerans*
- Isolated from wounds, urine, blood

- IMVIC: -, -, +, +
- TSI A/A, no H_2S, no gas
- *E. aerogenes* = (-) urease & (-) arginine decarboxylase
- *E. cloacae* = (+) urease (65%) & (+) arginine decarboxylase
- (+) Motility

- Opportunistic organisms
- Enterobacter infections occur following catheterization, incubation, urinary tract procedures.
- Infections; UTI, pneumonia, bacteraemia, and meningitidis

Diagnosis

- Specimens are collected according to the infection urine, sputum, pus, infected tissue etc.
- Microscopy;Gram negative rods,
- Motile
- Enterobacter gives similar colonies as Klebsiella but not so mucoid

TREATMENT

- Treatment depends on the site of disease and resistance pattern of isolate
- Cefotaxime, gentamicin

KEY POINTS FOR ENTEROBACTER

- Enterobacter spp. have many features in common with *Kelebsiella spp*.
- Enterobacter infections occur following catheterization, incubation, urinary tract procedures.

SERRATIA

- *Serratia* species are opportunistic gram-negative bacteria classified in Enterobacterales. *Serratia* are widespread in the environment, but are not a common component of the human fecal flora.
- The most common and primary pathogen species in the genus is *Serratia marcescens*.

Rare reports have described disease resulting from infection with

- *Serratia plymuthica,*
- *Serratia liquefaciens*,
- *Serratia rubidaea*,
- *Serratia odorifera,*
- *Serratia fonticola*.

Serratia spp.;

- Motile rods
- Grow well on laboratory media at 30-37 ^{o}C.
- *S. marcescens* produce pigment, **prodigiosin**, which ranges in color from dark red to pale pink.
- Utilize most carbohydrates with the production of acid and gas.
- Lactose (-), H_2S (-)

- IMVIC: -, -, +,+
- O and H antigen
- *Serratia* are capable of thriving in diverse environments, including water, soil, and the digestive tracts of various animals

Cause nosocomial infections including;

- Urinary and respiratory tracts
- Meningitis
- Wound infection
- Septicaemia
- Endocarditis.

- *Serratia* strains commonly resistant to cephalosporins. Resistance to ampicillin and gentamicin is variable, but many strains destroy these antibiotics enzymically. An aminoglycoside, such as gentamicin is usually the most reliable first-line choice.

KEY POINTS FOR SERRATIA

- *Serratia* spp. are oppurtunistic pathogens causing respiratory and urinary tract infections, wound infections, meningitis and septicaemia.
- *S. marcescens* produce pigment called prodigiosin.

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