

CORYNEBACTERIUM SPECIES

- Small Gram-positive pleomorphic (coccoid, club and rod forms) bacteria
- Stained smear reveals cells in palisades of parallel and angular clusters resembling Chinese letters
- Non-motile facultative anaerobes
- Catalase-positive, oxidase-negative
- Fastidious, require enrichment for growth
- Cause pyogenic infection
- Most pathogenic species are host specific
- Type species: *C. diphtheriae*, causes diphtheria in children

Diseases

- i. *Corynebacterium bovis*
Host (cattle): subclinical mastitis
- ii. *C. kutscheri*
Host (laboratory rodents): superficial abscesses, causes purulent foci in liver, lungs and lymph nodes
- iii. *C. pseudotuberculosis* (non-nitrate-reducing biotype)
Host (Sheep and goats): caseous lymphadenitis
- iv. *C. pseudotuberculosis* (nitrate-reducing biotype)
Host (horses, cattle): ulcerative lymphagitis, abscesses
- v. *C. renale* (type I)
Cattle: cystitis, pyelonephritis
Sheep and goats: ulcerative (enzootic) balanoposthitis
- vi. *C. pilosum* (renale type II)
Cattle: cystitis, pyelonephritis
- vii. *C. cystitides* (renale type III)
Cattle: severe cystitis, rarely pyelonephritis
- viii. *C. ulcerans*
Cattle: mastitis

Diagnosis

- Specimen: pus, exudates, tissue, sample, mid-stream urine
- Direct microscopy of Gram-stained smear may reveal coryneform bacteria
- Inoculate sample onto blood agar, selective media (McLeod's blood agar, Loeffler's medium) containing potassium tellurite, and MacConkey agar
- Incubate aerobically at 37⁰C for 24 to 48 hours
- Identification: no growth on MacConkey agar
- Colonial Characteristics:
 - *C. bovis*: a lipophilic bacterium. Small white, dry, non-haemolytic colonies
 - *C. kutscheri*: whitish colonies, occasionally haemolytic
 - *C. pseudotuberculosis*: small whitish colonies, surrounded by a narrow zone of complete haemolysis evident after 72 hours of incubation. Colonies become dry, crumbly and cream-coloured with age
 - Members of *C. renale* group produce small, non-haemolytic colonies after 24 hours incubation. Produce pigment after 48 hours of incubation
- Microscopy: Gram's staining and Albert's staining techniques
Albert's staining demonstrate metachromatic granule (inclusions)
- Biochemical tests
 - Nitrate reduction: *C. pseudotuberculosis* biotype
 - All pathogenic corynebacteria are urease positive except *C. bovis*

Differentiation of C. renale group

Feature	<i>C. renale</i> (type I)	<i>C. pilosum</i> (type II)	<i>C. cystidis</i> (type III)
Colour of colony	Pale yellow	Yellow	White
Growth in broth at pH 5.4	+	-	-
Nitrate reduction	-	+	-
Acid from xylose	-	-	+
Acid from starch	-	+	+
Casein digestion	+	-	-
Hydrolysis of Tween 80	-	-	+

- Enhanced haemolysis by *C. pseudotuberculosis* when inoculated across a streak of *Rhadococcus equi*

ACTINOMYCES ARCANOBACTERIUM AND ACTINOBACULUM SPECIES

- Gram-positive bacteria
- Require enriched media for growth
- Non-motile, non-sporing
- Morphologically heterogenous
- Anaerobic or facultative anaerobic
- Modified Z-N staining negative
- Some members have undergone changes in nomenclature
 - *Corynebacterium pyogenes* = *Actinomyces pyogenes* = *Arcanobacterium pyogenes*
- *Actinomyces species* have long filamentous morphology, although short V, Y, and T configuration also occur
- *Arcanobacterium* and *Actinobacterium* both have a coryneform morphology

Diseases

- *Arcanobacterium pyogenes*
Host: cattle, sheep, pigs
Conditions: Abscessation, mastitis, suppurative pneumonia, endometritis, pyometra, arthritis, umbilical infections
- *Actinomyces hordeovulneris*
Host: dogs
Conditions: cutaneous and visceral abscessation, pleuritis, peritonitis, arthritis
- *Actinomyces viscosus*
Host: dogs
Conditions: canine actinomycosis
 - cutaneous pyogranulomas
 - pyothorax and proliferative pyogranulomatous pleural lesions
 - disseminated lesions (rare)

- *Actinomyces bovis*
Host: cattle
Conditions: bovine actinomycosis (lumpy jaw)
- *Actinomyces viscosus*
Horses: cutaneous pustules
Cattle: abortion
- *Actinomyces spp* (unclassified)
Pigs: pyogranomatous mastitis
Horses: poll evil, fistulous withers
- *Actinobaculum suis*
Pigs: cystitis, pyelonephritis

Diagnosis

- Clinical specimens: exudates, aspirates and tissue samples from post-mortem
- Direct Gram staining of smear may reveal morphological forms of aetiological agent
- Inoculate blood and MacConkey agars and incubate at 37°C for up to 5 days. Different species have peculiar atmospheric requirement for culture
- Identification criteria
 - *Arcanobacterium pyogenes* produce a characteristic hazy haemolysis along streak lines after 24 hours of aerobic incubation. Pin point colonies are seen after 48 hours. Proteolytic, hydrolyses gelatine
 - *Actinomyces bovis*: adhere to agar media and produces no haemolysis
 - *Actinomyces hordeovulneris*: same as *A. bovis*
 - *Actinomyces viscosus*: produce two colony types
 - Large and smooth: V,Y, and T cell configurations
 - Small and rough: short branching filament
 - *Actinobaculum suis*: poor haemolysis on ruminant blood agar. Colonies have a shiny raised centre and a dull edge. It is urease positive

SPECIES DIFFERENTIATIONS

Characteristics	<i>Actinomyces bovis</i>	<i>Actinomyces viscosus</i>	<i>Actinomyces hordeovulneris</i>	<i>Arcanobacterium pyogenes</i>	<i>Actinobaculum suis</i>
Morphology	Filamentous branching, some short forms	Filamentous branching, short forms	Filamentous branching, short forms	Coryneforms	Coryneform
Atmospheric requirement	Anaerobic + CO ₂	10% CO ₂	10% CO ₂	Aerobic	Anaerobic
Haemolysis on sheep blood agar	±	-	±	+	±
Catalase production	-	+	+	-	-
Pitting on Loeffler's serum slope	-	-	-	+	-
Granules in the pus	Sulphur granules	White granules	No granules	No granules	No granule

- Granules in lesion is caused by *A. bovis* contains characteristic clubs. Club colonies are also produced by *Actinobacillus ligniersii* and *Staphylococcus aureus* botryomycosis

RHODOCOCCUS EQUI

- Gram-positive aerobic bacteria
- Non-motile catalase-positive, oxidase-negative
- Weakly acid fast
- Grows on non-enriched media
- Rod or coccibacillus in shape
- Produces pigments, colonies are pink
- It forms capsule. Produces large, moist, viscid/mucoid colonies

Diseases

Foals of 1 to 4 months of age: suppurative bronchopneumonia and pulmonary abscessation

Horse: superficial abscessation

Pigs, Cattle: mild cervical lymphadenopathy

Cats: subcutaneous abscesses, mediastinal granulomas

Diagnosis

- Specimens: tracheal aspirates, pus from lesion
- Inoculate blood and MacConkey agar
- Incubate aerobically at 37 °C for 24 to 48 hours
- No growth on MacConkey
- Does not ferment carbohydrate
- Does not haemolyse on blood agar. It is cAMP test positive. (enhanced haemolysis) with *S. aureus*
- Most strains are urease and H₂S positive

Tutorial Questions

- 1 Describe the type of colouration produced when *Listeria monocytogenes* colonies are viewed under oblique illumination
- 2 What is the significance of Anton's test in the diagnosis of *Listeria monocytogenes*
- 3 Describe the cold enrichment procedure for the diagnosis of *Listeria monocytogenes*
- 4 What is the aetiological agent of diamond skin disease of pigs
- 5 List two selective media for the isolation of *Corynebacterium spp*
- 6 What staining technique is employed for the demonstration of *Corynebacterial metachromatic granules*

PSEUDOMONADACEAE

- Pathogenic members that infect animals include:
 - Pseudomonas aeruginosa*
 - Burkholderia mallei*
 - Burkholderia pseudomallei*

- Gram negative rods of medium size
- Obligate aerobes
- Oxidase-positive and catalase-positive
- *Pseudomonas species* and *Burkholderia pseudomallei* are motile by polar flagella
- *Burkholderia mallei* is non-motile and require 1% glycerol for enhanced growth
- *P. aeruginosa* produces pigments which diffuse into culture media
- Pigments of *P. aeruginosa* include:
 - Pyocyanin: blue-green
 - Pyoverdin: greenish-yellow
 - Pyorubin: red
 - Pyomelanin: brownish-black

Diseases

- *P. aeruginosa*: causes opportunistic infection in many species of animals
 - Cattle: mastitis, metritis, pneumonia, calve enteritis, dermatitis
 - Pigs: Ear infection, respiratory tract infection
 - Horses: genital tract infection, pneumonia, eye infection
 - Sheep: mastitis, pneumonia, otitis media, fleece rot/ suppurative dermatitis (predisposing factor: heavy rainfall)
 - Dogs and Cats: pneumonia, ulcerative keratitis, cystitis, otitis externa
 - Minks: haemolytic pneumonia, septicaemia, farmed minks very susceptible
 - Rabbits: pneumonia, septicaemia
 - Reptiles: necrotic stomatitis, especially in captive reptile (found in oral cavity of snakes)
- *Burkholderia mallei*: glanders (a contagious disease of equidae characterized by the formation of nodules and ulcers in the respiratory tracts or on the skin)
- *Burkholderia pseudomallei*: causes melioidosis-chronic debilitating disease with disseminated abscesses in many organs of the body
- *Pseudomonas fluorescens* and *P. putida*: pathogens of freshwater fish

Diagnosis

- Sample collection: based on observed clinical signs and lesions. Samples may include pus, respiratory aspirates, ear swab, mastitic milk, discharges, blood (for serology) etc.
- Inoculate blood agar and MacConkey agar plates
- Incubate aerobically for 24 to 48 hours at 37°C
- *B. mallei* grows on media containing 1% glycerol and also on MacConkey agar
- Identification criteria:
 - Colonial morphology
 - Microscopy
 - Biochemical reactions
- Serology
 - Complement fixation test and agglutination technique for *B. mallei* detection
 - Slide agglutination, ELISA, CFT, indirect haemagglutination test used for detection of *B. pseudomallei* serum antibodies
- The mallein test: an efficient field test for screening and confirmation of glanders in animals. Mallein is a glycoprotein extract of *B. mallei*
 - It is injected intradermally just below the lower eyelid
 - A local swelling with mucopurulent ocular discharge is evident after 24 hours in positive cases
- *P. aeruginosa*: produces pigments detectable in media that contains no dye e.g. nutrient agar. It also has a characteristic fruity, grape-like odour

Comparative features of the Pseudomonadaceae

Feature	<i>P. aeruginosa</i>	<i>B. mallei</i>	<i>B. pseudomallei</i>
Colonial morphology	Large and flat with serrated edges	White and smooth becoming granular and brown with age	Range from smooth and mucoid to rough and dull becoming yellowish brown with age
Haemolysis on blood agar	+	-	+
Diffusible pigment	+	-	-

production			
Colony odour	Grape-like	None	Musty
Growth on MacConkey agar	+	+	+
Growth at 42°C	+	-	+
Motility	+	-	+
Oxidase production	+	±	+
Oxidation of carbohydrate:			
Glucose	+	+	+
Lactose	-	-	+
Sucrose	-	-	+

ENTEROBACTERIACEAE

- Members are Gram-negative rods about 3 µm in length
- Oxidase-negative, catalase-positive
- Ferment glucose and a variety of other sugars
- Non-sporing facultative anaerobes
- Reduce nitrates to nitrites
- Mostly enteric organisms
- Motile members possess peritrichous flagella
- Grow well on MacConkey agar because they tolerate bile salts
- Categorised into two broad groups based on lactose fermentation
 - Lactose fermenters e.g. *E. coli*, *Klebsiella spp*
 - Non-lactose fermenters e.g. *Salmonella spp*, *Proteus spp*
- Major animal pathogens (cause both enteric and systemic diseases)
- Examples:
 - *E. coli*
 - *Salmonella* serotype
 - *Yersinia spp*
 - *Y. pestis*

- *Y. enterocolitica*
- *Y. pseudotuberculosis*
- *Y. intermedia*
- *Y. kristensenii*
- *Y. frederiksenii*
- *Y. ruckerii*: pathogen of fish
- Opportunistic pathogens cause disease outside the GIT
- Major pathogens, cause disease in both enteric and non-enteric locations

Yersinia species:

- Yersiniae stain bipolar on primary isolation
- Yersiniae are intracellular organisms localizing in macrophages
- *Y. pestis*:
 - It is pleomorphic
 - It produces little or no turbidity and small deposit in broth culture
 - Haemin required for aerobic growth on nutrient agar
 - Two forms of colony: smooth and rough
 - Causes plaque: bubonic plaque, (septicemic, pneumonic sylvatic forms).
Characterized by lymphadenitis
 - Virulence factor F1 or fraction I (capsular/envelope heat-labile protein), V (protein), W (lipoprotein), F (factor antigens)
 - Probably produces toxin
 - Virulence strains kill mice or guinea pigs following intraperitoneal or subcutaneous injection with as low as 10 viable organisms
 - Transmission: Wild rat (through flea) to town rat (through flea) to humans

Diagnosis

- blood sample, materials from lymph nodes
- grow on blood agar and selective media

- Fluorescent antibody test on cerebrospinal fluid and in aspirates

Note:

- Colonies of non-lactose fermenting bacteria are alkaline due to utilization of peptone in medium. They are pale
- Colonies of lactose fermenters are pink due to acid production from lactose
- Somatic (O), flagellar (H), and capsular (K) antigens are used for serological identification and classification of the enterobacteriaceae

Differentiation and Identification of the Enterobacteriaceae

	<i>E. coli</i>	<i>Salmonella</i> serotype	<i>Yersinia</i> species	<i>Proteus</i> species	<i>Enterobacter</i> serotype	<i>Klebsiella</i> <i>pneumonia</i>
Clinical importance	Major pathogens	Major pathogen	Major pathogen	Opportunistic pathogen	Opportunistic pathogen	Opportunistic pathogen
Cultural characteristics	Some strains haemolytic	-	-	Swarming growth	Mucoid	Mucoid
Motility at 30 ⁰ C	Motile	Motile	Motile	Motile	Motile	Non-motile
Lactose fermentation	+	-	-	-	+	+
IMV _i C test						
Indole production	+	-	V	±	-	-
Methyl red test	+	+	+	+	-	-
Voges Proskauer test	-	-	-	V	+	+
Citrate utilization test	-	+	-	V	+	+
H ₂ S production in TSI agar	-	+	-	+	-	-
Lysine decarboxylate	+	+	-	-	+	+

Urease production	-	-	+	+	-	+
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Yersinia pseudotuberculosis

- Causes infection in many animals including guinea pigs, mice, rats, rabbits, chicken, turkey, pigeons, and canaries
- Sporadic cases reported in horses, cattle, sheep, goats, pigs and cats
- Produced in necrotic nodules in ileum and caecum as well caseous necrosis of mesenteric lymph nodes and omentum
- Grows on blood agar, MacConkey and Salmonella-shigella agar at 37°C and at room temperature (22°C – 28°C)
- Samples of isolation of organism: liver, spleen, heart blood

Yersinia enterocolitica

- Grows on blood agar, Salmonella-shigella agar, desoxycholate citrate agar (DCA) and MacConkey agar
- May require enrichment in phosphate buffered solution (pH 7.6) or peptone broth at 4°C for 3weeks
- Must be differentiated from *Pasteurella*

Note the following characteristic of *Pasteurella*: MR negative, Oxidase positive, no growth on MacConkey except *Manhemia haemolytica*

- *Yersinia enterocolitica* grows at 4°C unlike other enteric bacteria
- Pig is a major reservoir
- Isolation requires enrichment them subculture on agar then do identification tests.

Proteus

- *P. vulgaris*
- *P. mirabilis*
- Pathogenic role doubtful
- May cause diarrhoea in young animals

- Otitis media in dogs
- Often causes infection only when found outside the intestinal tract
- Associated with chronic urinary tract infections

Diagnosis

- Produces characteristic smell and swarms on solid media

Klebsiella

K. pneumoniae

- Pneumoniae in humans
- Klebsiella and Enterobacter cause neonatal meningitis in children
- Opportunistic infections in animals
- Pneumonia in fowls, metritis in mare and sow
- Mastitis (chronic) in cow
- Complicate air-sac infection and pullorum disease in poultry
- Other species: *K. ozaenae*, *K. rhinoscleromatis*

Providencia

P. stuartii, *P. rettgeri*, *P. alcalifaciens*

- Involved in urinary tract infection, sepsis, pneumonia and wound infections
- Hospital infection

Morganella

M. morganii

- Hospital infection
- Implicated in summer diarrhoea in children

Biochemical differentiation of *Proteus* species

	<i>Proteus vulgaris</i>	<i>Proteus mirabilis</i>	<i>Providencia rettgeri</i>	<i>Morganella morganii</i>
Maltose fermentation	+	-	-	-
Mannitol	-	-	Delayed	-

fermentation				
Indole production	+	-	+	+
Gelatin liquefaction	+	+	-	-
H ₂ S production	+	+	-	+
Citrate utilization	-	-	+	-
Urease production	+	+	-	+

Salmonella

Selective media:

- Desoxycholate citrate agar: slightly opaque often with central black spot
- Brilliant-green agar: *S. typhi*, *S. gallirum*, *S. pullorum*, *S. cholerae-suis* and *S. typhi-suis* do not grow on the agar. Colonies are pale-pink usually surrounded by a pink zone. Colonies have a translucent dew-drop appearance
- Wilso and Blair agar: colonies are black
- Salmonella-shigella agar: colonies are pale or colourless
- Hektoen enteric agar: blue-green with black centre
- Motile except *S. galinarium* and *S. pullorum*
- Enrichment media:
 - Selenite F. broth
 - Tetrathionate broth
 - Rappaport broth

Reactions of Members of Enterobacteriaceae in Triple Sugar Iron (TSI) agar

Species	pH change		H ₂ S production
	Slant	Butt	

<i>Salmonella</i> serotype	Red (alkaline)	Yellow (acid)	+
<i>Proteus mirabilis</i>	Red	Yellow	+
<i>P. vulgaris</i>	Yellow	Yellow	+
<i>E. coli</i>	Yellow	Yellow	-
<i>Yersinia enterocolitica</i>	Yellow	Yellow	-
<i>Y. pseudotuberculosis</i>	Red	Yellow	-
<i>Y. pestis</i>	Red	Yellow	-
<i>Enterobacter aerogenes</i>	Yellow	Yellow	-
<i>Klebsiella pneumoniae</i>	Yellow	Yellow	-
<i>Shigella</i> species	Yellow	Red	-

Shigella

- Non-motile
- Non-sporing
- Non-capsulated
- Oxidase-negative, catalase-positive
- *Shigella dysenteriae* type I is catalase negative
 - Species
 - *Sh. dysenteriae* (Tropics): dysentery in human and monkey (shigellosis, colitis)
 - *Sh. flexneri* (Tropics): dysentery in human and monkey (shigellosis, colitis)
 - *Sh. boydii* (Tropics): dysentery in human and monkey (shigellosis, colitis)
 - *Sh. sonnei* (temperate): dysentery in human and monkey (shigellosis, colitis)

Diagnosis

- Sample: fresh stool
- Small colonies on DCA and MacConkey agar
- *Shigella dysenteriae* type I does not grow on DCA
- No growth on Wilson and Blair medium

- Grow on S-S agar and Hektoen enteric agar producing pale and green colonies respectively
- May be inhibited to a certain extent by selenite F broth

Biochemical reactions:

Glucose fermentation	Positive (acid only)
Lactose fermentation	Negative
Sucrose fermentation	Negative
Mannitol fermentation	Variable
Indole production	Variable
MR reaction	Positive
Voges-Prostkauer	Negative
Citrate utilization	Negative
H ₂ S production	Negative
Urease production	Negative
Motility	Negative

Biochemical differentiation of *Shigellae*

Test	<i>Sh. dysenteriae</i>	<i>Sh. Flexneri</i>	<i>Sh. boydii</i>	<i>Sh. Sonnei</i>
Glucose	Acid (A)	A/A and G (gas)	Acid	Acid
lactose	-	-	-	Late fermenter
Mannose	-	Acid	Acid	Acid
Sucrose	-	-	-	-
Dulcitol	-	-/A	-	-
Xylose	-	-	-	-
ONPG test	-/+	-	-	+
Indole	Variable	Strain variation	Variable	-

ONPG: Orthonitrophenol (-β-D-galactopyranoside)