

Species of *Bacillus*:-

Bacillus anthracis

Bacillus cereus

Bacillus subtilis

Bacillus mesentericus

Bacillus mycoides

Bacillus megaterium

Bacillus stearothermophilus

Morphology and staining :-

1- Gram +ve bacilli, endospore producing rods up to 10 mm in length.

2- All strains are motile exception *Bacillus anthracis* and *Bacillus mycoides* non-motile and posses capsule can seen stained with poly chrome methylene blue dye reveal chains of square ended surrounded by pink capsules .or by Giemsa stain (Mcfadyeans reaction), purple- staining rods surrounded by red capsules.

Cultural characteristics :-

1-aerobic bacteria or facultatively anaerobic. the important media are

a-**Nutrient agar**:- the growing colonies are big in size, flat, dry with irregular edge. This characterized appearance is called medusa head (curled hair) under microscope. *Bacillus cereus* colonies are similar to those of *Bacillus anthracis* but are slightly larger with agreenish tinge.

Other types of *Bacillus* colonies are dull ,rough, wrinkle led and strongly adherent to the agar and colonies become brown with age.

b-**Blood agar**:- all strains are β -hemolytic except *Bacillus anthracis* which is non-hemolytic .

c- **poly myxin- lysozyme- EDTA-thalious acetate agar (PLET).selective media.**

Biochemical test

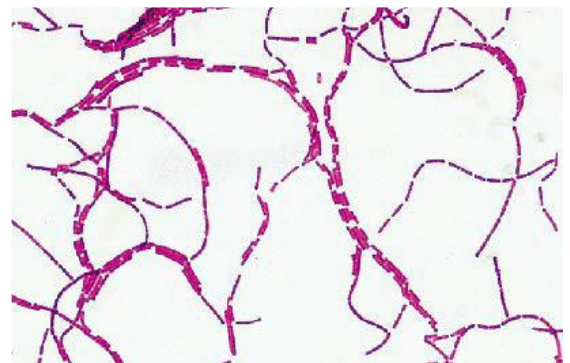
Species	Motility	β -hemolytic	Urease	Citrate	Starch hydrolysis	Gelatinase	Nitrate reduction	Voges proskauer	glucose
<i>Bacillus anthracis</i>	-	-	-	+	+	+	+	+	+
<i>Bacillus cereus</i>	+	+	-	+	+	+	+	+	+
<i>Bacillus subtilis</i>	+	+	-	+	+	+	+	+	+
<i>Bacillus mesentericus</i>	+	+	-	+	+	+	-	-	+
<i>Bacillus mycoides</i>	+	+	-	-	+	+	+	-	+

Diagnosis :-

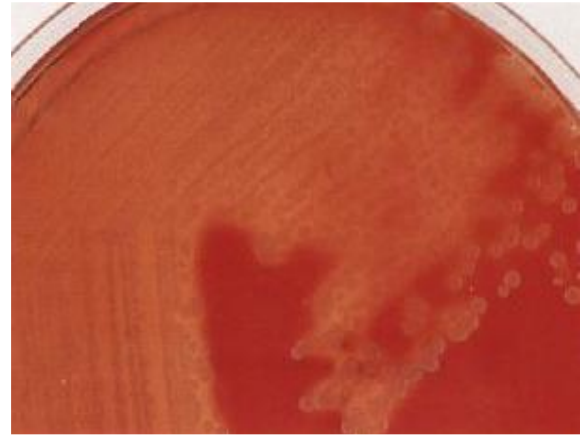
- 1-clinical signs depend on type of diseases and animal.
- 2-blood smear staining by Giemsa stain or polychrome methylene blue.
- 3- Inoculating the specimen on blood agar and selective media for bacillus .
- 4-Selective media :- polymyxin- lysozyme- EDTA-thallos acetate agar (PLET)
- 5-Biochemical test
- 6- pathogenicity tests on mice and rabbit .
- 7-**Thermo-precipitin test or Ascoli test:** - is a thermo precipitation test designed to detect Ag of bacillus anthracis in biological materials such as hides. Homogenized material is boiled and clarified by filtration. the examine make in capillary tubes. Positive result formation precipitation line between meeting Ag with anti sera during 15 minutes.
- 8- Agar gel immunodiffusion, complement fixation test, ELISA, PCR and immuno fluorescence test.



Bacillus subtilis



Bacillus megaterium.



colonies of *Bacillus subtilis* on blood agar are smooth, round, and surrounded by a zone of beta hemolysis

Clostridium

Species :-

Clostridium tetani

Clostridium botulinum

Clostridium chauvoei

Clostridium septicum

Clostridium novyi (*Cl. oedematiens*)

Clostridium perfringes (types A,B,C,D and E)

Morphology and staining :-

1-Gram +ve rods

2-endspore producing rods, the spore are oval shape terminal or sub terminal location causing swollen or non swollen bacilli.

3-All strains are motile by peritrichate flagella except *Clostridium perfringes* that is non-motile and posses capsule in animal tissues.

Cultural characteristics :-

Clostridium. spp are anaerobic. requirements varies among the species ,but they all prefer an atmosphere containing between 2-10%co₂.most clostridium require enriched media that include amino acid, CHO, vitamin and blood or serum. Optimum growth of the pathogenic species occurs at 37c. the important media used are :-

- 1- **Blood agar and MacConkey agar:-** should be inoculated and incubate an aerobically condition containing 2-10% co₂ as this enhance their growth. An anaerobic jar with a catalase an anaerobic indicator and envelope delivering H₂+CO₂ is usually satisfactory.
- 2- **Cook meat agar (selective media).**

3- **Cook meat broth or thioglycollate broth:-** boiling to expel absorbed O₂ and sub cultured on to blood agar under an atmosphere of H₂ + CO₂.

Biochemical test:-

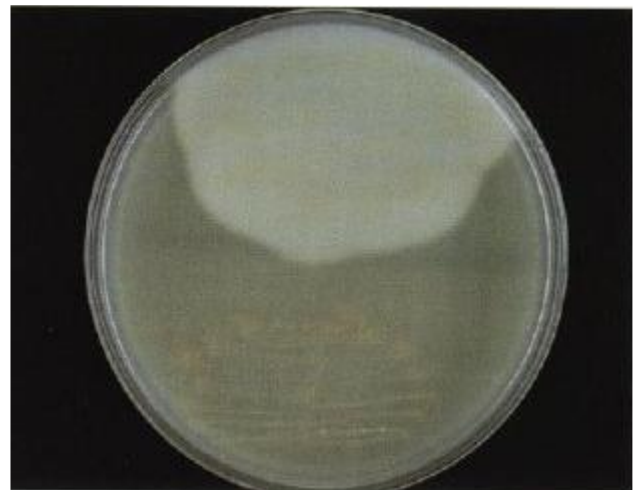
species	motility	Gelatine	Nitrate	Indole	Casein hydrolysis	Urease	Glucose	Maltose	egg yolk agar
<i>cl.tetani</i>	+	+	-	+	-	-	-	-	-
<i>cl. botulinum</i>	+	+	-	-	+	-	+	v	-
<i>cl. chauvoei</i>	+	+	+	-	-	-	+	+	-
<i>cl. septicum</i>	+	+	+	-	+	-	+	+	-
<i>cl.novyi</i>	+	+	-	-	-	-	+	+	+
<i>cl.perfringens</i>	-	+	+	-	+	-	+	+	+

Diagnosis :-

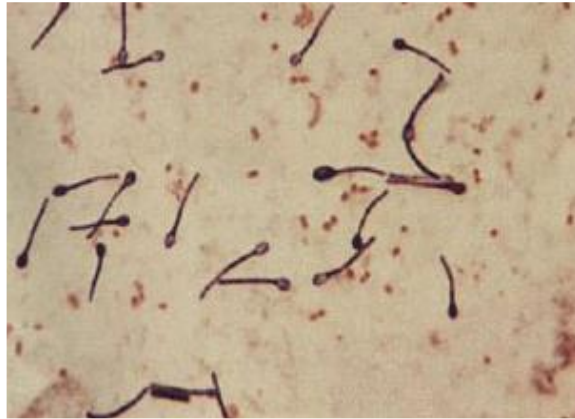
- 1- Gram stained smears from specimens.
- 2- Spore stain.
- 3- Selective media.
- 4- Biochemical test.
- 5- Serological test.
- 6- Neutralization or protection test:- the tests using specific antitoxin. The animals are give antitoxin at least 2 hours before inoculation with the material containing toxin. Demonstration of the activity of tetano spasmin in amouse.
- 7- Naglar test:-
- 8- This test used to distinguished between types clostridium that ability to secret Lecithinase (alph- toxin) that react with Lecithin present egg yolk medium lead to formation opalescent precipitation round bacterial colonies on the half plate which not contain antitoxin .while the other half plate that containing antitoxin. non formation opalescent.



cl. perfringens Spores are elliptical, and central or subterminal, cells slightly swollen



Clostridium perfringens on egg yolk medium



Clostridium tetani the spores are terminal position