DEPARTMENT OF VETERINARY MICROBIOLOGY AND PARASITOLOGY

COLLEGE OF VETERINARY MEDICIN

UNIVERSITY OF AGRICULTURE, ABEOKUTA

VPM 401: VETERINARY BACTERIOLOGY

LECTURE NOTES

DR. M. A. OYEKUNLE (SP 1071)

DR. O. E. OJO

DR. M. AGBAJE

DR. M. A. OYEKUNLE

Introduction

What is bacteriology?

Bacteriology is the study of bacteria

Why do we study bacteria?

We study bacteria in Veterinary Medicine or medicine because bacterial diseases are among the most important and common problems that animal and fish keepers/managers must deal with. Therefore, the veterinarian must be equipped to know about these organisms. Because infections frequently involve more than one system, veterinary microbiologist/bacteriologists have generally resisted the systemic approach to teaching infection diseases.

However, student may develop tables to assist himself in system orientation to infections agents.

We study these organisms to know which disease they are involved so as to find a treatment. Therefore, the approach to their study will include knowing fully about them.e.g. Their

- History
- Habitat
- Characteristics – Colonial/Culture characteristics
    - Cell morphology
    - Staining characteristics

- Biochemical characteristics
- Genetic characteristics

- Among others

The Actinomycetes

- They consist of a group of filamentous microorganisms occupying an intermediate position between bacteria and fungi.
- Their identity as bacteria was confirmed by:
  * Their prokaryotic cellular organization
  * Their cell wall chemistry
  * Their nitrogen metabolism
  * Their sensitivity to antibiotics and phages.
- There are two major groups of actinomycetes
  * Aerobic actinomycetes
  * Anaerobic actinomycetes
- They cause infections in animals and humans.
- There are also a large number of nonpathogenic species

1. Genus *Actinomyces*

- Are pleomorphic Grain Positive coccobacitli, rods, filament, branching or non branching cells.
- Non-notile, non-spore forming.
  membrane of the oral and nasal cavities and the genital tract.
- Important species are:

A. Israeli	human actinomycosis	obligate anaerobic
A. bovis	cattle actinomycosis	obligate anaerobic
A. viscosus	Dog periodontal disease	facultative anaerobic
A. hordeovulnesis	Human chronic supprutive Dog infection	facultative anaerobic
A. naeslundi	Human Periontal infection Dental caries	facultative anaerobic
A. pyogenes	Animals Pyogenic infection	facultative Anaerobic

Disease- Actinomycosis

- **Laboratory diagnosis (Actinomycosis)**
  Direct examination
  - Small amount of pus placed in petridish.
  - This is washed with water to expose small sulphur granules.
  - Transfer granule to a slide, add a drop of 10% NaOH, add cover slip and crush by gentle pressure.
  - Characteristic ray-fungi is seen with club shaped margins under low power if actinomycosis.
  - Then remove cover slip, spread and stained by Grams.

- If Actinomycosis, branching Gram positive filaments are observed.

- **Isolation and Cultivation**
  - Can be cultured on blood agar, brain heart infusion agar and thoglycollate broth.
  - An atmosphere containing 5-10% Co2 preferred for incubation.
  - Colonies are white, rough, nodular and adhere tenaciously to the medium and difficult to remove.
  - Gran stained smears from growth on media revealed masses of Gram positive rod and slightly branched filaments.

- **Identification**

Based on characteristic sulphur granulas

Demonstration of gram positive filaments

- **Treatment**
  - Drainage and antibiotic therapy

2. Genus *Nocardia*

- Non-motile, nonspore forming, grain positive rods which sometimes show branching.
- Partially acid fast, aerobic.
- Spits sugars by oxidation.
- Are important part of the soil and water flora.
- A number of the members of the genus cause a variety of diseases in both normal and immunoconipromised humans and animals.
- Mechanisms of pathogenesis complex and not well understood but include the capacity to evade or neutralize the myriad of antimicrobial activities of the host.
- More than 40 species have been described.
- Important species include

N. asteroides	-	Human and animals
N. bransiliensis	-	Human
N. cavaiae	-	Human, bovine mastitis, guinea pig
N. farcinica	-	Cattle

Mode of Infection

- By inhalation, through wounds, hands and feet of laboratory workers.
- Usually exogenous

Laboratory diagnosis

Direct examination

- Grain strained smears of pus/lesims reveal Gram positive branching filaments with or without clubs.
- Stains partially with ZN stain.

- Giensa stain can also be used.

Experimental animal: guinea pig susceptible

Isolation and Cultivation

- Organism grows on blood agar or any other enriched media.
- The colour of the colony varies from chalk white to deep orange.

Identification

- Based on demonstration of typical organism, colonial, cultural and morphological characteristics.

Treatment

- Various drugs useful including sulphonamides and antibiotics.

3. Dermatophilus congolensis

- Gram positive branching filamentous rods, aerobic and nonspore forming, non acid fast.
- Produce motile zoospores.
- Unique medically because natural growth cycle is restricted to the living layer of the epidemics of animal and human skin.

Pathogencity: Causes dermatophilosis in cattle and dermatophilus infection in other animals which is characterized by scabs formation on the skin.

Laboratory diagnosis

Specimen- Infected Scab

Direct Examination

- Many procedures employed in making impression smears.
- But better if impression smear is made from the moist concave undersurface of freshly removed scabs.
- Stains well with dilute carbol fuchsin or methylene blue stain, Gram stain or preferably 1:10 dilution of Giemsa strain for 30 minutes.

Isolation and Cultivation

Organism grows well on media containing blood or blood product.

Colony : -

- Small, rough, graywhite colonies appear in 24-48 hours of incubation.
- Colonies are yellowish to orange.
- Produces B haemolysis on sheep or horse blood agar. On human blood, haemolysis is narrow and hazy.
- Motile zoospores are formed as a result of the septation of hyphal element

- Zoospores possess polar flagella.
- Gram positive, branching hyphal elements in various stages of segmentation are seen.
- Two colony forms can be demonstrated.

(i) Rough - grows into the agar and difficult to remove and emulsify in water or saline.

(ii) Smooth- easy to remove from plate and emulsify in water or saline.

Antigenic Components

- Five (5) antigenic types demonstrated using agar gel precipitating test.

Treatment

Use of various drugs, chemicals and concoitious are in practice.

4. *Mycobacterium*

- Are Gram positive (Not easily stained by Gram method acid fast, small rods, non-motile.
- Filamentous and branching forms occur.
- They don't strain readily, but when they do so strain with basic dyes.
- They resist decolourization by acid.
- There are more than 50 Mycobacteria species including many that are saprophytes.

i. Runyon Classification of Mycobacteria (Runyon's group)

Classification	**Organisms**
Tuberculosis complex	*M. tuberculosis*
	M. bovis
	M. africanium
Photochromogens	*M. asiaticum*
	M. kansasi
(Produce pigment in light)	*M. marium*
	M. simiae
Scotochromogens	*M.flavescens*
	M. gordonae
(produce pigment in the dark)	*M. scrofulaceum*
	M. szulgai
Non-chromogens	*M. avium complex*
	M. celatum
(No pigment produced)	*M. haemophilum*
	M. gastri
	M. genovense
	M. malna

ii. Rate of growth

- Rapid growers
- Slow growers

iii. Anonymous mycobacteria

Are atypical unclassified mycobacteria that have been recovered from animals and man.

iv. Saprophytic or non pathogenic mycobacteria

Mycobacteria considered to be non pathogenic or not previously identified are now becoming epidemiologically important particularly in the AIDS era because of their high resistance to antibacterial agents.

v. New species

Laboratory diagnosis of tuberculosis

- Based on (i) Microscopy
  (ii) Culture
  (iii) Immunological test
  (iv) Molecular characterization
  (v) Others

Specimen: Different samples may be used depending on the clinical picture of the disease.

5. Genus *Actinobacillus*

- Gram negative, small rod, non-motile, non-spore forming, aerobic and fermentative.
- Rarely grows in filaments, and if so, filaments show some branching.
- Has tendency for bipolar staining

Important species include

A. plearopjneumnoiae - pig

A. equulin- Horse (fals) and occasionally pig

- joint illness, navel illness

A. suis – pig

A. seminis – sheep (ram) – affecting ram epididymus

- Natural infections with *A.liquieresii* occur in both cattle and sheep and are characterized by infections granulous containing pus affecting the soft tissue in the region of the head e.g. tongue.

Laboratory diagnosis

Granula/Pus specimen examined in the same manner as in actinomycosis.

- Small gram negative rods demonstrated in the lesion.

Isolation and Identification

Specimen
- Pus or necrotic material from early lesions.
- Natural seeded on blood or serum agar.

- Lucubated at 37oC under 10% CO2 accelerated growth.
- Subucultured strains grow well in air.
- In media contained fermentable carbohydrate long almost filamentous form are seen.

Colonies
- may be mucoid or stringy when freshly isolated.
- can be white, grayish-white, yellowish or bluish in colour.

Cultural
- Are aerobic to facultative anaerobic

Characterictis
- Are micro aerophitic on primary isolation.

Biochemical characteristics – Acid but no gas from carbohydrate when fermented.

Pathogenicity
- Pathogenic to animals.

Some species can affect humans disease produced by A. liqnieresii can be similar to that produced by Actinomyes and Maihemia haemidytics.

Differentiation of the species of the Genus *Acticiobacillus* using biochemical characteristic

A.liquieresii	+	+	+	-	+	-	+
A. Equuli	-	-	+	-	+	+	+
A. Seminis				-	-	-	-
M. haemolytics	+	+	+	-	+	-	+

6. Genus *Mycoplasma*

- Are bacteria
- Members of the genus are characterized by the absence of a cell wall.
- They are pliable and can pass through the pores of filters that retain bacteria.
- Most members have sterol in their membrane which provides added strength and rigidity protecting the cells from osmostic lysis.
- They are among the smallest form of life.
- Their genomes are thought to be the minimum size for encoding the essential functions for a free living organisms.
- Are facultative anaerobic or obligate anaerobic.
- Are pleomorpohic.
- Because they have cell membrane, RNA and DNA, they differ from viruses.
- Mycoplasma can resemble fungi because some produce filaments that are commonly seen in fungi.
- It is because of these filaments that scientists named them mycoplasma i.e. mycomeans “fungue”.
- They stain poorly, but giensa can be used to demonstrate it inn tissues.
- Many are unable to move because they lack flagella but some can glide.

Cultivation and Cultural features

- Mycoplasmas have low biosynthetic ability.
- Therefore they need rich medium containing natural animal protein (blood serum) and in most cases sterol compounds.
- Mycoplasma colonies on solid media produce a characteristic “frierd egg” appearance.

Cell morphology

- Coccobacilli, coccoal forms, ring forms, spiral and filaments seen in smerars stains poorly, but, giensa can be used.
- Size 50-60 to `00-250 mm, diameter 0.3-0.8mm.
- Parasitic mycoplasmas contain 10-20% lipid, relatively low content of nucleir acid compared to other bacteria.
- May grow in chicken embryo.

Viruses and Plasmids of Mycoplasmas

- 14 viruses identified to infect mycoplasma
- 6 in *Acholeplasma*
- 4 in *Mycoplasma*
- 4 in *Spiroplasma*
- There is evidence of integration of viral genomes into mycoplasma chromosomes.
- Release of virus is continuous and not accompanied by cell lysis.
- Plasmids detected in Mycoplasma, Acholeplasma and Spiroplasma.
- Acholeplasmataceae – does not depend on sterol for growth.
- Anaeroplasmatacae – strict anaerobes

The Bacteriodes