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Book Review

Microbiology: A Fundamental Introduction Second Edition

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Bacterial Descriptions

Bacterial Identification and Cultural Methods

Family Staphylococcaceae

Staphylococci

The *Staphylococci* are gram positive cocci that occur singularly in pairs and in clusters, are approximately 1μm in diameter. They are a non-motile, aerobic to facultative anaerobe, non-spore forming cocci, that are halophilic to haloduric, and are catalase positive [157,201,202]. They may be found in man and animals and occur as flora of the skin, mucus membranes, respiratory tract, vagina and genital-urinary tract [7,31,225,228].

Although the *Staphylococci* are gram positive cocci, they must be distinguished from similar gram positive cocci, such as the *Planococcus*, *Stomatococcus* and *Micrococcus*. These microorganisms may be found in the environment, and also are catalase positive [203]. Colonies should be incubated for 18 - 24 hours. They generally appear a cream colored, and may appear a golden yellow, and buttery looking on culture media at times. Small-colony variants can occur rarely, but approximately a tenth of the size of normal size colonies [203,225].

Staphylococci cause a number of skin infections, as well as wound infections. These skin infections may be filled with fluid, and or pus. The infection of the follicle is called furuncles. Furuncles are typically boils that infect the hair follicle [204,228]. "Scalded skin syndrome" is another skin infection that is caused by *Staphylococci*. The infection is due to an exfoliative toxin, or staphylococcal exfoliative. The infection may be the bullous type which is localized, and may lead to the generalized form. The infection usually begins from the face, neck, groin, and extends to the trunk. Within 48 hours there is a rapid spread with the cutaneous form, and a bright red lesion where there is peeling of the skin [203,204,220,225].

Enterotoxins are molecules that are heat stable, and are the frequent cause of food poisoning. There are a number of serological groups, A through E, G and I, that are heat stable and can cause peristalsis, and induce vomiting up to 2 to eight hours. The toxin can cause inflammation of the intestinal tract, changes in the white cells, and may cause lesions throughout the intestinal tract. There are a number of common food products, that can be a source of food poisoning, namely: bakery goods, custards, potato salad, processed meats, and ice cream. These products may be left out at room temperature, and thus contribute to the presence of enterotoxin contaminated foods [7,46,203,221,225].

Toxic shock syndrome toxin-1 or TSST-1 includes a number of exotoxins that have antigenicity, and are pyrogenic (fever inducing), and are what is referred to as superantigens. They are superantigens in the since, that they greatly affect the T-lymphocytes. They induce the rapid cell division, without an antigenically specific immune response. Some symptoms include high fever, vomiting, diarrhea, and vomiting for several days, and in life treating cases, shock and death may result [46,203,221,225].

Protein A

Protein A is a cell wall component. It has the ability to bind to the Fc region of all IgG subclasses except IfG3, and functions by interfering in opsonization, and the uptake of polymorphonuclear cells, the activation of complement, and elicits an immediate and delayed–type hypersensitivity reaction. The presence of protein A provides the basis for the co-agglutination test for the identification of "gonococci, streptococcal grouping and bacterial antigens in fluids) [7,46,146,203,221].

Capsule

Some strains of *Staphylococci aureus* produce a polysaccharide capsule that may prevent the phagocytosis by neutrophiles, and is particularly important for the adherence to prosthetic devices such as, pacemaker leads, peritoneal catheters, and intravenous lines. The capsule does contribute the virulence. Type 5 capsular antigen is associated with staphylococcal bacteremia and arthritis, whereas type 8 has been linked with the production of toxic shock syndrome toxin [46,146,203,221,228].

Other infections include pneumonia, and wound infections which could lead to osteomyelitis. With pneumonia, there may be complications in the form of secondary bacteremia or endocarditis. Bacteremia's can lead to a serious meningitis, and some entance pericarditis [46,201,203,221].

Culture Characteristics

Staphylococci aureus again is a gram positive coccus that is about one micrometer in diameter. In culture, they appear as colonies that are cream colored, white and comingly a golden yellow, with a butter-looking" appearance, when grown on 5% Sheep Blood Agar. They are approximately 4 - 8 millimeters at 18 - 24 hours of incubation. They can be present as small-colony variants requiring 48 hours of incubation to appear. These small variants are about one tenth the size of normal colony size, of most *S. aureus* in culture. Most laboratories us 5% Sheep Blood Agar for screening isolates from various sources. However, there are a number of selective agars for the isolation of *Staphylococci*, Along with Blood Agar, Mannitol Salt Agar, Phenylethyl alcohol Agar, Colistin Nalidixic Agar, Baird Parker Agar, and CHRO-Magar (125,221,222,223,224,225,226,239).

Most of the *Staphylococci* have been traditionally separated into groups, based on their ability to for a fibrin clot according to the coagulase test. A positive clot is formed in EDTA plasma. The enzyme involved in the coagulase test is staphylocoagulase. Those species that are coagulase positive include: *S. aureus, S. intermedius, S. delphini, S. lutrae*, and some strains of *S. hyicus*. Both *S. lugdunensis* and *S. schleifer* may be confused as coagulase positive [203]. There are two types of coagulase, namely free and bound coagulase. Free coagulase is a protein that is released into the medium. Whereas bound coagulase or clumping factor is a part of the cell wall, and reacts with coagulase reacting factor (part of plasma) resulting in a complex, which reacts with plasma fibrinogen to form of a fibrin clot [7,202,225]. The coagulase negative *staphylococci* include (CoNS) the major species *S. epidermidis, S. saprophyticus* and *S. lugdunensis*, whereas *S. epidermidis* is the most common nosocomially acquired cause of urinary tract infections (7,201). It has also been associated with intravascular catheters, shunts, and implantation devices. *S. saprophticus* has been found linked to urinary tract infections of younger women. Other opportunistic coagulase negative *staphylococci* are *S. warneri, S. capitis, S. simulans, S. hominis* and *S. schleiferi*. These microbes have been with a number of infections such as endocarditis, septicemia and wound infections [7,202,203,225,226].

Identification Methods

Again, the *Staphylococci* are gram positive cocci that occur singly, in pairs, and in clusters (grapelike clusters). They may be distinguished from the genus Micrococcus, based on the fact that Micrococci are strict aerobes, and will not grow facultatively [28,203,227]. *Staphylococci* may also be differentiated from the similar micrococcus, by the Microcase disk test, by Remel, Lenexa, Kan [203]. The major-

ity of *staphylococc*i are negative, whereas micrococcus are positive [203]. However approximately 5% of *S. aureus* do not form clumping factor, and thus making performing a tube coagulase necessary for confirmation [28,203,227].

The PYR test (L-pyrrolidone-β-naphthylaminde) can distinguish *S. aureus* (negative) from *S. lugdunensis, S. intermedius*, and *S. schleiferi* which are PYR (+). All the clinically significant staphylocci are positive for the production of acetoin (VP test), with the exception of *S. intermedius*, which may produce a delayed VP test [203]. Urine cultures that may be clinically significant can be screened with the Novobiocin sensitivity test. *S. saprophyticus* is resistant to nobobiocin. A simple lawn of the organism is laid down on a culture medium such as Mueller Hinton Agar (de la Maza., *et al*. figure 1-19), and a zone size of < or equal to 7 mm is considered novobicin resistant. A 5 µg novobicin disk is applied to a lawn of possible *S. saprophticus*, to determine either its resistance or sensitivity [203,225,228]. Antibiotic testing should be followed according to the Clinical and Laboratory Standards Institute (CLSI). Commercial tests should be adhered to by the manufacturer's procedural guidelines, (Note, See figure 1-10) on 225 p. 8. [203].

There are a number of identification commercial kits for differentiating *S. aureus* from the CoNS. Some use latex beads, that are coated with antibodies that are specific for *S. aureus* (monoclonal antibodies), and for example, Staphyloslide (BD Diagnostic systems). Staphaurex (Remel, Lenexa, Kan.), BactiStaph also from Remel, and Prolex (Pro-Lab Diagnostics: Cheshire United Kingdome). These kits have high specificity and sensitivity, than conventional testing methods for clumping factor [203]. In general, these kits are particularly practical for identification of MRSA, where a weak coagulase test is difficult to interpret (203). In regards to *S. aureus* and intermediate-resistance to vancomycin resistance (VISA), the first case occurred in 1996. "True" Vancomycin-resistance was reported in the United States in 2002 [203].

Methicillin-Resistant *Staphylococci*

Historically, some *staphylococci* have been characterized as methicillin resistant, although methicillin is not currently being using therapeutically. *Staphylococci* that are Nafcillin and or Oxacillin resistant, have classically been considered methicillin resistant [203]. *S. aureus* that are resistant to these penicillin's have been called MRSA, whereas *S. epidermidis* is referred to as MRSE (methicillin-resistant Staphylococcus epidermidis) [203].

Macrolide Resistance

The testing of clindamycin sensitivity, some situations may require testing both for erythromycin resistance, and clindamycin for susceptibility. When a normal disk sensitivity is performed for clindamycin, additional erythromycin disk may be added to the same sensitivity plate, the presence of the additional erythromycin disk may induce the display of antibiotic resistance for clindamycin, by a *S. aureus* isolate [203]. This sensitivity test is called a D test, a test using a "modified double-disk diffusion method" (D test) [203].

Streptococcaceae

Streptococci and Other Gram Positive Cocci

The *Streptococci* are gram positive cocci, that are microaerophilic to facultative anaerobic, nonmotile, and catalase negative. Some are considered aerotolerant anaerobes with their inability to utilize oxygen, in the presence of available oxygen. Some are capnophilic, grow best with an increased CO_2 atmosphere and may be homofermentative. Those that are also homofermentative, are so because they form lactic acid as their predominate acid, during the fermentation of glucose [178,202,229,332].

Other gram positive cocci belonging to the family Streptocociaceae include, the genera: The *Streptococci, Lactococci, Pediococci* and the *Enterococci*. The *Lactococci* were historically classified in the N *streptococci*, whereas the enterococci were previously classified in the group D *streptococci* [7,28,178,202,229,230].

The cell wall of *streptococci* contain peptidoglycan, and teichoic acid. The cell wall of *streptococci* may also possess a c- substance or c polysaccharide which is antigenic. As such it can be tested for serologically. The streptococci have historically been classified by these group specific c polysaccharides, based on the work in the 1930's by Rebecca Lancefield. Today the *streptococci* are routinely separated into groups based on Lancefields classification, and the serological basis of their group specific C carbohydrates, Groups A-D, F and G [7,67,230,231].

Hemolysis of 5% Sheep Blood Cells

Streptococci in the laboratory have also been grouped based on the hemolytic reactions when growing on 5% sheep blood agar. *Streptococci* that produce a hemolysis that completely lyses sheep blood cells are called beta hemolytic (β), whereas those that only cause partial lysis are called alpha hemolytic (α). No lysis of blood cells has been referred to as gamma hemolysis [29,230,231]. The important βeta hemolytic *streptococci* for man are *S. pyogenes, S. agalactiae, S. dysgalactiae subsp. equisimilis*, and the *S. anginosus* group some of which are alpha or gamma hemolytic [67,230,231].

Cell Wall Components and Antigenicity

The cell wall of *S. pyogenes* is very close in structure to other *streptococci*. The Group A antigen responsible for its Lancefield group is due to the presence of the M protein, which also contributes to its virulence. The M protein is heat and acid resistant, and is not only found in the cell wall, but the cell surface and present on fimbriae. Its virulence lies in its ability interfere with phagocytosis, and is also involved in bacterial attachment [232]. In fact, there are a number of virulence factors (disease causing) associated with the group A streptococi, namely protein F (fibronectin-binding protein), lipoteichoic acid, hyaluronic acid capsule, toxins, and enzymes. Both protein F and lipoteichoic acid may function as adhesions, regulate the movement of ions through the cell envelope, particularly magnesium ions. The presence of the hyaluronic capsule is a important weapon which prevents the enhanced opsonization by phagocytic neutrophils [7,67,230,231,232].

Virulence Enzymes

Streptococcus pyogenes also possesses enzymes such as streptolysin O, streptolysin S., deoxyribonuclease (DNase), streptokinase, hyaluronidase, and erythrogenic toxin. All these enzymes are thought to be important agents of virulence, although their exact role has not been substantiated. Streptolysin O is the hemolysin (lysis of R.B.C's) that is produced by strains of A, C and G, and is heat stable, but oxygen label and is strongly immunogenic [7]. The anti-Streptolysin O (SLO) test is often used to predict the presence of "Scarlet fever". It is used as a means to determine, Streptolysin S also a hemolysin is oxygen stable, but heat labile, and typically can lyse white blood cells. However, it is non-immunogenic and does not stimulate a immune response [7,67,230,231]. Hyaluronidase has the ability to dissolve the ground substance, which holds tissues together, and is often referred to as "spreading factor" [7,67,230,231].

Clinical Infections

S. pyogenes the most frequent infection caused by Group A streptococcal are namely, pharyngitis and tonsils. Group C and G are also a cause of pharyngitis. Acute pharyngitis is often referred to as "Strept throat" with common symptoms of fever, nausea, possibly vomiting, and maybe abdominal pain [67,230,231,235]. They can be the cause of many pyodermal infections, which include impetigo, cellulitis, erysipelas, wound infection or arthritis. A pyodermal infection can be defined as an infection where there is pus formation [142,236]. Cellulitis is characterized as a spreading redness and inflammation of skin and subcutaneous tissue causing tissue damage [7,220,230]. Erysipelas is a spreading "acute cellulitis" of the skin and subcutaneous tissue, resulting in "reddish patches with raised margins" [7,142,220,230,234].

Impetigo consists of spreading fluid filled vesicles which bust and become crusted lesions. Wound infections caused by *S. pyogenes* are not caused by infections, but are often caused by an accidental injury to the skin or deeper tissue [235]. A very serious infection "necrotizing fasciitis" is a rapidly progressive necrosis of the skin, subcutaneous tissue and fascia [7,204,220,230,235]. Toxic Shock Syndrome was previously linked to the use of vaginal tampons. Growth can occur in the vaginal area where the Group A *Streptococci* toxins are formed, and may enter the blood stream, resulting in shock due the presence of Super Antigens [204,220]. Rheumatic Fever is believed to be a complication that may occur after infection with *Streptococcus pyogenes*, in which auto-antibodies that are formed against Streptococcal M protein. This results in antibodies attacking the heart, and heart valves [142,230,236]. Acute glomerulonephris which is also a sequelae of "Strept throat," and believed due to the deposit of antigen-antibody complexes on the individual kidney units the glomeri during some infectious diseases [7,142,204,220,230,236].

Typical Identification Methods Used

In addition to testing for hemolysis, the *streptococci* have been distinguished based on disk tests formed using 5% sheep blood agar, where isolates are tested in for sensitivity, or resistance to various chemically impregnated disks. Case in point, the Group A Streptococi are sensitive to a bacitracin (A disk) in which a lawn and isolate is streaked on a 5% sheep blood plate, and a A disk is placed in the middle of that streaked lawn. Any clear zone around the A disk is considered and positive test., Any isolates that are bacitracin sensitive, are considered as *S. pyogenes* [230]. The Group B Strept or *S. agalactiae* is resistant to bacitracin, and will grow all the way up to the disk. The use of 5% Sheep Blood Agar has also had the incorporation of SXT (combination of sulfamethoxazole and trimethoprim into the formulation of the medium. This is used for the screening of throat cultures and identification of Group A and Group B *streptococci*. This medium is particularly useful, in that it tends to retard the growth of other respiratory flora [7,230]. Another important test for *S. pyogenes* is the PYR test (pyrrolidonyl-α-naphthylamide hydrolysis test. Both Group A *Streptococci* and Enterococci, Aerococci, and Gemella are positive for this test. The PYR test detects the activity of the enzyme L-pyrrolidonyl arylamidase, which acts on the substrate N,N-dimethylaminocinnamaldehyde. Suspect colonies of *S. pyogenes* are cultured at 35^{0}C over night, a distinct red disk color is considered a positive test [7,230].

The Group B *Streptococci* or *Streptococci agalactiae* may be presumptively identified on the basis of the CAMP test. The CAMP Test is a acronym for the first letters of the researchers who developed the test, whom are Christie, Atkins, and Munch-Peterson. In this test, a 5% Sheep Blood Agar Plate is vertically streaked with a known β-lysin producing culture of *Staphylococcus aureus*, and in which a suspect isolate is streaked at right angles to the first streak. However, the streaks do not touch or intersect each other. With a positive test, the Group B *streptococci* producing its own hemolysin, is enhanced in the presence of β-lysin produced by the *S. aureus*. If there is enhanced hemolysis, and a arrow head zone of hemolysis, the isolate belongs to the Group B *Streptococcus agalactiae* [7,230]. A second test the hippurate hydrolysis test can also be performed to confirm a isolate as a Group B *streptococci*. In this test, a four day old culture growing with 1.0% sodium hippurate (the substrate). The enzyme hippuricase splits the substrate into sodium benzoate and glycine. If the test is positive, A dark grayish color is formed in the form of a precipitate [7,67,230,231]. Other tests for the gram positive cocci would include the Leucine Aminopeptidase or (LAP). This test in conjunction with the PYR can help to differentiate the several genera of gram positive cocci. The test is useful for distinguishing *Aerococcus* and *Leuconostoc*, from other gram positive cocci. Both the genus *Streptococcus*, *Enterococcus* and *Pediococcus* are PYR positive, whereas *Aerococcus* and *Leuconostoc* are PYR negative [7,67,230,231].

The VP (Voges-Proskauer test) has been used for the detection of acetoin is also helpful to distinguish the small colony β-hemolytic anginosis group containing group A and C Lancefield antigen, from the large colony β-hemolytic pyogenic strains displaying the same antigens. A Red color confirms a positive VP test, with the anginosis group giving a positive red color [7,67,230,231]. The β-D-Glucuronidase (BGUR) is an enzyme that is found in the large colony β-hemolytic Groups C and G *streptococci*. The group C and G are able to act on the substrate methylumbelliferyl-β-D-glucuronide, and form a fluorescent compound that can be detected with long-wave UV light (See 231 Fig. 2-23 p. 21). Therefore, the Group C and G can be distinguished based on their ability to produce a positive BGUR test (7,67,230,231).

Bile esculin and salt tolerance are important tests, particularly in the differentiation of the Group D *streptococci* and the genus Enterococcus. The Enterococci are bile esculin (+) (black due to bile hydrolysis), and they are also PYR (+), and able to grow in trypticase soy broth (TSB) containing 6.5% NaCL [67,230,231]. The Group D *Streptococci* are also bile esculin, PYR (+), however they are unable to grow in 6.5% NaCl [67,230,231]. Whereas the Group A *Streptococci* are PYR (+), but are sensitive to a bacitracin disk with 0.04U. The Viridans *Streptococci* like the enterococci are resistant to a optochin disk at > 14m in diameter, but are bile esculin (-), unable to grow in TSB with 6.5% salt [230]. The test using the Optochin disk has primarily been used to presumptively identify the *Streptococcus pneumoniae*, which is a primary cause of pneumonia. However, the PYR test is the most useful test methods for differentiating similar gram positive cocci [67,230,231].

Corynebacteriaceae

Gram Positive Bacilli

Corynebacteria

The *Corynebacteria* are a large group of gram positive bacilli that are nonmotile, aerobic to facultative anaerobic, catalase, non-acid fast, asporogenous, and are metabolically chemoorganotrophic bacteria [7,28,178]. They are commonly found in the soil, water, and they make up part of our common microbiome [7,157]. They have been commonly described as "club shaped or coryneform", due to their pleomorphic shapes Tortora [30]. The organisms may vary in their size and shape, from their coccoid to rod shaped morphology. With coccoid forms microscopically range from 1-2 μm in diameter, to 6 μm in length [157]. They are said to be pleomorphic, since they may have the appearance of V forms, Chinese letters, and or parallel rods that may palisade [28,157,178,237].

The principle pathogens are *Corynebacteria diptheriae*, *C. pseudotuberculosis* and *C. ulcerans*, all of which may exhibit pathogenicity when a lysogenic phage is associated within their DNA, allowing all to express a toxin [7,157]. The toxin produced by *C. diptheriae* is a exotoxin, which is primarily associated in the nasopharynx, larynx and trachea, and may affect various areas, resulting in the loss of breathing, and affixation [309,310]. Although Cutaneous diphtheria can result in skin infections, these are more commonly seen in the tropics, and immigrants [309,310]. *C. diptheriae* can be cultured in a variety of culture media such as, Sheep Blood Agar, Chocolate agar, Loeffler slants, as well as selective media for example, Tinsdale and or Cystine-Tellurite blood agar [157]. On Tinsdale or Cystine tellurite blood agar, colonies appear black or brown or with brown halos [311]. The halo is the result of the enzymes activity of cytinase [237]. The organism produces metachromatic granules that are called Babes Ernst granules. When they are stained with methylene blue, they microscopically give its beaded appearance [7,237]. The presence of the diphtheria toxin can be identified based on an immunodiffusion test. Three parallel streaks of a known strain (control) of diphtheria, a negative control, and a unknown isolate, are place at right angles to a central strip of antitoxin impregnated filter paper, in order to determine whether an unknown isolate is capable of producing the diphtheria toxin. If an isolate produces a line of identity at the juncture where the antitoxin and where the bacteria toxin meet, that isolate is confirmed as a diptherial toxin producer [237]. Other methods for detection of diphtheria toxin are the ELIZA method and PCR assay [237].

Corynebacterium jeikeium

Another *Corynebacterium* the JK bacteria were named after Johnson and Kaye, and now called *Corynebacterium jeikeium* [237]. Besides being normal skin flora, it has been associated with a number infections associated with immunodeficient individuals. Particularly those that are intravenous drug users, have undergone surgery, catherization, or introduction of prosthetic devices [237]. Microscopically they may resemble *streptococci*, since they may appear as beaded rods, and or "wedged-shaped" [309].

In culture media on sheep blood agar, they are nonhemolytic, lipophilic organisms appearing as "small, pinpoint colonies, whitish, smooth with metallic sheen; and prefer blood agar rather than chocolate. Biochemically, they are aerobic, urease and nitrate negative, do not ferment carbohydrates, and express multiple antibiotic resistance [309].

Corynebacterium urealyticum

Corynebacterium urealyticum is a organism that is morphologically similar to *C. jeikeium*, and has been associated with infections of the urine, with a greater than 8 pH, as well as with peritonitis, endocarditis, pneumonia, and bacteremia with underlying urinary tract infection [309]. Culturally, the organism is lipophilic, and forms colonies that are pinpoint and grayish white on sheep blood agar [157]. Biochemically, it is urease positive, and does not ferment glucose [157,309].

Corynebacterium pseudotuberculosis and *C. ulcerans*

C. pseudotuberculosis and *C. ulcerans* seem to be closely related, *C. diptheriae*, but differ in that they both are fermentative, urea positive, reverse CAMP positive, and ferment both glucose and maltose [157].

Corynebacterium xerosis

C. xerosis is normal flora of the throat and conjunctivae, and has been associated with vertebral osteomyelitis, pneumonia, wound infections, and endocarditis, particularly following aortic valve replacement [7]. The organism resembles the diphtheria bacillus morphologically, since they may present barred forms, and granular forms may occur [7,309]. Culturally, they appear 1-5 μm in diameter, with "irregular edges, appear dry, and slightly yellow [157,309]. Biochemically, they are catalase (+), they ferment glucose, maltose and sucrose, pyrazinamidase is formed, variable in nitrate reduction, and urease negative [7,157,309].

Corynebacterium striatum

Corynebacterium striatum is a non-lipophilic diptheroid, that requires up 48 hours or more for culture. Some strains form a yellow pigment on blood agar. Winn *et. al* (75) describes *C. striatum* as forming "grayish-white, moist, smooth, nonhemolytic, and with entire edge" on sheep blood agar. They have been associated with pleuropulmonary infections, as well as endocarditis. Biochemically, they reduce nitrates, are pyrazinamidase (+), alkaline phosphatase (+), ferment glucose and sucrose, but are esculin (-), urea (-), and gelatin (-), See Table 5-1 p. 36 [157,75,309].

Arcanobacterium

Arcanobacterium are gram-positive bacilli that are highly pleomorphic, and exhibit branching" [157]. The organism is microscopically similar to the morphology of *Corynebacterium diptheriae*, and all three species are βeta hemolytic [157,309]. There are three species of Arcanobacterium, namely; *A. haemolyticum*, *A. pyogenes*, and *A. bernardiae*. *A. haemolyticum* characteristically forms a narrow zone of beta hemolysis, with colonies in addition exhibiting a small dark dot with a pit in that center, and a pit under the colony as it is removed [237]. Infections have been associated in wounds, soft tissue, endocarditis, and osteomyelitis. However, their colonies are small, and less than 0.5 mm. The microorganism is a fermenter, and is capable of fermenting glucose, and maltose, but not sucrose, mannitol or xylose. *A. haemolyticum* is lipase, lecithinase positive, and demonstrates a reverse CAMP test [237]. *A. pyogenes* is larger than other *corynebacteria* microscopically, up to 6 μm in length, and characteristically forms V and branching [157]. It ferments glucose, maltose, but differs from the other two species by fermenting xylose, See Table 5-1 p. 36 [157]. *A. bernardiae* microscopically does not branch but differs by its fermentation of glucose and maltose [157,309].

Nocardiaceae

Rhodococcus equi

Rhodococcus equi is a gram-positive bacillus that is aerobic, nonmotile, acidfast, and extremely pleomorphic, and may be partially acid-fast [309]. They may form rods that branch into mycellia, and or fragment giving rise to coccoid forms [7]. On sheep blood agar it can sometimes resemble *Klebsiella*, and characteristically forms colonies that may be mucoid, with a buff, or pink orange colored colonies

[7,150]. Biochemically it oxidizes glucose but does not utilize other carbohydrates, and is variable in nitrate reduction and urease hydrolysis [7,28,157,237,309].

Rothia

Rothia dentocariosa is a gram-positive microorganism that is nonmotile aerobic to facultatively anaerobic, and is pleomorphic, forming "rudimentary mycelia that may fragment into rods to coccoid forms [7]. Colonies on sheep blood agar appear as "whitish, raised, smooth, and may produce a spoke-wheel form under 5% CO_2 with incubation for 48hours incubation at 37°C. The key biochemicals, it is nonhemolytic, catalase (+), Voges Proskauer (+), esculin (+), reduces nitrates to nitrites, and forms acid in glucose, See Table 5-1 p. 36 [157,237,309].

Oerskovia

Oerskovia xanthineolytica is a gram-positive microorganism that is non-acid fast, and is highly branched, which may fragment into motile rod-like forms [312]. They form very yellow colonies on blood agar, and are aerobic to facultative anaerobic, catalase (+), oxidase (-), all strains are positive for reducing nitrates, gelatin, starch, and DNA [7,309,312,313].

Mycobacteriaceae

Mycobacteria

Mycobacteria are gram-positive bacilli, that are straight or slightly curved, and aerobic to microaerophilic, acidfast, nonsporulating bacteria, that are acidfast. They contain mycolic acid in their cell walls, and may appear microscopically as coccoid to branching rods. They appear under gram stain, as uniform, beaded and or branched rods [7]. Nutritionally, they are not in general fastidious, and are able to utilize a variety of sugars, hydrocarbons, and amino acids [7]. As far as metabolism is considered, they are considered to be respiratory, but increased atmospheric CO_2 may enhance their growth. However, they are slow growers in culture, may require from 1 to 6 weeks for them to grow in culture [7,28,81,234].

The Mycobacteria may be placed into various groups based on whether they are rapid growers, the type culture medium, the require temperature, the presence of pigment, as well as the affect light exposure. The rapid growers are usually able to form colonies in less than 7 days, while those that form orange carotene pigment after light exposure are called photochromogens, requiring more than 7 days for incubation. Those such as *M. tuberculosis* which do produce a buff color, and fail to form pigments are considered nonchromogens, and also require greater than 7 days for incubation [75,99,310].

The Mycobacteria that belong to the *Mycobacteria tuberculosis complex* include the following: *M. tuberculosis*, *M. bovis*, *M. bovis bacille Calmette-Guerin*, *M. africanum*, *M. canettii*, *M. caprae*, and *M. mungi*. The *Mycobacterium avium complex* (MAC), includes the two species, *M. avium* and *Mycobacterium intracellulare* [75,99,310].

The most prominent Mycobacteria in terms of disease are the three common species, which are *Mycobacterium ulcerans*, *M. tuberculosis* and *Mycobacterium leprae*. *M. leprae* is an "obligate intracellular pathogen, that is the cause of leprosy, is a nonculturable microorganism, and referred to as "Hansen's disease" [75,99,310]. It requires a optimum temperature of < 37°C for growth [235,310]. It has an affinity for the cooler areas of the body, and is able to be taken up by macrophages, which leads to a future invasion of the myelin sheath, of the peripheral nervous system [235]. Damage to the peripheral nerves is the result of a cell mediated response [235]. Leprosy again cannot be cultured by routine methods, but in the past it has been inoculated into the footpads, of nude mice [235]. It may be diagnosed by skin biopsy, in conjunction with the bacterial preparation or lepromin, and the lepromin test [75,99,235,310].

Mycobacterium ulcerans is also acidfast but may form filaments that appear longer microscopically, but without banding or beading. They were named in honor of Australian town, as well due to its cutaneous ulcer called "Bairnsdale ulcer" [314]. The organism grows optimally at 33°C, and in culture it forms colonies that are "rough, lightly buff, or nonpigmented, convex to flat, with irregular outline, similar to *M. tuberculosis* [7,234,314].

Mycobacterium tuberculosis

Mycobacterium tuberculosis is a gram-positive microorganism that is acidfast due to the presence of mycolic acids present in their cell wall. Microscopically they can be seen as slender rods, and contain cord factor, which may exhibit activity similar to endotoxin [7,75,99]. *M. tuberculosis* is the cause of tuberculosis, and can be transmitted either by aerosols, or by ingesting contaminated milk products [75,99,333]. Primary tuberculosis is a respiratory disease, where fine particles much reach the lungs, and enter macrophages there, in order to be able to multiply [314,333]. The pathology is due to sensitivity to mycobacterial antigen. In the lungs, there is an accumulation of lymphocytes, macrophages, inflammation, fibroblasts, giant cells, and hypersensitivity, which results in the body's formation of a granuloma [333]. Over time, there is the development of caseious materials which are laid down as the result of necrosis, resembling "solid, or semisolid amorphous material" [333]. Specimens have traditionally decontaminated by the treatment with 2% NaOH, and N-Acetyl-L-cysteine and depending on laboratory, other agents may also be used in conjunction of chaustic, and subsequently plated on culture media at 30°C [75,99,314]. Still today the most common way to diagnose tuberculosis is through the application of the Kinyoun or Ziehl-Nielsen acid fast stain, tuberculin skin test, culture of biopsy, gastric aspirates, cerebrai spinal fluid, and urine specimens [75,99,333]. Other special methods of identification include the use of the Bactec, DNA probes, and Nucleic acid amplification methods [75,99,333]. There are several types of culture media for the isolation of M. tuberculosis, for example, Lowenstein-Jensen medium, and the selective media of Middlebrook 7H10 and Middlebrook H11. Colonies on Middlebrook H11 appear "dry, wrinkled, rough, thin, irregular periphery, and buff in color" [75,99,234,314,333].

Nontuberculous Mycobacteria

The nontuberculous mycobacteria are also able to grow on Lowenstein-Jensen agar, as well as Middlebrook culture media [234]. Like the agents of tuberculosis, nontuberculous mycobacteria are characterized biochemically based on colony morphology, growth rate, temperature, nitrate reduction, hydrolysis of tween 80, iron uptake, arylsulfatase, pyrazinamidase, tellurite reduction, urease, as well as inhibitory tests of NAP (p-nitroacetylamino-β-hydroxpropiophenone), and T2H (thiophene-2-carboxylic acid hydrazide), sodium chloride tolerance, and growth on MacConkey agar [234,333].

These microorganisms are first separated into two groups based on the ability to produce a Yellow or Buff in the absence of light. Those that produce a orange pigmentation in the absence of light, and negative for tween hydrolysis include the species *Mycobacterium scrofulaceum*, whereas those species that are tween hydrolysis (+) are *Mycobacterium flavescens*, and *Mycobacterium gordonae*. While both *Mycobacterium szulgai* and *Mycobacterium flavescens* are nitrate (+), *M. gordonae* is negative. *M. szulgai* differs from *M. flavescens* in considered a Photochromogen, growing at 22°C and considered a Photochromogen found in Figure 26-8 p. 596 [333].

The second group after exposure to light of non-tuberculosis group, forms either a buff or yellow pigmentation after exposure light. The first branch that forms a new yellow pigmentation after exposure to light, is tested for nitrate reduction and catalase. If it can reduce nitrates, is positive for catalase and tween hydrolysis, it then is keyed out as *Mycobacterium kansasii*. While the same group but nitrate negative, next is screened for catalase, and those that are catalase (+) are further screened by tween hydrolysis. *Mycobacterium asiaticum*, is positive for tween hydrolysis, whereas those negative for tween hydrolysis, are identified as *Mycobacterium simiae*, see Figure 26-8 p. 596-597 [333].

The second branch after exposure to light that remains a buff pigmentation, is next tested for the requirement for niacin in addition to being catalase (-), nitrate (-), and catalase negative at 68°C is considered *Mycobacterium tuberculosis*. Those other buff colonies that are niacin (-), are next tested for tween hydrolysis. Those species that in addition to being tween (-), weakly catalase (+) at 68°C, nitrate (-), SQ catalase (-), are considered the *Mycobacterium avioum complex* and *Mycobacterium xenopi*. Others that are buff, and niacin (-), tween (+), nitrate (+) and 68°C catalase (+) are considered *Mycobacterium nonchromogenicum*. Whereas, the mycobacteria that are niacin (-), tween (+), nitrate (-), SQ catalase (+), are differentiated into *Mycobacterium malmoense* and *Mycobacterium gastri*. *M. malmoense* is catalase at 68°C positive, while *M. gastri* is negative, refer to Figure 26-8 p. 596 [333].

The MOOT or Other Mycobacteria

Group I, The MOTT or Other mycobacteria are classified into 4 groups, namely the Runyon Groups I-IV.

Group I includes the Photochromogens, which require greater than 7 days for colonies to appear. They produce a yellow to orange pigment, only in the light, not the dark, and require more than seven days for culture. Both *M. asiaticum*, *M. kanssii*, *M. simiae* and *M. marinum* are photochromogens [310].

Group II are called the Scotochromogens are slow growers on culture media, they also produce a yellow to orange pigment, but both in light and or dark, and include *M. gordonae*, *M. szugar*, *M. scrofulaceum* and *M. xenopi* belong to the Scotochromogens, See Box 26-5 p. 596-597 [310,333].

Group III are considered the Non-photochromogens, which are also slow growers, which are neither produce pigment either in the light or dark, and require greater than seven days to grow. They include the following species, *M. tuberculosis*, *M. avium-intracellulare*, *M. bovis*, *M. celatum*, *M. gastri*, *M. genavense*, *M. haemophilum*, *M. malmoense*, *M. terrae complex* and *M. ulcerans* [310,333].

Group IV include the mycobacteria that are rapid growers, that are able to grow in less than seven days, and include the *M. tuberculosis complex* or Mott Groups I-III, and include *M. phlei*, *M. smegmatis*, the *M. fortuitum complex*, and *M. ulcerans*, *M. chelonae*, *M. vaccae*; with materials from (Figure 26-8 p. 596) (310,72).

Listeriaceae

Listeria species and Similar Bacteria

Listeria is a motile gram positive bacillus, which is a non-spore forming, aerobic to facultative anaerobic, and is psychrophilic, and capable of multiplying at refrigeration temperatures of 4°C [7]. Some microbiology laboratories analyze tissue for Listeria, by culturing it in broths at refrigeration temperatures called cold enrichment. The broth is subcultured at weekly intervals [7,28,29,67,75,245].

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The organism under gram stain may appear as rods (bacilli) and or coccobacilliary, and may palisade (line up like matchsticks. The microorganism is oxidase and catalase positive, and is approximately 0.5µ in width by 0.5 - 2.0 µ-meters in length. It is also capable of hemolysing red blood cells, and is beta hemolytic [7,245]. It is very similar to *Streptococcus agalactiae* in that both organisms are CAMP (+) and hydrolyze sodium hippurate (#230 p. 336 table 15-3). It can be differentiated between both the Enterococci and *S. agalactiae* based on the tests for motility and catalase. The later microorganisms are nonmotile and catalase (-), whereas *Listeria monocytogenes* is motile and is catalase (+), See Figure 6-5 p. 47, CAMP test [7,29,230,231,245].

Typical Macroscopic tests

Along with *Listeria monocytogenes* ability to lyse red blood cells, it also exhibits a end over end motility, or tumbling motility, which can be seen in wet mounts, and or broth cultures grown at 22 - 25^{o}C or room temperature. It may also be tested for umbrella motility when inoculated into motility (low agar content) like "Difco motility agar." If the organism is inoculated with a sterile inoculation needle, with straight inoculation, the organism will exhibit what has been described as a umbrella motility. See Figure 7-9 p. 361 [7,237].

The organism was named in honor of Joseph Lord Lister, a British scientist that is considered the founder of aseptic surgery. It also receives part of its name based on its ability to invade monocytes, and cause the multiplication of white blood cells or monocytes [51]. Its ability to cause replication of monocytes is how it received its name [7,43,51,237,238].

The microorganism has been associated with a number of food-borne out-breaks, ranging from soft cheese, ice cream, vegetables, and salads and cheese [240,241,242,243] and became newsworthy in 1985. This outbreak occurred with the consumption of a "Mexican-style cheese. Other foodborne infections in the past, lead to a greater interest in Listeria, and better methods for its culture and identification [240,241,242,243,244].

Culture Methods

There are a number of culture media available for growing the genus Listeria. The organism will grow in many general purpose media such as trypticase soy agar, brain heart infusion agar, as well as 5% Sheep Blood agar, and Chocolate Agar. Select media have been developed both for recovery from food and meat samples. Other more selective agar would include the medium used initially in the nineteen eighties, called McBrides Agar [46,239]. It was primarily used for the isolation of Listeria from foods. Lithium Chloride and phenyl-ethyl alcohol were the selective agents that are incorporated into the medium [88,226,239]. Colonies appear as a bluish-greenish sheen when grown on McBrides Agar, as well as trypticase soy agar when examined with oblique light (looking from a 45o angle [7,46,237,240].

Samples undergo an enrichment procedure using Listeria enrichment broth, and then treated with 0.0% KOH before streaking on Modified McBrides agar (MMA). Typically, colonies are tested for the tumbling motility as well as the umbrella motility. Samples are further tested for βeta hemolysis, as well as CAMP test. In the CAMP test two parallel vertical streaks down the middle of a 5%Sheep Blood agar plate are performed using a β-lysin *Staphylococcus aureus* on the left vertical streak, and a parallel right vertical streak with *Rhodococcus equi*. Test isolates are streaked between the vertical streaks to determine whether enhanced arrow head zone of hemolysis is enhanced either by the β-lysin of *S. aureus* or by the *R. equi* [237,240].

If an isolate is positive for the tumbling motility, umbrella motility, and is βeta hemolytic, those subsequent suspect colonies are inoculated to biochemical tests [240]. Both *L. seeligeria* and *L. monocytogenes* displays a enhanced hemolysis or a arrow zone of hemolysis with the β-lysin of *S. aureus*, whereas only *L. ivanovii* shows enhanced hemolysis by *R. equi*. It also produces a double zone of hemolysis when streaked on to a blood agar plate. *L. innoccua*, *L. grayi* and *L. welshimeri* do not display enhanced hemolysis with either *S. aureus* or *R. equi* [46,88,75,239,240] 5Win p. (766-773) [765-840].

Meats samples by the FSIS method (Lee and McClain), again a 25 gm. Sample is weighed into 225ml of sterile University of Vermont Medium (UVM), and incubated for 20-24 hours at 30oC. After incubation, a 0.1ml aliquot is inoculated to Fraser broth for 26 hours, and those secondary enrichment broths turned black or darkened, are streaked on to Modified Oxford Medium (MOX) for isolation. Typical Listeria colonies are blacken and are dew drop in appearance [78,334].

Biochemical Identification

All *Listeria monocytogenes* are gram positive bacilli that are catalase positive, exhibits tumbling motility, and umbrella motility, and are

β-hemolytic, with a positive CAMP test by the enhanced β-lysin of *Staphylococcus aureus*. They are biochemically oxidase negative both MR and VP positive, bile esculin positive, they are fermentative in OF media, and they do not reduce nitrates to nitrates. In regard to utilization of 1% carbohydrates, they ferment glucose and rhamnose only, and they fail to ferment xylose and mannitol [75,237,334].

Pathogenicity

L. monocytogenes its notoriety has been because of its ability to cross the placenta and cause, neonatal meningitis, hydrocephaly, septicemia, and central nervous damage [29,243,245,246]. The organism possesses a number of factors that allow it to be a successful pathogen. The organisms produce a surface protein called internalin, which induces epithelial cells to engulf the bacteria cells. Once internalized into a phagosome, they are able to prevent fusion with a lysosome to form a phagolysosome. Once reaching the cytosol, the organism has the uncanny ability with the use of ActA a Listeria surface protein, to mobilize themselves, to be budded off from macrophages, and other cells [75].

Erysipelotrichaceae

Erysipelothrix rhusiopathiae

Erysipelothrix rhusiopathiae is microaerophilic to facultative anaerobic to anaerobic rod. It is a gram-positive bacillus, and may form thin long filaments that are slightly curved with rounded ends. They are nonmotile, and grow between 15 - 42°C [7]. Infection is often due to some injury to the hands or fingers, as a result of damage during handling of fish and meat products (7,29,245, Biochemically *E. rhusiopathiae* is catalase, and oxidase negative, indole negative, does not hydrolyse esculin, negative in both the Methyl red and Voges Proskauer tests, and urea negative. However, its key characteristic is its ability to form H_2S in Triple Sugar Iron agar (7,28,29,178,237,245).

Bacilliaceae

Bacillus

The genus *Bacillus* consists of gram positive *bacilli*, that are aerobic to facultative anaerobic spore forming rods. They are considered chemoorganotrophic as their means of nutrition. They are approximately 0.5 µm in width to 2 - 6 µm in length. They are ubiquitous in nature being found in soil, foods and water, and some are pathogenic to man [7,28,61]. Parry., *et al.* [4] made step a forward by placing the genera of bacillus into three morphologic groups and 5 subgroups [7,28,61,178].

The taxonomy of the genus bacillus follows the typical cultural and biochemical tests that follow: The three groups are separated into to 3 groups based on the type and location of the endospore, whether the sporangium is swollen, the production of lecithinase, ability to the utilize citrate, as well as the ability to grow only aerobically, and or anaerobically. Also nitrate reduction, indole production, growth in 7% NaCl, casein and starch hydrolysis, gelatin liquefaction, and utilization of 0.5% carbohydrates with ammonium salt as base [7,61]. The majority of bacillus species are capable of growing mesophilically at 37°C. However, there are other species that grow thermophilic (for ex. *B. stearothermophilus*, and *B. coagulans*), others require psychrophilic temperatures for growth namely *B. aminovorans*, *B. insolitus* and *B. psychrophilus* [7,61].

Bacillus anthracis is a nonmotile spore former with having microscopically square ends, and is a direct pathogen. It forms a plasmid mediated toxin and a capsule, which chemically is a polypeptide called D-glutamic acid [7,237]. The capsule is resistant to enzymatic attack and is essential for its ability to cause disease, and accounts for its main ability to initial disease. It also has its ability the produce of its 3 toxins. These toxins are protein in nature, and are protective antigen (PA), edema factor (EF), and lethal factor (LF). Each toxin has its own important function in the disease process. Protective agent has its main purpose, which is to bind to receptors on host cells. In the process, the production of both PA antigen and edema factor (EF) form a complex, leading to an increase in cyclic AMP, and disruption of normal protein synthesis, cellular pathways, and leading ultimately to cell death [7,75,237].

There are three main types of clinical infections caused by Anthrax, namely; Cutaneous anthrax, Inhalation Anthrax, and Gastrointestinal Anthrax. The cutaneous form of anthrax is usually acquired due to some type of injury either through abrasions, or scraps while cleaning animals after hunting or insect bites [237]. The Cutaneous form which is most frequently occurring, begins as a small lesion or papule that over time develops a darkened central area called a eschar. The lesion is painless and will begin to heal in 1 - 2 weeks. In most cases the infection is localized with no complications then no systemic involvement occurs [7,237].

Inhalation Anthrax is often referred to as "woolsorter's disease." The microorganism is taken up by macrophages, thru the reticular endothelial system, and are found in the hilar and mediastinal lymph nodes [75]. The illness begins with flu-like symptoms. Active infection occurs primarily in the lymph nodes. However, when spores germinate within the macrophages, they are not killed, and enter the general circulation. This leads to "edema and hemorrhagic necrosis of the mediastinum." Dissemination of the vegetative cells by the blood can lead to distribution to other body organs. The incubation period occurs from 1-3 days, and typical symptoms are fever, chills, substernal pain, difficulty in breathing and vomiting, and widening of the mediastinum. Other symptoms include abdominal pain, toxemia subsequently lead to cyanosis, septic shock and death. These severe symptoms can last for 24 hours [7,75,237,247].

Both Oropharyngeal and Gastrointestinal Anthrax are acquired by the ingestion of the vegetative cells, or endospores from foods (usually meat), that have not been properly cooked. Symptoms begin with fever, nausea and bloody vomiting. There more severe symptoms such as abdominal pain, vomiting, and fever. This form of anthrax has a high mortality rate [75,237,247,248].

Cultural Characteristics

Anthrax can be cultured on 5% Sheep Blood Agar (SBA), some isolates will grow on phenylethyl alcohol agar (PEA), but poorly [237]. The D-glutamate acid capsule can be stimulated when isolates are grown with an atmosphere with increased CO_2, or culturing with media containing bicarbonate and serum [53,237,248]. On SBA, it forms nonhemolytic colonies that can be described as "gray, and flat with an irregular margin, with outgrowths of long filamentous projections of bacteria." The colonies have been described as the "Medusa head". Picked colonies with an inoculation loop are difficult to uproot, since they adhere greatly to the agar surface. "They have been describes as having a beaten egg white appearance." The organisms will grow aerobically and anaerobically, is nonmotile and nonhemolytic on SBA [237]. The organism ferments glucose but is negative for mannitol, arabinose, and xylose. It will produce lecithinase when grown on egg yolk media, and will grow with 7% NaCl at a < 6 pH, and is sensitive to penicillin (10 U/ml) [53,237].

Bacilliaceae

Bacillus cereus

The genus *Bacillus* consists of gram positive *bacilli*, that are aerobic to facultative anaerobic spore forming rods. They are considered chemoorganotrophic as their means of nutrition. They are approximately 0.5 µm in width to 2 - 6 µm in length. They are ubiquitous in nature being found in soil, foods and water, and some are pathogenic to man [7,28,61]. Parry., *et al.* [4] made step a forward by placing the genera of bacillus into three morphologic groups and 5 subgroups [7,28,61,178].

Bacillus cereus is also a gram positive bacillus, and has a tendency to form chains. It forms ellipsoidal spores, and unlike *B. anthracis* is motile, catalase (+), and can grow aerobically to anaerobically, and is beta hemolytic (7). Microscopically cells are more rounded at both ends, See Figure B p. 26 [61]. The organism is noted as a cause of food poisoning. It is capable of causing two forms of disease, one called the diarrheal syndrome and second the emetic form [237]. The diarrheal syndrome is an intoxification and is often associated with the ingestion of meat and poultry. The emetic form is due to the ingestion of rice and Asian dishes [237]. Both the diarrheal syndrome and the emetic form are associated with the ingestion of enterotoxin, See Table 2 p. 19 [7,28,61,178,237].

Culture and Biochemical characteristics

Bacillus cereus colonies on sheep blood agar are hemolytic and display different morphologies, but appear often circular to irregular in shape, and with a "grayish to greenish appearance, as well as a ground glass appearance [247]. *B. cereus* produces the enzyme lecithinase (egg yolk media, is VP+, NO3+, grows in 7% NaCl, hydrolyses starch, casein, gelatin, tyrosine, and also is capable of growth in 0.001% lysozyme (74 p. 18). According to [237] Mahlen., *et al* (p. 373), *B. cereus* is identical culturally to *B. thuringiensis*. *B. thuringiensis* differs however, since it forms parasporal crystals [7,74,237,247].

Nocardiaceae

Rhodococcus equi (previously known as Corynebacterium equi) is a chemoorganotroph, and gram positive organism, that is aerobic and appears coccoid to coccobacilli in morphology. It is pleomorphic, and may appear as rods to branching mycelium. It is acidfast, and commonly is found in moist and soil environments, with an optimum growth temperature of 30°C. It rarely causes infection, but has been more common with the immune-compromised, and particularly AIDS patients (237). It produces an easily recognized pigment, a coral pigment on Sheep Blood Agar. However, it does not ferment carbohydrates. Its characteristic features are its ability to form filaments and gram positive morphology, and is considered a "diptheroid" [237]. It is also is used in the CAMP test, testing for enhanced hemolysis of some species of Listeria [7,28,78,237,250,251,256].

Nocardia

Nocardia are gram positive, aerobic, nonmotile, asporogenous, chemoorganotrophic filamentous bacteria, that are partially acidfast. They are acidfast due to the presence of mycolic acids in their cell walls. The filaments are approximately 1 μm in length, may be beaded, and may fragment into rods and or coccoid forms, depending the species. They are commonly found in the soil, and are pathogenic the man and animals. The organism also has its notoriety based in its ability to form what is referred to as sulfur granules [7,237].

The organism may be cultured on nonselective media such as Blood Agar, and or Chocolate Agar, although it will also grow on selective media such as, Sabourauds dextrose agar (without chloramphenicol), Lowenstein Jensen, and or Middlebrook Media. Generally, growth requires from 5 to 7 days for visible colonies to appear, when incubated from 25°C to 35°C [7,237,340].

The infections attributed to *Nocardia* are usually associated with debilitated individuals, particularly those predisposed due to pulmonary disease. Both the Cutaneous and Pulmonary forms of the disease do occur in those individuals, that are immunocompromised. The following species are prominent causes of the Nocardial disease, and are the following: *Nocardia asteroids*, *Nocardia brasiliensis*, and *Nocardia otitidiscaviarum* [237,340].

The Nocardia biochemically may be differentiated based on the their ability to break-down (decompose) casein, tyrosine, xanthine, starch, and urea. *Nocardia asteroids* is positive for only urea, while *Nocardia brasiliensis* decomposes both casein and tyrosine, and is positive for urea. *N. otitidiscaviarum* decomposes only xantne, and is also positive for urea, See 340 Table 8-1 p. 58 [237,340].

Gram Negative Bacilli

Family Aeromonadaceae

Aeromonas

Aeromonas is a gram negative rod to coccobacillus, that is Oxidase and catalase positive. It is polar flagellated, and belongs to the family Aeromonadaceae. It measures approximately 1.0-4.0 micrometers in length by 1.0 micrometer in width. It is commonly found in brackish waters, as well as fish and cold blooded animals, and is considered a human pathogen [7,28,257]. It has also been associated with retail stores, with foods such as meats, produce, fish, some spices, red meats and chicken. It can grouped into the two groups, the mesophilic group, and the psychrophilic group [7]. The mesophilic group consists of what are termed three complexes. The *A. hydrophila complex*

includes *A. hydrophila*, *A. bestiarum* and nonmotile strains of *A. salmonicida* [257]. The *A. veronii complex* includes *A. veronii biovar sobria*, *A. veronii biovar veronii*, *A. janaei*, *A. trota* and *A. schubertii*. The third complex consists of the mesophilic complex, and includes *A. caviae*, *A. media* and *A. eucrenophia*. The psychrophlic group consists of *A. salmonicida* which is a fish pathogen [252-257].

Aeromonas is responsible for a number of infections namely, gastroenteritis, wound infection, meningitis, and "life threatening infections such as septicemia, and necrotizing fasciitis" [255]. The microorganism is capable of growing on routine culture media. It can be distinguished from both Vibrio and Plesiomonas based on growth requirement for salt, and its ability to grow in the presence of 2,4-diamino-6,7-diisopropylpteridine, the Vibriostatic agent O 129. *Aeromonas* is not inhibited by O 129 at 150 µg, and does not require salt for growth. Both Vibrio and Plesiomonas are inhibited by the O 129 at a concentration of 150 µg, whereas Plesiomonas can be differentiated from Vibrio, by for both the test for gelatin hydrolysis, and mannitol fermentation. Plesiomonas is negative for both gelatin hydrolysis and mannitol fermentation. Whereas, both Aeromonas and vibrio are gelatin positive, See Table 20-2 and 20-5 p. 464 and 473 [102,255,257].

Culture Media

It is capable of growing on the common culture media used in the clinical laboratory, such as Sheep Blood Agar (SBA), as well as Chocolate Agar. The organism is hemolytic on SBA, and colonies appear as "round, raised opaque colonies, with entire edge, smooth, and often mucoid surface" [257]. The organism will grow on commonly culture media used for the isolation of Enteric bacteria, such as MacConkey Agar, XLD Agar, and Hektoen Enteric Agar. It will also grow on CIN Agar, which is initially formulated for the *Yersinia enterocolitica* (cefsulodin-irgasan-novobiocin agar), and forms pink centered colonies on that medium [257]. However, Aeromonas is a lactose fermenter, and as such may be missed when culturing for Enterics on this medium. The growth of aeromonads could be masked by the growth of other lactose fermenting microorganisms, when using common selective media, such as MacConkey Agar, XLD Agar, or Hektoen Enteric Agar, which are commonly used for the isolation of *Salmonella* and *Shigella* [187,237,255,257]. Another culture medium for the selective isolation of *Aeromonas* is ampicillin blood agar, which is used for its recovery from feces, and contains 20 µg/ml of ampicillin. However, *A. trota* will not grow on this medium, since it is inhibited by the presence of ampicillin as are other susceptible *Aeromonas* species [255]. Palumbo., *et al.* recommended the use of a Starch-Ampicillin agar for the recovery of *Aeromonas* from foods, and contained only 10 µg/ml of ampicillin. He also in his isolation procedure recommended the use of Trypticase Soy Broth as an enrichment broth which also included the addition of 10 µg/ml of ampicillin into the enrichment medium [56,258].

Biochemical Characteristics

According to Winn *et. al* table 8-14 p. 421, the species *A. media* is easily separated from other Aeromonas species, by the fact that it is nonmotile. The three species *A. caviae*, *A. media* and *A. eucrenophila* differ from other Aeromonas species by the fact they are all three Voges-Proskauer (VP) and Lysine negative. The *subspecies A. veronii biotype veronii* differs biochemically by being ornitine positive, and arginine negative. *A. jandaei* can be distinguished from *A. schubertii* and others by the fact that *A. jandaei* is both negative for the fermentation of sucrose and positive for mannitol. Lastly *A. trota* is biochemically similar to *Plesiomonas shigelloides* in that both are negative the VP test and Lysine decarboxylase positive, however *A. trota* differs from *P. shigelloides* in that it ferments inositol, and is negative for mannitol fermentation, See Table 8-14 p. 421 [56].

Enterobacteriaceae

Plesiomonas

The organism is a gram-negative rod and is 1 µm by 3 µm in length. It is polar flagellated and is both positive for Oxidase and catalase, and indole [255]. It occurs as singularly, occurs in pairs, and may present as filamentous forms. The type species is *Plesiomonas shigelloides*. The organism is ferments glucose and is a facultative anaerobe [257]. Because of its range of growth temperature, it is capable of growing at 35°C, as well as psychrotrophically. It is often found in the environment (soil), as well as fresh and estuarine waters, and infects cold–blooded animals [253,257]. The microorganism has been responsible food borne outbreaks of intestinal infections, particularly diarrhea, from the consumption of cooked and uncooked foods, particularly seafood such as oysters, clams or shrimp, and fish [28,56,255,257]. Serious infections such as bacteremia, meningitis have occurred primarily with the immunocompromised [151,255,257].

Culture Media

Plesiomonas seems to be closely related to *Shigella*, since the organism has similar biochemical characteristics, and may cross agglutinate with antisera for *Shigella sonnei*, *S. dysenteriae* and *S. boydii*. On Sheep Blood agar at a incubation of 35°C for 18 - 24 hours, forms "shiny, opaque, nonhemolytic colonies, that are slightly raised with smooth and entire edge" [257]. It is a lactose fermenter like aeromonads. Some strains may be inhibited on eosin-methylene blue agar (EMB), and or MacConkey agars [257]. The selective medium of chose is inositol-bile salts-brilliant green agar, where colonies appear white to pink, whereas the majority coliform bacteria appear either green or pink [257]. However, the organism is unable to grow on TCBS agar [257]. It will grow on CIN selective for Yersinia species, where it forms opaque colonies, and does not ferment mannitol which is the selective carbohydrate in this medium [222,255,257,277].

Biochemically

Plesiomonas is biochemically oxidase, catalase and indole positive, reduces nitrates to nitrites, fermenters various carbohydrates (not mannitol). It is also arginine, lysine, and ornithine decarboxylase positive, but differs from Aeromonas in being Dnase negative [255,257].

Vibrioaceae

Vibrio species is a gram-negative bacillus, that is approximately between 1.5 µm in width and 3 µm in length, and belongs to the Family Vibrioaceae. The organisms are polar flagellated, and lacks a sheath. Microscopically the organism exhibits a slight curve morphology, and has been described as being "curved or comma" shaped [28,76,86,257]. The microorganism is found in the nature particularly in aqueous environments, such as fresh, brackish water, and salt and or sea water. It occurs in those environments during the warm months of the year, and particularly the summer months when the water temperature exceeds 20°C. They may be associated with algae, plankton, fish and shellfish [67,102,257]. There may be a risk of food-borne infection, due to the ingesting of under cooked seafood, such as shellfish. This may lead to a very serious infection, for those individuals who are immunocompromised or immunodeficient [28,67,102,257].

The majority of Vibrios are sensitive to the vibriostatic agent (2,4-diamino-6,7-diisopropylpteridine) at 150 µgs [257]. Vibrios may also require salt for their growth, whereas similar organisms such as Plesiomonas and Aeromonas do not require salt as a growth requirement. Aeromonas is able to grow in the presence of the Vibriostatic agent O 129, while Plesiomonas is inhibited at 150 µgs. However, both Aeromonas and Plesiomonas do not have a requirement of salt for growth [257].

The vibrios may be grouped accordingly based on their salt requirement for growth, and the ability to perform a string test. *Vibrio cholera* characteristically forms a string of mucus like cells, when colonies are exposed to 0.5% sodium deoxycholate [257]. Those vibrios that require NaCl are referred to as halophilic, and include all vibrio species except *V. cholera* and *V. mimicus,* which are non-halophilic [257]. Of the most importance is *Vibrio cholera* the cause of cholera, as either endemic, epidemic, and pandemic. It has been placed into 3 subgroups based on their serology, namely the subgroups *V. cholera* O1, *V. cholera* O139 and *V. cholera* non-O1 (76). Six of the pandemics caused by *V. cholera* have been the *V. cholera* O1 (Classical cholera), whereas a seventh pandemic was caused by the El Tor biotype, a biotype of Classical Cholera in 1961 [257].

Cholera occurred again in India (Bangladesh), and also in parts of Asia in 1992, and the O139 serotype was responsible. In 2002, the O139 serotype appeared responsible for another epidemic in Bangladesh, and was the beginning of a 8th pandemic. This time it was the O139 serotype. Both the Classical O1 and O139 have similar serological characteristics, and both are able to produce a heat-labile cholera toxin. They are the most common causes of cholera, whereas the *V. cholera non-O1* (non-O1, non-O139) are the third most commonly isolated vibrios [67,102,257].

In terms of the infections, there are a variety of infections that are caused by vibrios. For example, *Vibrio parahemolyticus* is a cause of gastroenterictis, often associated with the consumption of seafood, and particularly of fish, oysters, and shellfish [257]. *V. vulnificus*

may be the cause more serious infections such as, wound infections, septicemia and most severe infections such as necrotizing fasciitis [257,259,260].

Transport Media

The Vibrios are not considered fastidious, however care should be exercised for the collection of specimens. A variety of specimens may be collected when possible for example, body fluids, pus and tissues for clinical specimens. Although swabs are acceptable, Cary-Blair transport media is best particularly to prevent sample dryness. However, to use of glycerol saline may not be used, since it may interfere with the recovery of vibrios [257].

Culture Media

Vibrios are capable of being cultured on both Nutrient Agar and 5% Sheep Blood Agar if they are supplemented with 0.5% salt [76,86,257]. They also grow quit well on Chocolate Agar, where they form medium sized colonies. Colonies appear characteristically as "smooth, opaque, and iridescent with a greenish hue" [257]. On MacConkey they frequently appear as non-lactose colonies, although *V. vulnificus* could be overlooked since it is a non-sucrose fermenter [257]. Characteristic colonies should be tested for the present of oxidase, and should be performed after subculturing to 5% Sheep Blood Agar [257]. A culture medium that is often used for the isolation of Vibrio species is Thiosulfate Citrate Bile Salts Sucrose (TCBS Agar). It is a selective medium, and inhibits most coliform bacteria, pseudomonads, and gram positive bacteria [7,257].

Biochemical Characteristics

Winn., *et al.* [56] lists in his Table 8-12 the key biochemical tests for differentiating the clinically significant vibrio species. In his table, he also lists the various species also into groups 1-6. Winn., *et al*. (p. 418) highlights the combination of biochemical tests that may be used to differentiate similar species [56]. For example, the Group 1 *V. cholera* and *V. mimicus* may be distinguished based on the combination of 1% NaCl. Both are able to grow in the presence or absence of 1% NaCl, however groups 2-6, are unable to grow with the presence of salt, See Table 8-12 p. 418 [56].

The Group 2 species, *V. metschnikovii* differs from the other groups based on its inability to produce a positive oxidase test, and reduction of nitrates to nitrite. The Group 3, *V. cincinnatiensis* differs from the other species listed, by its fermentation of myo-inositol. The Group 4 species, *V. holisae* is unable to decarboxylate arginine, lysine and or ornithine. Group 5 consists of the species *V. damsela*, *V. fluvialis* and *V. furnissii*, which characteristically are positive for arginine dihydrolase, but differ from group 4 which are arginine dihyrolase negative. The final group or group 6 key characteristics are the fact that they are arginine dihydrolase negative and lysine decarboxylase positive. The combination of arginine dihydrolase and lysine decarboxylase helps to key out these species of vibros from the other groups of vibrio species [151]. In terms of biochemical identification, Alsina and Blanch have remarkable dichotomous keys for the identification of Vibrio species [56,261,262].

Campylobacteriaceae

Campylobacter

The *Campylobacter*'s are nonsporulating gram-negative, microaerophilic, and motile microorganism. They are polar flagellated, and may exhibit a cork-screw or darting motility. The microorganism measures 0.9 μm in width to 5 μms in length, and appears microscopically with gram stain, either as gull, seagull or S shape morphology. When forming a gram stain on campylobacters, they should be counter stained with carbol- fuchsin, since they appear more visible with carbol-fuchsin as the counterstain [7,28,263].

The Campylobacters such as *C. jejuni* and others such as (*C. jejuni subps doylei*, *C. coli*, *C. lari*, *C. hyointestinalis*, *C. upsaliensis* are thermophilic (42°C), whereas *C. fetus* subsp. fetus is not thermophilic, and requires a mainly a 37°C growth temperature [7,263,264]. Campylobacters are carried from a variety of animals including food (meats, poultry, shellfish, fruits and vegetables) and water, as well as raw milk product [7,263,264].

Culture Media and enrichment

In the original method by J. Lovett., *et al.* 1984 and FDA, the use of Brucella broth and Campy Blood Agar with selective antibiotics (Skirrow's formulation) was the commonly used method in the food microbiology laboratory, using Campylobacter Isolatory agar "Skirrow's formulation" [173]. Today the procedural method for the *campylobacter* is have become more complex, using various types of enrichment techniques, selective antibiotics, for enrichment, and the use of selective culture media for isolation [264]. The current BAM method may entail some samples being centrifuged, and then pre-enriched in Bolton broth for 4 or 5 hours, and incubated microaerophillically. While other samples, may be pre-enrichmented at 30°C and 37°C, pre-enriched according to a prescribed time period [264]. Enrichments are subsequently next plated to selective culture media at 42°C. The types of enrichment broths and the time and temperatures used, depend on the type of product analyzed [264].

The use of a Campylobacter Enrichment Broth (Bolton Oxoid or Malthus Diagnostics), differs from the Brucella broth with antibiotics (vancomycin, 15 mg/liter, trimethoprim lactate 7.5 mg/liter and polymyxin B 10,000 U/liter), that was used in the Skirrow's' formulation. The current Bolton enrichment broth contains sodium cefoperazone 20 mg/liter, Trimethoprim lactate 20mg/liter, Trimethoprim 20 mg/liter, Vancomycin 20 mg/liter and cycloheximide at 50 mg/liter [173,264].

Culture Media

The recommended culture media according to the BAM procedure is the use of either Abeyta-Hunt-Bark (AHB Agar) or Modified Campy Blood free agar (mCCDA). On either culture medium, Campylobacter colonies appear as "round to irregular with smooth edges, and may appear thick, translucent white, with a spreading growth. Culture medium should be examined at 24 - 48 hrs incubation at 42°C for thermophilic campylobacters, and 37°C for *C. fetus* [13,264].

Biochemical Identification

As with the earlier method by Lovett., *et al.* [13], isolated colonies according to the BAM method should be gram stained (showing curved and gull shapes), and tested for corkscrew or darting motility [263]. Typical colonies cultured on Abeyta-Hunt-Bark agar appear as "round with irregular with smooth edges and may exhibit a spreading film-like growth [264]. Several biochemical tests are essential for the differentiation of the various campylobacter species, namely the following, the test for antibiotic inhibition (nalidixic acid and cephalothin disk test), growth temperature tolerance (25°C, 35 - 37°C, and 42°C), oxidase and catalase positive, growth on MacConkey Agar, biochemical tests using 0.1 dilute culture, inoculated into 1% glycine, 3.5% NaCl, H_2S from cysteine, growth reaction in TSI, nitrate reduction, and motility, See #264 Table 1 p. 7,17 [263,264].

All Campylobacters are gram-negative oxidase and catalase positive, microaerophilic, and grow at either 35°C or 42°C, with the exception of *C. fetus* which grows at 37°C [263]. Campylobacters are not capable of utilizing glucose, or other carbohydrates [264]. The Key biochemical tests for the identification of campylobacters are the following: growth at (25°C, 35°C, and 42°C), nitrate reduction, growth (3.5% NaCl), H_2S (lead acetate strip), H_2S (TSI), Oxidase, catalase, growth (MacConkey Agar), motility, growth in 1% glycine, glucose utilization, hippurate, and resistance to naladixic acid disks or cephalothin disks [263,264].

All seven species of campylobacters are listed in Table 1. According to the BAM [264] are able to growth at 35 - 37°C and 42°C, except *C. fetus* which grows at 35°C. The subspecies *C. jejuni subsp. doylei* is the only species that is nitrate (-), while other species reduce nitrates to

nitrites. *C. jejuni* differs from other campylobacters by not growing at 25°C, and the only species that is hippurate (+). All other species are able to grow on MacConkey Agar, with the exception of *C. upsaliensis. O*nly *C. hyointestinalis* is capable of forming H_2S on TSI (264). With respect to disk antibiotic testing, both *C. jejuni* and its *C. jejuni subspecies doylei* are sensitive to naladixic acid, and resistant to cephalothin. *C. ori* is sensitive to naladixic and resistant to cephalothin, whereas *C. coli* like *C. jejuni* is sensitive to naladixic acid, and resistant to cephalothin. *C. fetus* subsp. fetus differs in it being resistant of naladixic acid and sensitive to cephalothin. *C. hyointestinalis* like *C. fetus subsp fetus* is also resistant to naladixic acid, and sensitive to cephalothin, whereas *C. upsaliensis* is sensitive to both antibiotic disks [264]. A final note, Campylobacters are considered the main cause of intestinal illness, and are very infective. Infective doses range from 500 to 10,000 cells, depending on the strain, status of the host and may lead to meningitis, pneumonia, miscarriage, and serious form of Guillain-Barre syndrome [263,264].

Campylobacteriaceae

Helicobacter

Helicobacter is a gram-negative microaerophilic organism, that exhibits from rod-like to spiral, or curved morphology microscopically. It is motile by lophotrichous flagella, and often exhibits the spiral morphology from gastric biopsy specimens, and has been associated with, chronic gastritis, peptic ulcer, and gastric adenocarcinoma, and lymphoma. There are over 30 species of *Helicobacter*, however *H. pylori*, *H. fenneliae*, and *H. cinaedi* are considered pathogenic to man [265]. *H. pylori* has been associated with peptic ulcers, whereas *H. fenneliae* and *H. cinaedi* have been isolated from the feces of AIDs patients, and can cause proctitis, enteritis, and bacteremia. The microorganisms in general have been known to infect a number of animals, namely horses, rodents, chickens, dolphins, and wholes [28,265].

Culture Media

The organisms are very fastidious, and require a number of enrichments for their growth [264]. *H. pylori* will grow on chocolate agar, as well as brain heart infusion, or brucella agar with the addition of horse or rabbit blood. The organism can also be cultivated on Thayer-Martin Agar, Pyloric Agar, and Dent's medium [265]. It however is often detected by the Campylobacter-like organism (CLO) test, in which a specimen is placed in a gel containing urea. In the presumptive identification of *H. pylori*, *H. cinaedi* and *H. fennelliae*, identification should be based on the gram stain, a positive oxidase, and catalase. *H. pylori* differs from the other species, by producing a rapid urease test [265]. *H. pylori* appears in culture, as a "small, gray, and translucent forming colony. Most strains are nonhemolytic hemolytic [90,265].

Biochemical Tests

Like the campylobacters, the Helicobacters have similar biochemical substrates used to differentiate the species, and include the following: growth at 25°C, or 35°C and or 42°C, nitrate reduction, growth in 3.5% NaCl, H_2S (lead acetate strip), catalase, oxidase, growth (MacConkey Agar), motility, growth in 1% glycine, glucose utilization, hippurate hydrolysis, H_2S (TSI) and or sensitivity to either naladixic acid or cephalothin [257,264]. *Heliobacteria pylori* is catalase (+), nitrate reduction (-), TSI (H_2S-), urease (+), indoxy acetate (-), hippurate (-), but is incapable of growth at both 35°C and 42°C. It is also resistant to nalidixic acid (30 mcg) and sensitive to cephalothin (30 mcg). *H. fennelliae* is also catalase (+), but negative for nitrate reduction, urease, TSI (H_2S-), hippurate (-), is Indoxyl acetate (+), and does not grow at either 25°C, 35°C or 42°C, and sensitive to both naladixic acid (30 mcg) and cephalothin (30 mcg). *H. cinaedi* is positive for both catalase and nitrate reduction, but negative for urease, TSI (H_2S-), hippurate hydrolysis, variable for indoxyl acetate hydrolysis, and also does not grow at either 25°C, 35°C or 42°C, See Table 8-4 p. 400 and Table 20-8 p. 479 [56,257,264].

Campylobacteriaceae

Arcobacter

Arcobacter appear microscopically very similar to campylobacters, in that they also may exhibit spirally curved, and or with short S shape morphology. They may be distinguished them from campylobacters by being aerotolerant, as well as their ability to hydrolyze iodo-

acetate. They also have a wider growth temperature range, being able to growth at 15°C, 25°C, and 36°C but not at 42°C, as well as inability to hydrolyze hippurate. According to Ramees., *et al.* isolated colonies at 30°C on Blood Agar (7%) appeared whitish, round, convex and 2 - 4 mm in mm in size [28,56,266,267].

Pasteurellaceae

Haemophilus species

Haemophilus is a gram-negative nonmotile "pleomorphic" to coccobacilliary rod shaped microorganism. The organisms are facultative anaerobes, and appear microscopically approximately between 0.4 to 1-2 µm in length, and are very fastidious. Some *haemophilus* require the X or V factor, or both (X or hemin, V or Flavin adenine dinucleotide). They may acquire those factors by enrichment (Fildes enrichment), serum, and or agar medium, for example chocolate agar which provides those nutritional requirements [7,268]. Their optimum growth temperature is between 35° - 37°C, in conjunction with a CO_2 atmosphere of 5 - 10% [7,28,268]. All Haemophilus species with the "prefix" para only require the V factor, while both *H. influenza* and *H. hemolyticus* require both the X and V factors [268]. *H. ducreyi* requires only the X factor. *Aggregatibacter aphrophilus* (formerly *H. aprophilus*) requires neither X or V factors, See Table 22-1 p. 177, and Table 18-2 p. 402 [7,153,268,335].

The organism is nonmotile, oxidase and catalase positive, facultative anaerobic, and capable of reducing nitrates to nitrites, as well as fermenting carbohydrates, except lactose [7,153,268,335]. *Haemophilus* species have commonly been associated with the mucous membranes of the gastrointestinal, genital and respiratory tract of man and animals [7,153,268]. They have been responsible for a number of infections from mild upper respiration infections, to very serious infections such as epiglottitis, endocarditis and meningitis [153]. The majority of Haemophilus infections are not transmitted sexually, with the exception of *H. ducreyi*, which causes a soft lesion or chancre on the genitals, and can become very painful [153].

Cultural Morphology

The *Haemophilus* do not grow on culture media that are commonly used for many other types of microorganisms. This is because of their fastidious requirement for a number of nutritional factors for growth [268]. Chocolate agar is the common culture media that is often used for the recovery of various *haemophilus* species. Colonies of *H. influenzae* appear on chocolate agar, as "convex, smooth with entire edge on chocolate agar," with a mousy or bleach-like odor. Whereas *H. parainfluenzae* are smaller, light gray, and may have a matted appearance on chocolate agar" [62,148,268]. Both *H. aphrophilus* and *H. paraphrophilus* form colonies that are small (1mm in diameter) convex, granular, and yellowish in pigment [153,335]. The colonies of *H. ducreyi* appear as "small, flat, smooth, transparent to opaque colonies, that may be tan or yellow on chocolate agar [153,268,335].

Biochemical Characteristics

Along with the test for X and V factors, there are a number of biochemical tests according to Table 18-2 p. 402, that may be performed for the Identification of Haemophilus species. Along with the oxidase and catalase tests which are common for most species of haemophilus, with the exception of *H. ducreyi*, which is both oxidase and catalase negative. *A. aphrophilus* although variable in the oxidase test may be oxidase positive, but negative for the catalase test. Other biochemicals include hemolysis in rabbit blood, ONPG, enhanced growth in CO_2, and various carbohydrate substrates [268]. The *H. influenzae* and *H. haemolyticus* which require both the X and V factors, *H. hemolyticus* is negative for maltose, whereas *H. influenzae* is positive for lactose, both ferment glucose but are negative for sucrose, mannose, mannitol, maltose, xylose, lactose, esculin, lactose, but both are variable for fructose. While *H. influenzae* is positive for xylose, *H. haemolyticus* is variable (V) for both xylose, and indole. *H. haemolyticus* is negative for ornithine, whereas various biotypes of *H. influenzae* may differ in this particular substrate [268].

The *H. parainfluenzae*, *H. parahaemolyticus*, and *H. paraaphrohaemolyticus* require the V factor only, and all three ferment both glucose and sucrose. The biochemical pattern of utilizing those carbohydrates, and X or V factors, help distinguish them from other species of haemophilus. *Aggregatibacter segnis* requires the X or V factor for growth, and differs from the other three species, in its weak reaction in both glucose and sucrose. *H. ducreyi* however does not ferment any carbohydrates, or any of the biochemical tests list in table 18-2, other than the except that *H. ducreyi* may be enhanced in its hemolysis of rabbit blood agar and CO_2 enhanced growth (268). *A. aphrophilus* (formerly *H. aphrophilus*) requires neither the x or V factors, with growth is enhanced in CO_2, and is ONPG (+). It ferments glucose, sucrose, fructose, maltose, lactose, nitrate (+), but is variable for mannose, and negative for mannitol, xylose, esculin and ornithine, indole and urea [268].

Gram Negative Coccobacilli

Neisseria and Moraxella

Neisseria are gram-negative cocci in pairs with adjacent sides flattened. They are aerobic, nonmotile, nonsporulating and are oxidase and catalase positive. Neisseria may grow anaerobically, providing there is an alternate electron acceptor (nitrites), but they grow best in the presence of a reduced atmosphere of 3-5% CO_2, and as such are considered capnophilic [28,269].

Neisseria gonorrhoeae is considered a direct pathogen, and is not considered a part of the normal floral. However, *N. meningitidis* can be found as normal flora of the respiratory tract of carriers. Both species require iron, and have a mechanism for binding human transferrin to specific receptors [369]. All other *Neisseria* are considered opportunistic, whereas the species *N. weaver* is considered normal flora of the respiratory of dogs. *N. gonorrhoeae* causes a pyogenic (pus forming) infection, any where there are non-ciliated columnar and transitional epithelium. At one time (1530 AD), it was thought to be a early symptom of syphilis. But in 1767, a physician mistakenly inoculated himself with what he thought was gonorrhea, but unfortunately for him it was syphilis instead. Gonorrhea comes from the French word meaning "brothel [269]".

Virulence Factors

The pathogenic Neisseria species have a number of factors contributing to their pathogenicity, namely the following: they have receptors for chelating human transferrin. They also have a capsule which can interfere with phagocytosis. The presence of pili helps the microorganism in attachment to cell surfaces. Cell membrane proteins may also interfere with humoral, and cellular immunity [270]. Other contributing factors also include the possession of a different form of endotoxin called Lipooligosaccharide (LOS), which consists of a lipid A moiety, a core LOS, as well as a protease that "cleaves" IgA from outer membrane surfaces [269].

Some possess various pili types that are important in attachment membrane surfaces. Types1 and Types2 are more pathologic, since they help the organism to inhibit phagocytosis from neutrophils. Whereas Types 3-5, are considered avirulent. Types 1 and 2 do predominant during typical urinary tract infections (269). Other factors (Protein 1) include the presence of outer membrane proteins, that can help to channel nutrients, into and out of the cell. Protein 11 (OPa), helps to guard against a host inflammatory responses, and complement. Protein 25 (Rmp) protects the organism against serum proteins [269,270]. Also, significant, is the ability of the organism to possess the presence of IgA protease, that can cleave secretory antibody from mucosal surfaces [269,270].

When *Neisseria* are to be observed microscopically, gram stains should not be performed on pharyngeal specimens, where commensal *Neisseria* might also be present. Specimens from the urogenital tract, should be gram stained for the presence of gram-negative intracellular diplococci, which may also appear as kidney or coffee bean shaped in morphology. In the Microbiology laboratory, symptomatic males can even in conjunction with a positive culture (95% correlation), be clinically insignificant due to the presence of avirulate (nonpiliated) microorganisms [269]. With females there is only a 50% - 70% correlation between culture, and gram stain smears. Again, culture is still important for a definitive Confirmation [269].

Moraxella are pump gram-negative rods, to coccobacilli that are oxidase and catalase positive, indole negative, nonmotile and asaccharolytic. Although morphologically they may occur singularly, or in short chains, they are best described as gram-negative aerobic diplococci [82,84,270,298]. They are approximately 0.6 - 1.0 μm in diameter, and although considered nonmotile, may exhibit a twitching motility [7]. They are a nutritionally fastidious microorganism, requiring blood agar and or chocolate agar for growth in culture [7]. The organism is normal respiratory flora, however is an opportunist, and my cause disease in susceptible individuals. They are frequent inhabitants of the mucus membranes of the respiratory tract, and the genital tract [82,271]. They have been associated with some very serious diseases, namely endocarditis, meningitis, and may exhibit acute otitis media and sinusitis in children [28,82,269,298].

Culture Methods

The organism grows poorly or not at all on MacConkey Agar [77]. *Moraxella* Growth occurs after 48 hours on Sheep Blood Agar, it may exhibit what is referred to as the "wagon-wheel appearance." The organism may also be found growing on selective media, such as Thayer Martin and Modified Thayer Medium, and possibly New York City (NYC) Medium table 17-5 p. 385 [269,298].

Biochemically

In general *Moraxella catarrhalis* is the organism that is of particular concern, it may need to be distinguished between *Neisseria gonorrhoeae* and *N. meningitidis*. *M. catarrhalis* is nonpigmented, biochemically oxidase positive, catalase positive, reduces nitrates, and is positive for tributyrin hydrolysis and DNase positive, but is nonsaccharolytic, See table 17-5 p. 385 (7,269). Traditionally, *N. gonorrhoeae* and *N. meningitidis* have been identified by carbohydrate tests, where *N. gonorrheae* is positive for dextrose (glucose), *N. meningitidis* ferments both glucose and maltose. However, over the years this method can have erroneous results. There has been a shift to more accurate methods [269].

There are a number of more sensitive methods that are based on the isolation from selective media for *Neisseria*, and related organisms. For example, the chromogenic substrate method uses enzyme detection, as well as the coagglutination that relies on monoclonal antibodies, for the detection of *N. gonorrhoeae*. Others methods include the Modified Conventional/ chromogenic enzyme method, which uses enzyme substrate, and other biochemical tests for both *Neisseria* and *Haemophilus*. This method colonies may be picked from selective media. Finally, the Fluorescent Antibody technique can be used for the confirmation of *N. gonorrheae* [269].

Nonpathogenic Neisseria

Neisseria lactamica is also a part of the normal throat flora, and at one time was considered a variant of *N. meningitidis*, because of it fermentation of the sugar lactose. It is capable of growing on selective media, hydrolyses ONPG, and ferments both glucose and maltose with acid production. *N. cinerea* may be differentiated based on its inability to produce a polysaccharide from sucrose, and scant production of a yellow pigment. It can be biochemically distinguished from *Moraxella catarrhalis* by its negative reactions for nitrate reduction, DNase, and tributyrin hydrolysis, See Table 11-4 p. 600 and Table 17-9 p. 391 [269,270].

N. flavescens like most Neisseria species, it is part of the respiratory flora. On Blood and Chocolate Agar, it forms a smooth, yellowish colony at 35°C. Biochemically it is able to synthesize iodine positive polysaccharides from the carbohydrate sucrose, but unlike *M. catarrhalis* is unable to reduce nitrates, and negative for both DNase and tributyrin hydrolysis, Table 17-9 p. 391. *N. flavescens* differs from *N. cinerea,* by its ability to grow on Sheep Blood Agar, and or on Chocolate Agar at 22°C, and by its yellow colonies" [269,270].

The various biovars, of *N. subflava*, *N. subflava biovars*, *Neisseria mucosa*, and *N. sicca* are part of the "upper respiratory tract flora." The biovars are subdivided into the various biovars based on production of acid from fructose and sucrose, and the ability to synthesis iodine

positive polysaccharide from sucrose. They all reduce nitrites but are unable to reduce nitrates. *N. mucosa* differs from the *N. subflava* biovar's, by its ability to reduce nitrates to nitrogen gas, although they have similar carbohydrate patterns. Both *N. sicca* strains, and *N. subflava* are the same biochemically, and produce a "leathery colony on agar media," [270].

N. polysaccharea is also a part of the normal human throat flora. It is biochemically both oxidase and catalase positive, and morphologically is a gram negative diplococcus. Biochemically it is able to ferment with acid both glucose and maltose, but not fructose and or lactose. It also produces the enzyme amylosucrase, that allows the synthesize of an "acidic extracellular polysaccharide" from sucrose. In culture, it forms smooth yellow colonies on either blood agar or chocolate agar [270]. It also can grow on selective media such as Modified Thayer Martin Agar (TTM). *N. polysaccharea* can be differentiated from *N. meningitides* by its ability to produce iodine positive polysaccharide from sucrose, and is delta-glutamylaminopeptidase negative. *N. meningitidis* differs from *N. polysaccharea* by not being able to produce iodine positive polysaccharide from sucrose, and is delta-glutamylaminopeptidase positive [270].

Spirochaetes

The *Spirochaetes* are a group of microorganisms that are very small, only between 0.1 - 3.0 μm in width to 5 - 250 μm in length. They include the genera *Leptospira, Borrelia* and *Treponema,* and are motile by means of their periplasmic flagella, also referred to as endoflagella [7,28,272]. The organisms may be found in the environment (aquatic and soil) as free living parasitic microorganisms, but survive by their animal and or animal hosts [7,272]. The organisms may be seen if stained, with certain dyes (Warthin-Starry, Dieterle, and silver dyes, using monoclonal antibodies, as well as the fluorescent antibody technique [7,130,272]. Metabolically the microorganisms are considered chemoheterotrophs, and are capable of utilizing carbohydrates, amino acids, and long chain fatty acids. The oxygen requirements are dependent on the genera, some are aerobic, to facultative anaerobic, and or anaerobic. Both the *Leptospira* and *Borrelia* reproduce by binary fission, whereas the *Treponema* reproduce by transverse binary fission [7,336,272].

The Leptospira are thin microorganisms which are 0.1 μm in wide, to from 5-15 μm in length. Their coils are not as tightly packed as compared to Borrelia and Treponema [272]. The organism also morphologically have one or two hooks associated with its ends. There are approximately 200 serotypes, with *L. interrogans* considered as the clinically significant species, whereas all nonpathogenic species have been relegated to the species *L. biflexa*. Although seen by bright field microscopically, the organism can be seen with the technique of silver staining, as well as darkfield, phase contrast microscopy, and immunofluorescent microscopy [272]. Both Leptospira and Borrelia are cultural on selective media. Leptospira are considered obligate aerobes, and may be cultured on Fletcher's medium (semisolid), Struart liquid, or Ellinghauser-McCullough-Johnson-Harris (EMJH) semisolid media [272,336].

Pathogenic Potential

The microorganisms may gain access through small cuts, or even "intact mucosa." It is not clear where the initial multiplication site occurs [272]. Leptospira incite an inflammatory response, primary due to the presence of the *Leptospira* antigens. Its pathogenicity may be attributed to a reduced immune response, due to a weakened phagocytotic response, soluble hemolysins, and cell-mediated sensitivity to leptospiral endotoxin by the host [272]. There are a number of common symptoms, that are expressed as "fever, chills, headache, and malaise." The severe disease is called "Weils disease," and can result in kidney failure, vascular damage, and leading ultimately to death [272]. The microorganism can be contacted by animals, slaughterhouses, and particularly during recreational areas. The can be contacted through infected urine and from carriers [272].

Serological Methods

Patients with a blood infection typically form Ig M antibodies by the first week after the presence of disease. IgG Antibodies arrive about 30 days after the initial onset of disease, and remain high for months. The use of the serological test IgM ELISA has a sensitivity of 98% and a specificity of 90.6% [272].

Borrelia

The genus comprises of a number of organisms that exhibit a similar morphology, but may differ in their pathogenic potential. The microorganisms is 0.2μm in width by 3.0 - 20 μms in length, thin and tightly coiled [7,272]. Like *Leptospira, Borrelia* can be readily be seen by the light microscope, if properly stained. The species names of the genus *Borrelia* are based on the arthropod vector [7]. They are believed to affect the immune system by interfering with a pivotal intermediate in the complement cascade, namely the C4b-binding site, which is essential for both humoral and cellular (innate and adaptive immunity) immunity. *Borrelia* are also able to undergo surface antigen change, thus making antibodies useless [272].

Clinical Signs

Individuals can develop symptoms between 2 to 15 days after onset of exposure, with a tremendous influx of organisms, and can result in a bacteremia. The constant periods with fevers followed by less symptoms has become the reason the Borrelia's are often referred to as the "relapsing fevers" [7,272]. Symptoms include a sudden spike in temperature, along with headache, muscular pain, and feeling of weakness [272]. Again, the organism can be seen microscopically by Giemsa or Wright stain blood smears, within the febrile period of the disease [7,272,336].

Culture Isolation

The organism can be cultured by Kelly Medium, and or inoculation of animals (sucking rats or Swiss mice). However, inoculation as a means for culturing borrelia is not routinely performed. Several species of the following borrelia can be grown in culture, *B. recurrentis*, *B. hermsii*, *Borrelia parkeri*, *B. turicatae*, and *B. hispanica*. Because borrelia are able to disguise their surface antigens, serological testing may be difficult for diagnosis [272].

Borrelia burgdorferi the cause of Lyme Disease first occurred in Lyme, Connecticut, in 1975. The organism is transmitted by an arthropod vector the tick Ixodes. It is often relegated to the summer months, occurring between June and September, since more people are active in the outdoors [272]. There are three species that occur in the World, *B. burgdorferi* is found in the United States, while the other two a located in Asia both *B. garinii* and *B. afzelii* [272]. *B. burgdorferi* can be detected by the indirect fluorescent antibody and enzyme immunoassay, and Western blot. However false positive tests do occur, particularly if a individual might be infected with *Treponema pallidum*, then a rapid plasma regain (RPR) test should be used to rule out Lyme disease [272].

Treponema

The *Treponema* are microorganisms that may occur as "parasites or pathogens of the mouth, intestinal tract, and genital tract of man and animals" [7]. The organisms are considered pathogenic, and are 0.2 μm in width by 6 to 20 μms in length. They are very thin, and difficult to be seen by bright-field microscopy, they can be observed by dark field microscopy [272]. The *Treponema* are pointed at both ends, and have three "periplasmic endoflagella" that are inserted from end to end. The organisms also are surrounded by a shealth, and they are motile by the flexing and relaxing of their endoflagella [145,272].

There are a number of species many that are not pathogenic to man, however the pathogenic ones are more significant to man. The significant pathogens of the genus *Treponema* have been subdivided into the subspecies of *T. pallidum*, into *T. pallidum subsp. pallidum*, the cause of syphilis, *T. pallidum subsp. pertenue*, which is the cause of yaws, and *T. pallidum subsp. endemicum*, the cause of endemic syphilis, and finally *T. pallidum subsp. carateum*, the cause of pinta [7]. These 4 species share a common DNA homology, since they share common antigens [145,272].

Again, syphilis is caused by the microorganism *T. pallidum subsp pallidum*, and term is believed to based on a 1530 poem. In which a "mythical shepherd" called *Syphilis* was cursed with the disease by the gods as a punishment for his insurrection [272]. *Syphilis* although

commonly transmitted by sexual contact, can be contracted through open wounds, such as lesions from the lips of any individual infected, with either a primary or secondary active lesion. They also can be contracted by "transplacental transmission", to a developing fetus, resulting in congenital syphilis [7,272].

There are three stages of syphilis the first stage is called primary syphilis, whereas the 2nd and 3rd stages are secondary and tertiary syphilis. The Primary stage begins with a papule, becoming a ulcerative lesion, or chancre at the site of inoculation. The lesion is approximately 1 to 2 centimeters in diameter, and is usually painless and without tenderness. The chancre normally does heal, however in females the infection may not be noticed for some time, lesions commonly can occur in the cervix or vaginal wall. The organism can be contacted during anal sex as well [272]. Chancre lesions are teaming with the presence of the microorganism, and if the organism can gain access to the blood stream, this may lead to the development of secondary syphilis. The organisms may appear approximately 2-12 weeks after the initial infection [272]. During this time of the secondary stage there can be the presence of a rash on the mucosa and skin, and may also be demonstrated on the palms and soles. Patients during this stage may or may not experience symptoms, however they are highly contagious, and this stage may last for several weeks [272]. For a given time usually one year, patients may be free of symptoms, however approximately one third of those suffering from secondary syphilis, will undergo tertiary syphilis [272]. Tertiary syphilis involves damage to the central nervous system and the heart. During this stage, there are a numerous granulomatous lesions, that can occur in the skin, bone and liver, as well as cardiovascular damage, particularly aorta [272].

Because the treponemas are not culturable, identification lies predominately through serological methods, the fluorescent treponemal antibody technique, enzyme-linked immunosorbent assay (ELISA), the Venereal Disease Research Laboratory (VDRL), and RPR tests [7,145,272].

Enterobacteriaceae

The Enterobacteriaceae are gram negative bacteria that are 0.3 μm in width and 1.0 - 6.0 in length. They are motile by means of peritrichous flagella, and are rod-shaped bacilli in morphology [7]. They are widely distributed in nature (soil and water), and in man and animals. They are aerobic to facultative anaerobic, non-spore forming bacilli, and biochemically oxidase negative, catalase positive, and reduce nitrates to nitrites [7,336,337].

The enteric bacteria have traditionally been separated into their various tribes based on the IMViC test, which is the abbreviation for the following tests: Indole, Methyl-red, Voges-Proskauer and Simon's Citrate test. Although the IMViC test is simple in its approach to the identification of this family of bacteria, it stills provides a framework for teaching students of today, See Table 7-34 p. 694 [7,57,155,184,185,336,337].

The IMViC test provides a way to separate the enteric tribes, as well as into the various genera, that make up the family enterobacteriaceae. Both the genera *Escherichia* and *Shigella* have the IMViC pattern of biochemically being Indole and MR (+) and this combination help separate these two genera from the other enteric tribes. The genus Edwardsiella also presents this pattern, but differs in producing hydrogen sulfide, and is a non-lactose fermenter [57,155].

Klebsielleae

The Tribe Klebsielleae which consists of the genus *Klebsiella, Enterobacter*, and *Hafniae*, differ from the tribe Escherichiae and Proteeae, by the IMViC pattern of indole (-), methyl red (-), voges-proskauer (+), and Simons citrate positive, see Table 7-34 p. 694 [41,73]. Both the genus Proteus and Providencia belong to the tribe Proteeae. The genus *proteus* differs from *providencia* by biochemically producing both the enzymes urease and phenyalanine deaminase. The genus *providencia* however, differs from *proteus* in producing only phenylalanine deaminase. These two enzymes help to separate the tribe from the other enteric [57,155].

They typically are found as common inhabitants of the intestinal tract of animals and humans. The four main pathogens that may cause intestinal disease are *Escherichia*, *Salmonella enterica, Shigella* and *Yersinia*. Outside their normal body sites, Enterics may cause serious opportunist infections. In the case of urinary tract and respiratory infections, both may lead to more serious infections, such as, bacteremia, in immunocompromised individuals [185,275,337]. In general, the enterics are capable of growing on nonselective media such as nutrient agar [7], but in the clinical laboratory they have been traditionally cultured on 5% Sheep Blood Agar and numerous selective media, for example Hektoen Enteric Agar (HE) agar, Xylose Deoxycholate agar (XLD Agar), and Bismuth Sulfite Agar (BSA Agar) [7,185,275,337]. In addition to 5% sheep blood, they also are often cultured on the MacConkey Agar, and other selective media, and screened by slants of Triple Sugar Iron Agar (TSI) and Lysine Iron agar (LIA). These media help to recognize isolates that form clear colonies with possible hydrogen sulfide production, on selective media, as well as help determine the presence of lactose and non-lactose fermenters [7,181,185,275,337]. Isolates that produce an acid butt and alkaline slant, with H_2S inoculated to TSI are considered possible pathogens. In the food microbiology laboratory, LIA is also inoculated in addition to the TSI, and isolates are also screened with LIA, for colonies that also produce an alkaline reaction (purple through-out) the LIA agar slant (with H_2S+). Those isolates with both a characteristic TSI and LIA should be subsequently tested with specific antisera, as possible pathogens, and determine whether the need for identification methods [7,67,173,275,336,337].

On culture media the enteric exhibit various colony types, on blood agar colonies and oftenly appear as "gray, shiny, entire, convex, and opaque" [275,277]. Still many labs use MacConkey Agar, because of the ease of use, and ability to recognize lactose fermenters from non-lactose fermenters. Escherichia which is a lactose fermenter, characteristically produces colonies on MacConkey Agar that appear as pink colonies surrounded by a precipitation of bile salts [185,275,337]. They also appear salmon pink when cultured on Hektoen Enteric Agar (185). *Escherichia coli* produces a positive Indole and Methyl-Red (+) test, but is Simon citrate and Voges Proskauer (-) in the IMViC test [185,275,337]. *Escherichia* forms an acid/acid in TSI, whereas *Shigella* gives a acid butt and alkaline slant in TSI. Escherichia can be distinguished from its nearest neighbor *Shigella*, by the test for motility, and by the tests for acetate agar and mucate. *Escherichia coli* is positive for both substrates, whereas *Shigella* is negative for both substrates. *E. coli* is also motile while Shigella is nonmotile, and does not produce gas in carbohydrate media [25,275]. There have been a number of cases of Shigellosis in the Kentucky, particularly in the Louisville area, See Table 9 p. 59 (Farmer III) and 185 Table 11-1 p. 98 [85,185,275,308].

Edwardsielleae

In the tribe Edwardsielleae, the genus *Edwardsiella* like the above genera, it also produces an acid butt, and alkaline slant inoculated to TSI agar. It differs from *E. coli* and *Shigella* in forming hydrogen sulfide in appropriate culture media [11]. The Edwardsiella also is motile, and positive for the tests for Lysine decarboxlyase, and Ornithine decarboxylase, but arginine dehydrolase negative, See Finegold Chapter 27 Figure 27-2 p. 409 [7,85,275,277,338].

Klebsielleae

The Klebsielleae consists of the five genera, *Klebsiella, Enterobacter, Serratia*, and *Hafnia* and *Pantoea agglomerans* (formerly *Enterbacteria agglomerans*. The Klebsielleae exhibit a common IMViC pattern, in which they are Indole and Methyl Red negative but positive for Voges Proskauer and Simons citrate [278]. *Klebsiella* is nonmotile and produces large colonies on primary isolation media. On MacConkey Agar, they appear as "large, mucoid, red, with red pigment diffusely distributed throughout the surrounding media," since they are lactose fermenters. This can be demonstrated on any selective media containing the carbohydrate lactose. [275,277,278].

Klebsiella differs biochemically from the other tribe members, by being ornithine decarboxylase negative, whereas the other genera making up the tribe *klebsielleae* are ornithine decarboxylase positive (*Enterobacter*, *Serratia* and *Hafnia*). With the exception of the genus Pantoea (formerly *E. agglomerans*), which is ornithine decarboxylase negative, lysine, ornithine and arginine dehydrolase negative [277]. All the members of the genus *klebsielleae* also differ from the other tribes of enteric's in that all are positive for the Voges Proskauer, with the exception of a few species which may be found in Table 6-7 [277]. In the genus Klebsiella, *K. pneumonia* and *K. oxytoca* are important

causes of pneumonia, wound, and urinary tract infections. *K. oxytoca* and *K. pneumonia* are identified biochemically, they both are Simons citrate (+), Methyl Red (-), Voges Proskauer and Lysine decarboxylate (+), but differ only in the fact the *K. oxytoca* is indole positive. For the most part, they have been considered to be nonmotile, See reference 120, Table 12-1 p. 107 [275,278].

The genus *Enterobacter*, they may resemble Klebsiella when grown on MacConkey Agar, are biochemically Simons citrate (+), Methyl red (-), and like *Klebsiella* are Voges Proskauer (+), but unlike Klebsiella most species are ornithine decarboxylase (+). However, both *E. gergoviae* and *E. cloacae* are ornithine decarboxylase (+). Both *E. aerogenes* and *E. cloacae* have been isolated from wounds, urine, blood and cerebral spinal fluid [275]. They both ferment a number of carbohydrates, but *E. cloacae* is both arginine and ornithine decarboxylase (+), while *E. aerogenes* is Lysine and ornithine decarboxylase (+). *Enterobacter agglomerans* (currently *Pantoea agglomerans*) has gained particularly notoriety international because of its occurrence as a contaminate of "intravenous fluids" [275]. *E. gergoviae* differs from *E. aerogenes* in that it is arginine dehydrolase (-) and ornithine decarboxylase (+), and is yellow pigmented on sheep blood agar [272]. *E. sakazakii* (currently classified as *Cronobacter sakazakii*) is also a yellow pigmented microorganism, and has been a particular problem in the infant formula, since it has been a cause of meningitis in infants [275]. *Cronobacter sakazakii* biochemically is both arginine and ornithine decarboxylase (+), and ornithine decarboxylase (-) and is sorbitol (+), compared to *E. aerogenes*, which is lysine decarboxylase (+), See 275 Table 19-5 p. 436 and 277 Table 6-18 p. 264 [275,277].

Serratia

The genus *Serratia* are commonly found in the soil and water. They are biochemically indole (-), MR variable (ve), Simons citrate (+), VP variable. Some species are positive for five enzymatic tests namely: DNase, lipase, gelaniase, Lysine, and Ornithine decarboxylase (both *S. marcescens* and *S. liquefaciens*). Both *S. rubidae* and *S. fonticola* are malonate (+), giving a typical bluish-green color [7,277]. Some species form a brick red pigment, *S. marcescens* has been typically been noted for, both *S. plymuthica* and *S. rubidae* may also produce a red colony pigment [7,277,278]. The *Serratia* have been associated with a number of hospital acquired infections, such as respiratory infections, urinary, as well as injection solutions, See Table 6-19 p. 266 [7,275,277,278].

Hafnia

Hafnia is a genus that is associated within water and the soil. It is considered an opportunist, and is motile at 25°C, and nonmotile at 35°C. It is biochemically indole and MR (-), Simons Citrate and Voges Proskauer (+), and decarboxylases lysine and ornithine, but is arginine dehydrolase negative [7,275].

Citrobacter

Citrobacter are also associated in water an soil, and human and animal feces. The microorganism has been recognized as a cause of a variety of infections, such as respiratory infections, urinary tract infections, wounds and uncommonly meningitis. They have also been spread to others through intravenous fluids, and other clinical devices [7]. Citrobacter form similar colonies to *E. coli* on MacConkey Agar (pink in color), with a pink sheen of pigment around the colony [7]. Biochemically the *Citrobacter* are Simons citrate and methyl-red positive, lysine decarboxylase negative, and are considered late lactose fermenter. In the case of *C. diversus* and *C. gilleni* are malonate positive [7,278]. On TSI they generally produce a acid over acid reaction when inoculate to a TSI slant. According to de la Maza., *et al.* the most clinically significant species of *Citrobacter* are *C. braakii*, *C. youngae*, and *C. freundii* which has been reported as forming H_2S, See 277 Table 6-15 p. 280, Also 278 Table 12-4 p. 108, and Figure 12-3 p. 109 [7,85,275,277,278].

Salmonella

The *Salmonella* have been relegated into two species, *S. enteric* and *S. bongori*, with *S. enteric* separated into six subspecies of *S. enteric* susbsp. enteric [275].

The *Salmonella* have been relegated into two species, *S. enteric* and *S. bongori*, with *S. enteric* separated into six subspecies of *S. enteric* susbsp. enteric [275].

The genus *Salmonella* is a gram negative bacillus belonging to the Family Enterobacteriaceae, and as such have the following general characteristics: they are oxidase (-), catalase positive, peritrichously flagellated, facultative anaerobic, reduce nitrates to nitrites (NO_3^- to NO_2^-), and are capable of growth between 35°C to 35°C. They are widely distributed in nature, and are capable of being transmitted to man from food, water, the environment, and animals, See 275 Box-19-1 p. 428, 440-441 [7,173,275].

All *Salmonella* are considered pathogenic regardless of the source. The most severe form called "Enteric fever," and is caused by *Salmonella typhi*, which can cause septicemia, and systemic disease. Other Enteric fever types produce a "milder form" of the disease, with a shorter incubation period, and duration. The second form of the disease is Gastroenteritis, which is the most common form of "Salmonellosis", and is usually associated with foods, water and animals [40,85,189,173,174,275].

Historically, *Salmonella* has been classified according to the Kaufman White Scheme, based on the O (cell wall), and H (flagellar) antigen. The O antigen or lipopolysaccharide (LPS) somatic antigen appears to contribute to the disease capabilities of the microorganism [7,275]. The Lipopolysaccharide or LPS is the cell wall component called endotoxin. The endotoxin is composed of three components, the Core polysaccharide, Lipid A, and the O Antigen (Antigenic Side Chain), all of which are covalently linked. When looking at the lipopolysaccharide in a vertical fashion, the Lipid A is more towards the interior, with the Core Polysaccharide sandwiched between the O Antigen above it. The Lipid A is below the Core Polysaccharide, which is in the middle. The O Antigen consists of a long linear chair of oligosaccharide, and contributes to the immunogenicity, and specificity of the molecule. The Lipid A is a glycolipid that consists of disaccharides to which are attached the two phosphate atoms, and trailing fatty acids. The Core polysaccharide consists of a number of sugars that are linked to Lipid A. Lipid A has the ability cause toxicity and certain microbes are capable of stimulating B-cells to undergo cell division, and activation, See 177 p. 22-24 Figure 4-5 [7,175,177,178,179,189,290].

Most species produce hydrogen sulfide in culture media containing iron salts. Inoculating Triple Sugar Iron Agar, they give an acid butt/alkaline slant with H_2S, and alkaline or purple throughout inoculating Lysine iron. Inoculating Both TSI and LIA *Salmonella* produce abundant hydrogen sulfide. An isolate that expresses typical biochemicial reactions, and a positive agglutination test in O antisera with the Vi-antigen, confirms an isolate as *Salmonella* [180,183,189].

Salmonella in most microbiology laboratories use a pre-enrichment step in order to enhance the recovery of *salmonella*. This extra step involves the use of a enrichment broth, which may retard the growth of coliform bacteria, but however facilitating the growth the Salmonella. There are several types of enrichment broths for example, Selenite-F Enrichment Medium, Selenite Cystine Broth, Tetrathionate Broth, and Tergitol 7 broth by FSIS recognition program [173,180,181,182,183].

There are a number of selective agars for Salmonella such as: Brilliant Green Agar (BGA), Brilliant Green Sulfa A (BGSA), Salmonella-Shigella Agar (SS Agar), Xylose Lysine Deoxycholate Agar (LDA Agar), Hektoen Enteric Agar (HE Agar). With BGA Agar, Salmonella colonies typically appear pink, and may be surrounded by a red mirror. BGSA also forms red colonies that are typical in Salmonella appearance. On both XLD and He Agar suspect colonies appear color-less or red, with or without hydrogen sulfide production. Suspect colonies growing on Bismuth Sulfite Agar may appear as green colonies with dark black centers, brown with black centers, or black colonies with a silver mirror [85,154,183,184,185,186,187,189,337].

Salmonella are gram negative facultative anaerobic and biochemically are oxidase (-), catalase (+), reduce nitrates to nitrites, ferment glucose with or without gas, and are Methyl red positive, Simons citrate positive [275]. They are capable of decarboxylating the amino acids of ornithine, lysine (OD), and dehydrolysing arginine (AD). Along with fermenting glucose, they also ferment dulcitol with acid and

gas, commonly seen in the laboratory. In general, most procedures for Salmonella include screening isolates with a TSI slant and Lysine Iron Agar (LIA). The typically reaction in TSI is a acid butt and alkaline slant (cherry red slant), with abundant hydrogen sulphide (H_2S), and with a alkaline slant (purple throughout) with a LIA slant, and abundant H_2S. Suspect colonies are next tested for agglutination in Polyvalent A-I antisera, with the Vi antigen included. Isolates that agglutinate with the antisera by 1 minute, are considered presumptive Salmonella. Those positive isolates are next inoculated to conventional biochemical tests and or by commercial kits for a complete identification [173,183,189]. Please refer to the following tables, Reference 13 on Table VI-1 on p. VI-29, Reference 183 Table 4.95, and Reference 189 Table 1 on p. 7.14.

There are a number of genera that make up the family, and in 1985, 98 new species were added to family of "enteric bacteria" [85]. In 1988 during a South Central Association for Microbiology meeting Dr. Ewing provided descriptions of the various new members of the Family Enterobacteriaceae (273). At that time Dr. Ewing described, and listed the key biochemical tests needed to differential many of the newly named genera of enteric bacteria, some are listed here: *Buttiauxella* are Indole (-), MR+VP-, Simons Citrate (+), Lysine and Arginine (-), Ornithine (+), and sucrose (-), *Cedecea* are ONPG (+), Simons citrate (+), MRVP (+), esculin (+), lipase (+), DNA (-), gelatin-, Colistin Resistant, and Cephalothin sensitive, and Ornithine (-). *Kluyvera* are Indole (+), MR +/VP-, Simons citrate (+), Malonate (+), Esculin (+), and *Tatumella ptyseos* is Indole (-), urease (-), decarboxylase (-), phenylalanine (+), See 277 Table 6-25 p. 278 [85,273,277].

Brucellaceae

Brucella

Brucella are aerobic gram-negative obligate intracellular parasitic bacilli, that are nonmotile, and 0.5 - 0.7 µm in width, by 0.6 to 1.5 µm in length. They have been described as causing undulate fever in man. They are very fastidious microorganisms, that are unencapsulated, and often require the addition of blood, a 5 - 10% CO_2 atmosphere, and may often require a biphasic media for their culture [7,28,268,279]. *Brucella* also may require several weeks incubation, with blind subcultures to blood, and chocolate agar [279]. The organism frequently is phagocytized by monocytes and macrophages, and may be transported to the spleen, liver and bone marrow, which is a part of the Reticular Endothelial system (RES) [279]. Biochemically, they are oxidase, and catalase (+), and reduce nitrates to nitrites [279]. The various species have typically over the years differentiated based on urea hydrolysis, H_2S production, and sensitivity to certain dyes [7,268,279].

The organism in culture forms colonies that are "small, convex, smooth, translucent, slightly yellow, and become opalescent after 48 hours incubation at 35°C [280]. It may be cultured on serum-glucose, chocolate agar, and brucella agar. They are often associated with the exposure to various animals, either by cuts or abrasions, during examination of animals by veterinarians, hunters, and laboratory personnel [268]. They are recognized primary by a patient's history, current health, as well as serological testing [279]. The best specimen includes blood and bone marrow. Immunoglobulin M (IgM) is the antibody that occurs first, with IgG antibody appearing after 2 - 3 weeks [279]. Biochemically the species is recognised based on CO_2 requirement, H_2S formation (lead acetate), and growth in the presence of thionine, and basic fuchsin. The "typical strains" of *B. abortus* may be differentiated by its requirement of a CO_2 environment, and by its H_2S production, and growth in the presence of thionine and not fuchsin. *B. suis* hydrolyses urea rapidly, whereas *B. abortus* and *B. melitensis* are variable for urea hydrolysis. *B. abortus* will grow in the presence of basic fuchsin, but not thionine whereas, *B. suis* is opposite in that it grows in the presence of thionine, but not basic fuchsin [281]. Brucella are in general gelatin negative, indole negative, Methyl Red and Voges Proskauer (negative) [7,268, 279,281,282]. Please see reference 268 Table 18-6 p. 411, and Reference 281 Table 22-2 p. 170-171.

Francisellaceae

Francisella

Francisella is a very small gram negative coccobacillus, between 0.2 to 0.2 - 1.0 µm in size [282]. It is very pleomorphic, and is a obligate aerobe. It is nonmotile and is non-sporulating, it exhibits bipolar staining [28,268,282]. The microorganism has a "reservoir" in the animal population, with over a 100 species of vertebrates that can be a source of human infection. The number of microorganisms required for an

infective dose, is only 10 cells, if introduced through the skin (subcutaneously). However, after ingestion of infected materials, a gastrointestinal infection requires at least 10^8 microorganisms [282].

There are a number of species of *Francisella*, *F. philomiragia*, *Francisella novicida*, *Francisella noatunensis*, and *Francisella tularensis*. There are three subspecies, *F. tularensis subsp. holarctica* (type B), *F. tularensis subsp. mediaasiatica*, and *F. tularensis subsp. tularensis*. However, *F. tularensis* is the only species that has been associated with human disease. They have been referred to as "rabbit and deerfly fever," and etc [268,282]. The organism appears to be able to penetrate intact skin, however it most likely requires cuts in the skin, in order to gain entrance [268,282]. As far as the course of disease, the symptoms are chills, headache, and generalized pain. There are several forms of the disease, which include the ulceroglandular, glandular, oculoglandular, oropharyngeal, pneumonic, and typhoidal forms. However, the most common form in the United States is the ulceroglandular form, which is from tick bites from infected animals. Therefore, tularemia is considered a zoonotic disease, because of its presence in the animal population [268]. The organism is culturable, and may grow in sheep blood agar, and chocolate agar. However, it is a fastidious microorganism, and may require the addition of cysteine, or thiosulfate for growth within chocolate agar. It will also grow on Modified Thayer-Martin Agar, and buffered charcoal yeast extract agar [268]. Both MacConkey and EMB Agar will not support the growth of *Francisella*. Colonies may require up to 48 hours before they appear in a 5% CO_2 atmosphere, at a 37°C incubation temperature. Some laboratories prefer cysteine trypticase agar, supplemented with 9% chocolatized sheep blood agar. In general colonies appear "gray-white raised with a smooth appearance. Serologically the *Francisella* can be detected by slide agglutination tests, the Direct Fluorescent Antibody Technique, Monoclonal antibodies, and PCR [268,282].

Pasteurellaceae

Pasteurella

This microorganism is a gram-negative pleomorphic bacillus, that is nonmotile, and is a facultative anaerobe. It may appear rod-shaped to coccoid in morphology, and is 0.3 - 1.0 μm in width by 1 - 2 in length. However, they may occur singularly, in pairs, or in short chains [7,28]. They have a tendency to stain more intensely at both ends of the cells, and thus the term "bipolar staining." There are five serotypes (A, B, D, E and F), based on the presence capsular antigens. Pasteurella primarily cause wounds, and is often transmitted by scratches, and bites by animals [268]. The organism biochemically is oxidase and catalase positive, and reduces nitrates to nitrites, and utilizes the carbohydrates fructose, glucose, and mannose [268,283]. On Sheep Blood Agar and Chocolate Agar, they form small, smooth, non-hemolytic grayish colonies [268]. Most strains and particularly *P. multocida* do not grow on MacConkey Agar [283]. *P. multocida* typically produces an acid over acid TSI agar slant, or yellow throughout the slant, since it ferments glucose only weakly. The majority of the *Pasteurella* strains ferment glucose, fructose, mannose, and sucrose, and reduce nitrates to nitrites [7,268,283].

Chlamydiaceae

Chlamydia

The Chlamydia are considered "obligate intracellular parasites" [28,285]. They are non-motile, coccoid, and very pleomorphic. They range in size from 0.2 -1.5 μm in diameter [7]. As a gram negative bacillus, they do have an outer membrane that is type and species specific [7]. In the past, it was primarily cultured in cell culture, and in the yolk sacs of chickens [7]. The microorganism does exist in two major forms in its reproductive cycle, namely the elementary body (EB) which is infectious, and the reticulate body (EB), which reproduces by binary fission [7]. Once the elementary body has entered a appropriate cell, it will undergo various "structural changes," and becomes larger in size undergoing the change to the reticulate body after between 36 - 72 hours. Once the cycle is complete, reticulate bodies now convert back to elementary bodies, which accumulate until the cell bursts. Then again, elementary bodies are now able to infect other cells. *Chlamydia* is cultured in what is referred to is Shell vials [7,284].

Chlamydia trachomatis, *Chlamydia pneumonia*, and *Chlamydia psittaci* are the three species that are considered pathogenic to man, while the disease potential of other species has yet to be decided [284]. *Chlamydia trachomatis* expresses various serological types, A-K, Ba, and L1, L2 and L3. These are all based on the differences serologically in the "Major Outer Membrane Proteins" or (MOMP). *C. trachomatis* is responsible for the major cause of blindness in the world today [284]. Damage to the eye occurs over time due to poor sanitation, as well as scaring of the eyelids, and conjunctiva. This eventually results in the eyelids turning end word, the immune response is due of the microorganism [284]. *C. trachomatis* can be demonstrated in Hela cells, McCoy cells using giemsa as inclusions, which push the nucleus to one side, and appear a dark purple with a halo [284]. See reference 284 Figure 34-3 Giemsa in McCoy cells.

Chlamydia trachomatis

C. trachomatis is also a prominate cause of sexually transmitted disease, which affect the urethra, and cervix. This can result in infant births that may develop respiratory infections, and or ocular infections due to exposure to secretions. An infected mother may develop an inflammatory infection, that could lead to "pelvic inflammatory disease" [284]. The serotypes that are often involved in genital infections are serological types D-K. *C. trachomatis* is also responsible for Lymphogranuloma venereum, which is a serious systemic disease. The serotypes that are involved are L1, L2 and L3. These types of infections are common in Africa [284].

Chlamydia pneumoniae

C. pneumoniae is a cause of pneumonia that is acquired through respiratory secretions, particularly when individuals are confined and exposed at military barracks, or areas that house many people in one location [284]. It appears to be associated with the risk factors of asthma, atherosclerosis, strokes, Azheimers disease, and Multiple Scterosis. However, the role these conditions play in contributing to infection has not been substantiated [284]. The organism can be cultured in HeLa 229 cells, HL Cells, HEp-2 cells, or NCI-H 292 cells [286]. Tests for serology may be confusing and complicated. Although cell culture methods have been considered the preferred testing method in the past, today nucleic acid amplification tests (NAATs) are now the method of chose. Both *C. trachomatis* and *C. psittaci* may be cultured in HeLa and McCoy cells, as can *C. pneumonia*. Some use HEp-2 or HL cells as their preference for culturing *C. pneumonia,* See Figure 34-1 and Figure 34-3 [284].

C. psittici

C. psittici is a microorganism that has a reservoir in the animal population primarily birds, and especially parakeets [7]. It is the cause of psittacosis in birds, and ornithosis in man. Although the organism is the cause of pneumonia of the lungs, it can also cause a infection in other organs [7,286]. It has been diagnosed by serology, particularly complement fixation, and microimmunofluorescence test. Microimmunofluorescence test has been the method of choose, although PCR can detect the organism directly from clinical specimens [7,285,286].

Mycoplasmataceae

Mycoplasma

The genus *Mycoplasma* was originally discovered in 1989, by the French scientists named Nocard and Roux. They noticed cattle that were suffering from a disease called "pleuropneumonia," [7,28]. The mycoplasmal organisms were initially found in the pleural fluids of the cattle. They called the organisms "*Mycoplasma mycoides*, and described them as "rounded and stellate forms with radial, mold-like filaments," [337]. The microorganisms because of the pleomorphic morphology were at one time called pleuropneumonia-like organisms or PPLO [7]. Today they are called *Mycoplasma*. The *Mycoplasma* are nonmotile, facultatively anaerobic, and are chemoorganotrophic. They are devoid of a cell wall, but instead have a trilaminar cytoplasmic membrane, that contains sterol as well as phospholipids and proteins [7]. It does contain both DNA and RNA, and has a "flavin-terminated electron transport chain" [7]. In culture *Mycoplasma* are described as forming colonies with a "fried egg appearance [7,287].

The *Mycoplasma* are associated with respiratory infections, urinary and genital infections, but can be distributed to other organs with dibilitated individuals [287]. Both *M. hominis* and *Ureaplasma urealytic* have been found in the urinary tract [287]. *M. honinis* has been found in patients with "pelvic inflammatory and postpartum fever," whereas *U. urealyticum* may be important in nongonococcal urethritis, and inability to have children [287]. Other species are be involved in respiratory infections (*M. fermentans*) [287]. *M. genitalium* is considered the cause of "cervicitis and endometritis [287].

These microorganisms are capable of being be grown in culture, however the need for additional nutrients such as "nuclear acid precursors," and serum additives would be required for their culture. Both *M. pneumonia*e and *M. hominis* may be grown in Shepard's 10B urea broth, whereas both *M. hominis* and *U. urealyticum* can be cultured on the solid culture media A8 [287]. The broths should be incubated at 37°C in an atmosphere of 5 to 10% CO_2. In general, the Mycoplasma Ureaplasm are diagnosed based on serological tests, such as cold agglutininas in the case of *M. pneumonia*e, as well as using tests for example, enzyme immunoassays, indirect immunofluorescence, and indirect hemagglutination methods for mycoplasmas in general. However serological methods for mycoplasma infections of the genital tract, have not been affective [287].

Rickettsiaceae

The genus *Rickettsia* is named after Howard Taylor Ricketts, who discovered them as a result of his research on Rocky Mountain spotted fever in 1909 [7,28,339]. Unfortunately, Mr. Howard Rickets developed Rocky Mountain spotted fever, succored to the disease himself, while working in the laboratory [28,101,288].

The *Rickettsia* are coccobacilliary gram-negative bacteria, that are considered "obligate intracellular parasites" of man, vertebrates, and arthropod vectors such as ticks mites and fleas [7,101,288]. The microorganisms are non-flagellated, and nonpiliated, and range in size from 0.8 - 2 by 0.3 - 0.6 in µms in length [7]. The microorganisms have not been cultured in routine culture media, but have been recovered in yolk sacs of embryonated eggs, and by monolayer cell lines (HeLa. or HEp-2 cells), and small laboratory animals [286]. They may be stained by the Gimenez method, and reproduce by binary fission, and are found predominately in the cytoplasm of cells [7,285,289].

The organisms that form the typhus fevers are *R. prowazekii* and *R. typhi*, whereas the spotted fevers are called *R. rickettsii*, *R. akari*, *R. coronii*, and *R. africae*. With the spotted fevers, particularly Rocky Mountain Spotted Fever, the organism is transmitted by the saliva of the tick vector *Dermacentor variabilis*, while the tick is feeding [285]. The Rickettsia are able to infect adjacent cells, without damaging host cells. This can lead to "local blockage, and rupture of small blood vessels [101,288]. However, the significant damage by these organisms can cause an inflammation of the blood vessels, of the major organs of the body, the brain, heart, lungs and kidneys [104,285,288]. Early symptoms are flu-like (1st week), but afterwords typical symptoms include fever, headache, myalgia, nausea, vomiting and rash. The rash begins at the extremities, involving the palms and soles, and may extend to the trunk [285,289].

Both *R. typhi* and *R. prowazekii* are the cause of typhus fever, when bitten by infected fleas. *R. typhi* is often referred to as "Murine typhus or epidemic typhus, since it is transmitted by mice that contain the infected fleas. It is maintained in the rodent population by transfer of the organism from adult mouse, to infant or "transovarian transmission." Individuals often become infected when they scratch the infected wound. Humans may also be infected directly when bitten by an infected fleas [285].

Louse-Borne Typhus or "Epidemic typhus" is caused by *R. prowazekii*, however is transmitted by the human louse *Pediculus humanus* [285]. The main source in the United States is by flying squirrels, primarily in the eastern United States [285]. Although other parts of the world, particularly Africa, and Central American where there is poor sanitation, lends itself to the higher prevalence and incidence of infected body lice [285]. Serology is the current method using biopsies from skin samples, diagnosis by Nucleic Acid amplication techniques, although they may be cultured in embryonated eggs, tissue culture, cells may be stained by Giemsa or Gimenez stains. They may be confirmed by fluorescent antibody techniques. The Weil-Felix test has been used in the past, but other diagnostic methods are preferred [289].

The Richettsia that cause scrub typhus are now called *Orientia tsutsugamushi* (formerly *R. tsutsugamushi*), and is transmitted by chiggers, that are harbored by reservoir rats [285,289].

Coxiellaceae

Coxiella

Coxiella is the cause of "Q fever," it is a gram-negative pleomorphic microorganisms that is a nonmotile rod, and is considered an obligate intracellular parasite [7,28,286]. They range in size from 0.4 - 1.5 µm in wide by 0.4 - 1.0 µm. in length, and may be stained by Gimenez stain [7,286]. *Coxiella* may be transmitted by the bite of ticks, although *C. burnetii* is principally transmitted by aerosols, or infected milk [286]. In the case of *C. burnetii*, the organism is able to infect macrophages, and will initiate growth within phagolysosomes [289]. These microorganisms may exist as small variants, that are able to undergo binary fission [289]. The small variants are unable to divide but are infectious, whereas larger variants are noninfectious, but able to divide [289]. Again, most infections start through aerosols, however drinking raw cows, or goat's milk has been in the past the driving force for infectivity [289]. Symptoms include fever, chills, headaches, and myalgias. Liver involvement may occur, liver biopsies can detect a atypical pneumonia showing a "donut-shaped granuloma." Subacute endocarditis can be a consequence of those with heart problems [289]. Q-fever is usually diagnosed by indirect immunofluorescen-antibody assay, and enzyme-linked immunosorbent assay (good specificity and sensitivity), and direct immunofluorescence [285,286,289].

Nonfermenters

The nonfermenters have been described by Mahnon., *et al.* [204], as "bacteria that are unable to utilize glucose, or other carbohydrates in the absence of oxygen." Nonfermenters have typically been screened by the use of OF media (Hugh and Leifson's), in an effort to determine how an organism utilizes glucose, and or how various carbohydrates are oxidized or fermented [7,204]. In this method tubes of media are inoculated, while one is overlaid with a 1 cm of sterile mineral oil. Tubes that show growth only at the top of the open tube are considered oxidative, whereas organisms growing in both tubes are considered facultative anaerobic. Those microbes that produce only alkaline products are said to be nonoxidizers. Triple Sugar iron agar is also a culture medium for screening nonfermenters, since it is possible to detect those organisms that form alkaline products, and or others that are fermenters [7,145,290].

Most of the prominate work on nonfermenters began in the early nineteen fifties, and credited to the determination, and ingenuity of Elizabeth King at the Center Disease Control, in Atlanta Georgia. Her Scheme has lead the way for a better understanding of the architecture of very large, and confusing group of microorganisms [145]. Elizabeth King based her identification of the nonfermentative bacteria, on separating those organisms in to groups primary on their ability to grow on MacConkey agar, produce cytochrome oxidase, catalase, and whether fermenters or oxidizers by OF media, as well as nonoxidizers. These parameters thus allowed similar microorganisms to be grouped accordingly, not only on morphology, but also on many typical conventional biochemical tests which include motility, pigmentation, and ability to grow on selective culture media [81,145].

There have been numerous approaches that have been published in the literature by many investigators, or researchers on the culture, and identification of the nonfermenteratative bacteria, examples would include Pickett., *et al.* [291] in his scheme in 1970, Oberhofer TR [292] in 1977, and Weaver E and G Hollis [293]. Weaver and Hollis., *et al.* later published their second edition book the nonfermentative bacteria [81,271,291-294,331].

Pseudomonadaceae

Pseudomonas

One of the most prominent nonfermenter of the genus Pseudomonas is *Pseudomonas aeruginosa* [28,81]. It is the most common nonfermenter that is isolated in culture, in the clinical laboratory. It is like other nonfermenters, a gram-negative bacillus to coccobacillus [292].

They are motile by polar flagella, oxidase, catalase, and are oxidizers of variety of carbohydrates, such as glucose [81,294,331]. Most are able to grow in MacConkey agar, however there are some exceptions.

Pseudomonads in general are aerobic, nonsporulating, gram-negative bacilli, some of which able to reduce nitrates to nitrite or all the way to gas [81,290,294,331]. *Pseudomonas aeruginosa* produces a fruity or grapelike odor. They form colonies that appear flatten and spreading, and may exhibit a metallic sheen on 5% Sheep Blood Agar, See Figure 7-2 and 7-6 p. 138 [294]. *P. aeruginosa* is a fluorescent microorganism as is *P. fluorescens* and *P. putida*, which may be detected by growing on sheep blood agar, or another medium such as, Mac Conkey agar, and or Mueller-Hinton agar [81]. Also, fluorescence can be detected by the use of King's Medium A, and King's Medium B, and or Pseudomonas P, and Pseudomonas F agar. *P. aeruginosa* may produce both pyocyanin (blue-green pigment), and pyoverdin (yellow pigment). Both *P. fluorescens* and *P. putida* produce the pyoverdin. P. aeruginosa may be distinguished from the later species on the ability of *P. aeruginosa* to grow at 42°C, hydrolyse arginine (+), ability to utilize acetamide (+), as well as the substrate gluconate (+), but is negative for gelatin hydrolysis. Both *P. fluorescens* and *P. putida* do not grow at 42°C, and are negative for acetamide, and gluconate hydrolysis. *P. fluorescens* differs from both *P. aeruginosa* and *P. putida*, in that it is gelatinase positive, See 82 Table 7-3 p. 318 and 81 Figure 21-2 p. 487 [81,82,271,294,295]. *P. stutzeri* another pseudomonad forms a flat, and smooth colony, that is characteristically wrinked on 5% Sheep Blood Agar [295]. *P. sutzeri* biochemically hydrolyses starch, reduces nitrates to nitrogen gas, is able to grow in broth with 6.5% salt, is gelatin negative, and grows at 41°C, See 271 Table 7-3 p [7,81,82,294,295].

Burkholderiaceae

Burkholderia

Burkholderia pseudomallei (formerly *P. pseudomallei*) forms a wrinkled colony on sheep blood agar, and resembles *P. stutzeri* [28,81,82,271]. It is motile and can exhibit bipholar staining. It is the cause of melioidosis, which is a "granulomatous pulmonary disease," and can be capable in forming abscesses in the lungs and tissues. The organism is a coccobacillus, that is positive for oxidase, catalase, exhibits bipolar staining, and is motile by multitrichous flagella, and may form a pungent odor [271,296,297]. It is able to grow at 42°C, and is lysine decarboxylase (+), grows on MacConkey Agar, and biochemically in OF media, produces acid in glucose, lactose, maltose, mannitol, reduces nitrates to nitrogen gas, Simons Citrate (+), and arginine hydrolyse (+) [82,271,295,296]. See 82 Table 7-7 p. 323-324. The microorganism is typically found in soil and stagnant water. It has been found in Thailand, Vietnam and parts of Northern Australia, and is endemic in China and Taiwan [81,82,145,271,296,297].

Burkholderia mallei (formerly *Pseudomonas mallei*) the organisms is the cause of glanders, which primarily affects various types of animals, such as horses, mules, and donkeys. Like *B. pseudomallei*, it is considered a "potential bioterrorist agent." It is considered a "Obligate parasite of animals" [82,271]. The microorganism is a gram-negative coccobacilliary rod, that is nonmotile, and is sometimes pleomorphic. It is nonpigmented and is unable to grow at 42°C, and may not grow on blood agar or MacConkey Agar [81]. However, on brain-heart infusion agar, colonies appear grayish white, later translucent after 48 hours. Biochemically, the organism is able to utilize glucose, xylose, gelatinase (+), and is arginine hydrolysis (+), See 82 Table 7-7 p. 324 [7,295,296]. Table 6 p. 677.

Moraxellaceae

Acinetobacter

Acinetobacter is a aerobic gram-negative coccobacillus to diplococcus, that is nonsaccharolytic, nonmotile, oxidase negative, catalase positive, and nitrate negative [7,28,81,296]. The organism is approximately 1 - 1.5 µm by 1.5 - 2.5 µm in length, and is considered a nonfermentative microorganism [298]. Acinetobacter is found in the soil and water, and is considered an opportunist pathogen, and is associated in the hospital environment, and nosocomal infections [7,82,271,298]. It has been involved in a number of infections such as wounds, catheter, respiratory and urinary tract infections [298]. In culture, the organism can be cultured on 5% sheep blood agar, and forms colonies

that are "smooth, opaque, and grayish white to yellowish in color [298]". Some strains of *acinetobacter* form a "purplish hue" on MacConkey Agar, which can be easily mistaken for a lactose fermenter [81,298]. The two-prominent species are *Acinetobacter baumannii* and *Acinetobacter lwoffi*. *A. baumannii* is saccharolytic, while *A. lwoffi* is asaccharolytic. *A. baumannii* has been particularly a problem for soldiers who have contracted wound infections, after returning to the states following duty in Irag [299]. *A. lwoffi* appears has a lesser in ability to cause disease, and is more considered a contaminate, and or a colonizer [7,81,82,298,299].

Xanthomonadaceae

Stenotrophomonas maltopilia

Stenotrophomonas maltopilia (formerly *Pseudomonas maltophilia*) is a gram-negative bacillus, that is a oxidize negative nonfermenter, which may occur rod shaped, or "slightly curved," and is polar flagellated (multitrichous) [7,28]. *S. maltophilia* is found in the environment in water, sewage and on plants [7]. It can be found in the respiratory tract as a colonizer, and is considered "normal human microbiota" [81]. It is approximately 0.5 - 1 µm in width by 1.8 - 5 µm in length, and is somewhat smaller in size compared to *Burholderia mallei* [7,297], and as the name implies, it is able to oxidize both glucose and maltose, and reduce nitrates [7,297]. Some strains may give off an ammonia-like odor, from colonies growing on sheep blood agar [297]. It is unusual in that unlike most pseudomonad-like organisms, it is oxidase negative [297]. Colonies may appear a pale yellow, or a lavender gray, on Sheep Blood agar [82,271]. In other biochemical tests, it is lysine decarboxylase positive, but arginine dihydrolase and ornithine decarboxylase negative. It also is DNase positive, oxidase and catalase positive, esculin and gelatin positive. It has caused a number of infections, such as pneumonia, endocarditis, wound infections, cellulitis, bacteremia, respiratory infections, but rarely meningitis and urinary tract infections, See 82 Table7-10 p. 333 [7,81,82,271,297].

Burkholderiaceae

Burkholderia cepacia

Burkholderia cepacia is a gram-negative rod that is commonly found in soil and water, and is lophotrichously flagellated [7,28]. It causes a rot on plants, and is the cause of a foot rot in man [81,82,271]. The microorganism has been isolated from the sputum of cystic fibrosis patients as well [81,82,271]. It has particularly been a problem in the hospital environment, where it can be a source of contamination of water, moist surfaces, various detergent solutions, disinfectants, intravenous solutions, nebulizer solutions, and respiratory equipment [81,82,271]. The organism can be easily culture on a variety of culture media namely: 5% Sheep Blood Agar, MacConkey Agar, Pseudomonas Cepacia Medium (PCM), Oxidative-fermentative base + (polymyxin B, bacitracin, lactose), as well as *Burkholderia cepacia* selective agar (BCSA) [81,82,271]. *B. cepacia* forms colonies on blood agar that are may be "yellow or green, and is nonfluorescent in color" [81]. Biochemically they are able to oxidize glucose, and most strains oxidize maltose, lactose, and mannitol. Most strains of *B. cepacia* are lysine decarboxylase (+), ONPG (O-nitropheyl-β-D-galactopyranoside) (+), ornithine decarboxylase (-), but fail to reduce nitrates to nitrates [7,81,82,271,297].

Burkholderia glacioli

Burkholderia gladioli is a former pseudomonad (*P. marginata*), that was recently reclassified into the genus *Burkholderia* [81,82,271]. It is a pathogen of plants, although it has been recovered from blood and tissue of patients, particularly immune compromised, such as as cystic fibrosis patients [81,297]. It has one or two polar flagella, and may be cultured on blood agar, and MacConkey Agar [81,297]. On culture media, it forms a "diffusible nonfluorescent yellow pigment. Biochemically it is weakly oxidase (-), catalase (+), oxidizes glucose, and mannitol, but is negative for maltose and lactose [82,271]. It is also arginine dehydrolase (-), lysine and ornithine decarboxylase (-), but is very urea positive [300]. It can be confused with *B. cepacia*, since both organisms can produce a bright yellow pigment on Oxidative Fermentative Media (OFPBL Agar), colonies may appear wet runny, and mucoid, See 297 Table 3 p. 7 [82,271,297,300].

Alteromonadaceae

Shewanella putrefaciens

Shewanella putrefaciens (formerly *Pseudomonas putrefaciens*) is a gram-negative bacillus that is a aerobic chemoorganotroph, that is found in water, the marine environment, and various environmental sources. It is motile by means of a polar flagella [7,28,82,84,271,298]. On blood agar, it forms colonies that are about 2 - 3 millimeters in size, and are "brown to orange tan pigmented," although some may appear orange tan in color [60,271]. As they were once recognized as a pseudomonad, in that relation they were considered unique, able to produce hydrogen sulfide in TSI and KIA media [81,82,271]. Biochemically, it is oxidase (variable), DNase (+), Ornithine (+), some biotypes grow in 6.5% NaCl, and both positive for sucrose and maltose (+), Refer to reference 300 Table 5 biochemical Shewanella p. 19. [81,84,298,300].

Sphingomonadaceae

Sphingomonas

Sphingomonas paucimobilis is (formerly *Pseudomonas paucimobilis*) a gram-negative bacilli that appears morphologically as a slender rod, that is motile by polar flagella, and forms a strong yellow to orange pigment in culture [84,298]. The microorganism can be isolated from the environment such as, water, swimming pools, hospital equipment, and clinical infections [81]. The organism does not grow on MacConky Agar, and is oxidase (+), ONPG (+), esculin (+), DNase (+), indole (-), and is positive for glucose, maltose, sucrose, and xylose in OF media, and negative for OF rhamnose (-), and indole (-) Refer to reference 300 Table 5 p. 19 [81,84,298,300].

Ralstoniaceae

Ralstonia

Ralstonia pickettii (formerly *Pseudomons pickettii*) are gram-negative bacilli, that is associated with sterile fluids, found in hospital use. It has been isolated from urine, abscesses, wounds, respiratory specimens, and blood. It has also been found from respiratory specimens of cystic fibrosis patients [28,81]. It can cause bacteremia, meningitis, endocarditis and osteromyelitis [81,297]. The microorganism is motile, is oxidase (+) grows in cetrimide agar at 42°C, Simons Citrate (+), urea (+), reduces nitrate to gas, but is esculin (-), gelatin (-), starch (-), is lysine decarboxylase (-), ornithine decarboxylase (-), arginine dehydrolase (+), and forms acid from OF glucose, fructose, galactose, mannose, and xylose [300,302]. The biochemical characteristics were found from the journal article by Fujita., *et al* [302]. The organism was isolated from a patient's blood culture, and has a identical biochemical pattern, as the type strain *P. pickettii* ATCC 27511 culture, See also 297 Table 17-1 p. 143, Also 300 Table 3 p. 7 [300,302].

Delftia

Delftia acidovorans (formerly in the genus Comamonas) is a gram-negative bacillus that is slightly curved, and is 0.5 to 1.0 μm by 1.8 - 5 μm in length. Appying the gram stain, they tend to have a washed out or "moth eaten appearance," due to the appearance of hydroxybutrate inclusions [271,297]. The organism is also are motile by the presence of a tuft of polar flagella. Like other nonfermenters, they are widely distributed soil, water, as well as food, hospital equipment, and clinical specimens [81,82,271]. In culture, on sheep blood agar, they appear as "round tan colonies [297]. They can be found in association of nosocomal infections of bacteremia, corneal ulcerations, endocarditis of intravenous drug users, and cystic fibrosis patients [81,297]. Biochemically the microorganism is similar to the alkaline pseudomonads, since it fails to oxidize OF glucose media. It is oxidase (+), reduces nitrate to nitrites (+), utilizes acetamide, and is weakly positive for OF fructose and mannitol. This organism has a unique property, in that it forms a "orange indole reaction" with Kovacs reagent, and is biochemically negative for gelatin, esculin, starch hydrolysis, See 297 Table 17-1 p. 143 [82,271,297,300,304].

Alcaligenaceae

Alcaligenes

Alcaligenes is a microorganism that is a gram-negative coccobacillus to coccus, that can been found in the soil, water, and clinical specimens. The organism is 3.0 µm in length, and is motile by up to 12 peritrichous flagella (7,28,81). They are obligately aerobic, catalase (+), some my grow anaerobically by reducing nitrates to nitrites, whereas others can grow chemolithotrophically by utilizing hydrogen (H_2). The genus alcaligenes are considered asaccharolytic, but are able to alkalinize organic acids, and some amides [7,81]. They are able to grow on blood agar, and MacConkey Agar. *Alcaligenes faecalis* produces a fruity odor, and as well as a "green hue" or green discoloration of the media [81,301]. Biochemically, *A. faecalis* is oxidase (+), grows on MacConkey Agar, is able to reduce nitrates to N_2, indole (-), esculin (-), able to utilize acetamide, malonate, 6.5% NaCl (+), and is negative for OF glucose and xylose. *A. denitrificans* is identical to *A. faecalis* biochemically, except it does not grow in 6.5% NaCl. *A. piechaudii* is also oxidase (+), and grows on MacConkey Agar, reduces nitrates to nitrates not to gas, does grow in 6.5% NaCl, but is malonate (-), See Table 7-15 p. 341 [7,82,271,303].

Caulobacteriaceae

Brevundimonas

Brevundimonas is a group that are similar to other alkaline pseudomonads, in that they are "nonreactive" or weakly reactive in carbohydrates [82,271]. The organisms are 0.6 µm in width by 1.0 µm in length, and are polar flagellated [60,271]. *B. vesicularis* produces colonies that form a orange to brown color, accumulate Poly-β-Hydroxybutrate (PBHB), and are esculin (+), and positive in OF maltose. Most strains fail to grow on MacConkey Agar [82,271,295]. *B. diminuta* is considered a contaminate, although has been involved in blood, cerebrospinal fluid, and wounds [81]. The organism produces a brown water soluble pigment, when inoculated to tyrosine agar. Biochemically, it is oxidase (+), and can oxidize OF glucose and maltose and is oxidase (+), but is indole negative, and most colonies are white. It differs from *B. vesicularis*, in that it commonly grows on MacConkey Agar. Most strains of *B. vesicularis* produce an orange intracellular pigment, hydrolyse esculin, which differs from *B. vesicularis*, See Table 4 p. 17 [81,82,271,295,297,300].

Alcaligenaceae

Achromobacter

Achromobacter is a microorganism that is curved, and or hooked at one end of the cell, and motile by polar flagella [28,305]. They have been associated in contaminated solutions, as well as nosocomal infections, and the immune impaired [301,305]. *A. xylosoxidans* differ from *A. denitrificans* and *A. piechaudii*, in that it is capable of oxidizing both OF glucose and xylose, whereas *A. piechaudii* differs from the former species, in that it reduces nitrates to nitrites [300]. The organism is aerobic oxidase and catalase (+), and will grow on routine culture media such as, Sheep Blood agar, Chocolate agar, MacConkey Agar, and SS Agar [301]. It produces two colony types, one large and mucoid, and another shiny, glistening and opaque [305]. Biochemically they are oxidase and catalase positive, reduces nitrates to gas, hydrolyse esculin, phenylalanine (+), produces H_2S in TSI (triple sugar iron agar), See 300 Table 6 p. 21 and 82 Table 7-15 p. 341 [81,82,271,300,301,305].

Flavobacteriaceae

Flavobacteria

Flavobacteria are gram-negative bacilli that range in size from 0.5µm by 1 - 3 µm in length. They are nonmotile, aerobic and are chemoorganotrophic, and morphologically cells have rounded ends. They are commonly found in water, soil, milk, meats, and clinical specimens [7]. They often form soluble nonfluorescent yellow to orange pigments, which may be "flexirubin type, and spread on the agar surface [7,28,81,84,298]. *F. odoratum* has been transferred to the genus Myroides, whereas both *F. meningosepticum* and *F. indologenes* were moved to the genus *Chryseobacterium*, 300 see Table 8 p. 22 [298,300]. *F. meningoseptica* has been reclassified into another genus called *Elizabethkingia meningoseptica*, which was originally isolated by Elizabeth King in 1960 [28,306]. It is a slender rod that is nonmotile, is

aerobic and nonsporulating [28,306]. It has historically been associated as a cause of neonatal meningitides, endocarditis, pneumonia, and nosocomial infections. On sheep blood agar, it forms a pale yellow to salmon pink colonies, that are nonhemolytic [7,81,84,298,306]. They are also have variable growth on MacConkey Agar, and are oxidizers [306]. Biochemically, they are oxidase, catalase, and indole positive, ONPG (+), esculin (+), DNase (+), and oxidize glucose, and mannitol [300]. *Chryseobacterium indolgenes* and *C. gleum* formerly (CDC group 11b) are organisms that are interesting in that are thinner towards the center of the cell with bulbous ends, and both produce a yellow orange flexirubin water-soluble pigment [28,81,82,84,271,298,300]. They both biochemically are nonmotile, catalase (-), oxidase (-), indole (+), esculin and gelatin (+), however *C. indologenes* is beta hemolytic, and doesn't grow at 42^{0}C. *C. gleum* is α-hemolytic, and is capable of growth at 42^{o}C [81,82,84,271,298,300].

Flavobacteriaceae

Myroides

Myroides odoratus and *Myroides odoratiminus* were formerly classified in the genus Flavobacteria [28,81,82,271]. These organisms are microscopically small gram-negative bacilli, that have been associated in such clinical specimens such as wound, sputum, blood, and ear [81,82,271]. They characteristically form flat spreading colonies that are yellow pigment, and produce a fruity odor similar to *Alcaligenes faecalis* (81,82,271). Like many nonfermenters, they are asaccharolytic, and are oxidase (-), catalase (-), urease (-), gelatinase (+), indole (-), and reduce nitrates are reduce to nitrites, See Reference 82 Table 7-18 p. 349 [81,82,84,271,298].

Sphingobacteriaceae

Sphingobacterium

Sphingobacterium multivorum and *Sphingobacterium spiritovorum* originally belonged to the genus Flavobacterium [28,81]. Both are nonmotile and yellow pigmented, and are able to oxidize OF glucose, and may display a gliding motility [81,82,271]. Biochemically, they are oxidase and catalase (+), esculin (+), but differ from other Chryseobacterium, and Weeksella, by their failure to produce indole. They have been isolated from urine, blood, and from the hospital environment [81,82,271]. Both species are similar biochemically, but *S. spiritovoran* produces acid from mannitol, ethanol, and rhamnose, whereas *S. multivorum* does not [81,82,271].

Weeksella

Weeksella virosa is a gram-negative bacilli that is nonmotile, and produces a pale yellow pigment to butterscotch pigment, See Table 8 p. 22 [81,82,271,300]. Colonies tend to be very "sticky, mucoid" in consistency, and difficult to pick up from culture agar media [81,82,271]. They do not grow on MacConkey agar, and are sensitive to penicillin and polymyxin B. They have primarily been associated with the urinary tract infection of females, and biochemically, they are oxidase (+), indole (+), but are all gelatin, starch, esculin, ONPG, Dnase, and urea negative, Also See 82 Table 7-17 p. 347 [81,82,271,300].

Methylobacteriaceae

Roseomonas

Rosemonas species are nonfermentative bacilli that characteristically occur rod shaped to coccoid in morphology, and forms a pink pigment on sheep blood agar [28,81,82,84,271,298]. *Rosemonas gilardii* is the more significant species in terms of disease causing capability [81,82,271]. Morphologically, they form plump coccoid rods that appear singularly or in short chains, and are capable of growing in a variety of culture media such as, sheep blood agar, chocolate agar, buffered charcoal yeast extract (BCYE), MacConkey agar, Modified Thayer Martin agar, as well as Sabourauds agar. On agar, colonies are typically "pinpoint, runny pale pink to red, mucoid, raised, entire, and mucoid in appearance." Biochemically, the organism is weakly oxidase (+), catalase (+), hydrolyses urea, and is variable in the OF oxidation of glucose, and negative in OF methanol [81,82,84,271,298,300].

Methylobacteriaceae

Methylobacterium

Methylobacterium mesophilicum (formerly *Pseudomonas mesophilica* and *P. extorquens*) is a gram-negative bacillus that is polymorphic, and has the morphological appearance as rods that are swollen, vacuolated, and may appear partially gram-positive. They are motile by polar flagella, and grow at 25°C - 35°C [28,81,298,307]. The genus has been described based on its ability to utilize methanol as its sole carbon source, and formation of a pink pigment [81,84,298]. The microorganism is found in the environment in soil, plants, water, and hospital equipment. It has also been isolated from throat swabs, bronchial washes, and blood specimens [81]. *M. mesophilicum* like *M. zatmanii* have been recovered from a number of nosocomial infections such as, lung, bone marrow, "peritoneal dialysis-associate peritonitis," and septicemia [81,84,298,307]. The organism is a slow grower in culture media, and may take up to 4 - 5 days for the presence of isolated colonies [81,84,298]. It is capable of grow on a widely of culture namely: Sheep Blood Agar, Chocolate agar, MacConkey agar, Sabouraud dextrose agar, Thayer Martin agar, and Buffered Charcoal Yeast extract Agar [81,84,298]. Biochemically it is oxidase (+), catalase (+), urea (+), and is OF xylose (+), but indole, esculin, OF maltose, mannose and sucrose negative. They organism is decarboxylase negative, nitrate variable, ONPG negative, simons citrate variable, and unable to utilize sucrose, and does not utilize glucose either aerobically or anaerobically [81,84,298,307].

Trends in Microbiology

Foods and Contamination problems

As far back as 2004 there have been a number of articles on the contamination problem with Dry infant formula. It became apparent when the article by sacbee.com became available. It was about the fact that a particular microorganism was causing serious infections, of infants eating dry infant formula. Nearly one in every four 5 lb containers could be found to contain the food borne bacteria, which can attack the brain causing brain damage. A scientist at the FDA explained that it was not possible to remove that contaminating microorganism (*Enterobacter saksaki*i), currently known as *Cronobacter sakasakii* with the current food technology. Little new information has been released for the current status of *Cronobacter*, in its incidence origin in dry infant formula [71,278].

Other food problems would include the presence of Arsenic found in rice associated products, such as rice, apple juice, chicken, and pet food [72]. According to a Dartmouth Scientist Brian Jackson (Analytical Chemist), even exposure of even less than 10 milligrams over long term exposure, can be a "insidious threat," even at concentrations of parts per billion (ppb), "a drop in a swimming pool" [72].

Water Problems

The inorganic form (most toxic form) is related to a increased risk of cancer and other life treating diseases. In 2001, the EPA changed the limit of arsenic from 50 ppb to 10ppb of arsenic in water [72]. Animal studies by Dartmouth's Hamilton found that arsenic exposure at 10 ppb, could compromise the immune system of test mice. When water samples were taken from a well from New Hampshire's arsenic rich bedrock, the well water was found to contain arsenic levels at 1ppm (1,000 ppb) [71]. Births could be of low weight, and possibly more susceptible to childhood diseases at these high levels [72,74]. There have also been problems with the run off of fertilizers, resulting in Bloom formation by Cyanobacteria [72,74].

Evidence that well water can pose a serious problem can be realized when one reviews the cases of well water contamination. In Taiwan a case of blackfoot disease, resulting in gangrene from arsenic contaminated well water. Arsenic levels exceeded 800 ppb, with some wells reaching the 1 part million [72]. Bangladesh in the 1970's also experienced many cancers (lung, bladder and cardiovascular problems), and diabetes when they resorted to using ground water containing arsenic levels of up to 500 ppb [72,188].

There have a number of issues with public health, namely the presence of Arsenic in rice, apple juice, chicken, pet food, as well as Melamine in Baby formula, and pet food. Not the least, the presence of Mercury in the presence of seafood. There have been a number of bacterial contaminants in foods, that can pose serious health risks, for example, the presence of *E. coli* in spinach, lettuce sprouts, bologna, beef, hazelnuts, and frozen cookie dough [72]. *Salmonella* seems to be in just about everything, for example, spinach, sprouts, tomatoes, mangoes, papayas, cantaloupe, chicken, beef, pistachios, wheat cereals, peanut butter, dog food and many others [72].

From the 1980's and the present, Listeria has become a major player. Just like Salmonella it seems to contaminate everything. Work in labs on Listeria, became very serious particularly after 1986. At that time, it seemed to becoming considered a major player, when it was it was isolated in Brie cheese, then ice-cream. It next was found in coleslaw, then later in that year there began to be reports of its presence in meats [237]. Recent reports have also been on the Norovirus, which can be spread through human contact, and from eating tainted foods. The virus can be found as a cause of outbreaks, as a result of consuming leafy greens, fresh fruit and shellfish [72,76-78,237,251].

In 1951, a women entered the John Hopkins clinic in Baltimore, Maryland, although she appeared to healthy her cells would say otherwise. Her name was Henrietta Lacks. A lesion was found on her cervix, and was found to be cancerous. Her cells that were recovered would lead to a new way of treating disease. Although her cancer would cause her death, her cancer myeloma cells would not die. Her cells would provide hope for all the generations to come. In the near future, Scientists by chance will fuse these cells with others, and eventually with plasma cells (antibody producing), leading to a new type of cell called a "hybridoma cell". The hybridoma cell had both properties of the cancer cell (continuous division), as well as the property of producing antibodies, what is now know as monoclonal antibodies [79].

Malaria even today is still a major disease problem worldwide. Malaria is cause by the female anopheles mosquito. In malaria when mosquitoes take a blood meal, they also can introduce infective sporozoite as well. Sporozoites enter the blood, and in the liver that convert to merozoites which are infective for red blood cells. As merozoites multiple by multi-division, the red blood cells eventually will burst, allowing more and more merozoites to infect even more red blood cells. This results in the extreme loss of red blood cells, resulting in anemia and shock [128,170].

After a number of cycles of merozoite formation, the malarial parasite will begin to produce sex cells or gametophytes. Opposite gametophytes can fuse, and can result in the formation of sporozoites, and subsequently another anopheles mosquito can pick a sporozoite, along with their blood meal. Vaccines are often difficult to fashion, since the malaria parasite has the way to overcome the immune system, by its ability to change its outer cell surface or glycocalyx. It therefore has been difficult to fashion a vaccine, because of the fact that its glycocalyx can change many times [128,143,170,171].

In the Jan/February Discover magazine reported as of 2000 that there had been a 25% reduction in the incidence of Malaria, from 219 million to 74 million. This reduction was due to the combining the use of chloroquine with the artemisnin (plant derived). Unfortunately, however the majority of artemisnin, comes from China, and is therefore subsequently subject to the production conditions there. The manufacture and processing of the drug requires about 18 months [128,131,170-172,209].

Recently a new process has made possible genetically to insert a gene for producing the drug into a yeast. The drug will not replace the need to naturally produced artemisnin, but Sanofi (Drug company) expects to produce between 50 and 60 tons of the drug in 2014 [128,170-172].

In a different incident in 2009, the FDA made a recall of peanut butter, peanut paste and peanut oil. In the outbreak, more 500 people had become sick, including 8 deaths. It was from Peanut Corp. of America a plant in Blakely, Georgia [150,173,190]. Also in 2009, WLKY News reported of peanut products that were sent to Schools in Louisville, Kentucky, with the possibility of being contaminated with insecticides and Salmonella. The same company Peanut Corp. of America also shipped tainted product to California, Minnesota and Idaho and Kentucky [191].

There also was a recall of dry Pet food products produced by Mars Pet care US in a plant in Everson, Pa. Sept. 13, 2008. MSN.com reported that this company Peanut Company knowingly shipped salmonella-tainted products Tues. Jan. 27, 2009 [192].

There have been numerous outbreaks and recalls since the year 2000. Recalls on, meats, Cantaloupe, Tomatoes, and the list goes on and on. 1Federal Inspectors., (2001). Potentially Tainted Peanut Products Sent to Schools [193-196].

Salmonella is also a major concern for the poultry business, since there are estimated 4 million infections, and 500 deaths occur each year. There are about 1,800 or more serotypes of Salmonella, and each are antigenitically different [197]. In 2008, Astronauts tested the virulence of Salmonella on board of the Space shuttle Atlantis. They found that mice that were injected with Salmonella in space were more pathogenic, than the same organism that was inoculated on the ground on earth [198]. A knew serotype of Salmonella was discovered by researchers named Marie Bugarel from the department of Animal and Food Sciences in Texas. The new serotype was named after the city Lubbock, Texas and called *Salmonella lubback* [199]. In Georgia, researchers found that there appeared to be an increased risk of the presence of Salmonella after a rainfall [200].

Rapid Methods

Rapid Microbial Identification Methods

There has been much controversy with the current method of dispensing antibiotics by doctors. Patients today are insistent when it comes to receiving treatment for whatever symptoms there are experiencing. Doctors are therefore pressured to prescribe more and more antibiotics, in order to satisfy the needs of their patient. However, this can lead to a over-use of antibiotics, instances where patients supposedly would otherwise recover with even without the use of antibiotics [90,207].

The problem is that cultures and sensitivities may require from 24 - 48 hours before lab results become available. This leaves doctors concerned that a patient could be in danger of a possible life treating medical situation [90,207].

With the advent of new promising biosensor technologies, doctors could look forward to same day lab results. This new technology could be faster, and more reliable results in shorter time. The biosensor method entails the use of Polymerase Chain Reaction (PCR), in combination of Mass Spectrophotometry. A blood sample is taken at certain areas of the DNA, and snipped by a polymerase enzyme, making small snips, or pieces of DNA at specific locations along the DNA. Therefore, making multiple copies of targeted sections is made using a process called PCR [207].

As a result of the snipping of the DNA, primers are created with a short number of nucleotides, that serve as a template for generating double stranded segments. A mass spectrometer now is used, to determine the molecular weight of each strand with its primer. With the use of a Mass spectrometer and a mathematical formula, the nucleotide sequence of the prime marker, and the weight of the sequence of the microbe can be determined. The microbial sequence is now compared to the sequences of 2680 microbial species, in order to determine an identification [207].

Superbugs

Superbugs are microorganisms that are resistant to almost all prescribed antibiotics. Although we may not realize it, between 20 - 30% of us are colonized by *Staphylococci aureus*, within our nose. *Staphylococci aureus* its notoriety has been due to its ability to cause necrotizing fasciitis, with the organism is often referred to as a "fleshing eating bacteria" Koo., *et al* [205].

Staphylococcus aureus may be more apparent where we live, and in the environment where we play. According to Dr. Daum, "this superbug can occur with children in daycare centers, in waste water, prisons, on the grid iron, and where children and adults play sports [205,206,208].

In 2007 the CDC reported there were 19,000 deaths from MRSA that year, more than the number of cases of HIV/AIDS. Hospital patients on average stay ten days longer in the hospital, with an additional cost of 30,000 for those additional days [210].

As soldiers began to return to the states, they may also be able to carry with them a threat in the form of multi-resistant microorganisms. A number of multi-resistant microorganisms are now showing up with military personal, and other civilians namely; with *Acinetobacter baumannii, Staphylococcus aureus* MRSA*, Pseudomonas aeruginosa, Klebsillae pneumonia, Clostridium difficile, and Vancomycin Resistant Enterococci.* It was found in one Hospital in Maryland that nearly 40% of protective gowns and gloves can be contaminated with *A. baumannii*. The study was conducted in 2009 [211,212].

According to Washington (AP), MRSA has acquired another mechanism for being successive parasite. It is often associated with health care facilities, where patients are more susceptible to infection, being debilitated. This is even more apparent with emergence of more aggressive strains. These strains are called "community-associated MRSA, or CA-MRSA, where severe infections can result in death [211,212]. In a recent article from the Courier-Journal, community hospitals within the eastern united states saw a tremendous increase in antibiotic resistant superbugs [211-27]. What is also a critical concern besides the MRSA is the Carbapenem-resistant Enterobactereace, that are almost resistant to all antibiotics [213].

Recent Developments

Dalvance is a recently approved drug for MRSA skin and soft tissue infections. This drug is administered by injection. A second drug oritavacin appears to be on the fast track in clinical trial [213].

Gut flora

Originally in 1987 it was found that *Veillonella parvula* is a microorganism that is found in the digestive, respiratory, and urinary tracts. By 1898 Bifidobacteria was found to be part the intestine flora. The Microbiome has now been estimated to be between 900 to 2680 different species [213,215]. According to Canny., *et al.* [219] there are 500 species that have been identified from the large intestine. The majority of the genera are obligate anaerobes, and would include the following genera: Bacteroides, Anaerobic Gram-positive Cocci, *Peptostreptococcus* species, *Eubacterium, Lactobacillus* species, and *Clostridium*. However, the dominant was Bacteroides [215,219].

In an examination of possible "microbial factors" that potentially serve as regulators in the intestine. It was found that these factors are involved in the regulation of the immune system. They help to create a balance within the intestinal tract. The "segmented filamentous bacteria" are involved in the induction in the production of intestinal Th17 cell, helper cells that are critical to fighting pathogens. Bacterial testing determined that probably a filamentous microbe is anaerobic microorganism of the genus *Clostridium* [214].

Of concern is the fact that Th17 cells can promote autoimmunity. It was found that T-regs can suppress an immune response. What was also learned was the fact that *Bacteriodes fragilis* is capable of inducing T-reg production in the gut [213,214]. It seems relevant that certain intestinal flora can affect the balance of the intestinal tract, as well as release factors important in the regulation of the immune system in the gut [214]. This new information may provide a better understanding of autoimmune diseases [213,214]. *Prevotella* is also a prominent microorganisms in the intestines and has a role in reducing inflammation in the gut [215,218].

Various Receptors

The body has a vast variety of cells, but even though each cell may be unique in its self, all cells must be able to be able to communicate with its neighbors, as a well as outside environmental influences, such as viral, bacterial, fungal, or by the presence of parasites. Our cells must be able to communicate, and they do this by the presence of receptors on their surface. These receptors can have a great bearing on the vitality of cells, ability to recognize foreign antigens, as well as to promote damage, inflammation, or cellular repair [232,37].

According to the AM Piccinini., *et al.* toll-like receptor 4 are (TLR4) found on the surface of macrophages, and may engage in their "classic pathogen-killing macrophage state," when exposed to endotoxin. However, when a fragment of tenascin-C is used, macrophages can be induced to perform in "tissue repair mode." Prior to the current time, it was not understood how the same ligand could function, one in a pathogen killing mode, and yet also induce tissue repair [37,317].

Albert-Ludwigs-Yniversitat Freiburg noted that T-cells may also posess receptors, that are able to "switch their conformation, and this can be seen in the fact that cholesterol can bind inactive receptors, and in doing so, present a immune response. Or it can trigger a conformation change that will allow attack on any foreign agent [317]. Other Toll-like receptor agonists that are similar in function to invasive bacteria, may try to trick the immune system, into attacking cancer cells. This is because they seem to be able to work in combination with the chemotherapeutic agent cyclophospamide and TLR agonists, and help in the "activation of monocytes" [319].

Mixed Epitopes

An interesting aspect when antigens are processed, instead of only generating one type of epitome, the fragments are expressed with the histocompatibility complex on infected cells, and then they may also be snipped together, in order to form new totally different epitopes [318]. These newly discovered epitopes should provide a greater understanding of biochemically, and genetically how the immune system functions, as well as better insight for immune therapy for treating autoimmune disease, and cancer treatment [318]. Finally, other therapies that include a modified experimental chemical called DON (6 diazo-5-oxo L norleucine) may be a way to treat brain cancers, since this chemical is able to enter the blood-brain barrier. It is also able to block the ability of tumors to actively acquire nutrients in the environment, as well as blocking the glutamine pathway, used for building proteins [320]. It appears that microbes are also important in the regulation of glucose metabolism through "cross-talk," where improved glucose tolerance was correlated to higher levels of the organism Akkermanisa mucinipila in conjunction with lower levels of a type of interferon called IFN-y [321].

As of the year 2000, we have entered a new era using the similar coding methods from the Colosus Program of World War II (1944), to similar ideas, to making microbes do the computing for us (1944). Today not only do we use the ones and zeroes from computers of today to do our bidding, but today scientists are able to tell cells how to transcribe the message from the programs (proteins), we want and need. This can be done by inserting pairs of genes that act at the very molecular level, in this way they serve much like a circuit, which can turn particular genes on and off (Boolean operation, and, or, not, or xor) in a similar fashion much the way computers function today [315].

These operations may seem sinister from the onset, but a deeper understanding of the value of such manipulations, will provide greater incite not only how our cells, and genes function, but also will they provide a method for greater understanding how natural toxins, and man made toxins affect our cells and body, by flagging chemicals as they enter the body. Microorganisms may also be programmed to travel throughout our body, in order to detect and either activate or calm down key agents like macrophages in our immune system, or defeat the enemy there. Engineering microbes may make it possible to transfer chemotherapeutic agents for treating cancer, autoimmune diseases, by either turning genes off on or off the proteins they form, as well as agents that will cross the blood-brain barrier [315,325].

Microbiology is not stagnant but is actively growing exponentially drawing from new advances in computer coding, chemical methods, at the cellular level, in microbial genetics, microscopes (detail at atomic level), cell culture and culture methods, as well as immunological methods. These advancements have lead to a better understanding how cells communicate with each other, and to various microbes, shorter times for patient recovery. Due in part to better microbial identification, better methods for culturing microorganisms, and with the discovery of newer and more affective chemotherapeutic agents. This has lead to better therapies for the treatment of immunological disorders, autoimmune diseases, cancer and Alzhemier's disease. Microbiology will continue to lead in the treatment of infection disease, and will be on the forefront of new discoveries, for now and in the future [315,320-330].

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