GRAM-NEGATIVE BACTERIAL PATHOGENS

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I. INTRODUCTION

Gram-negative pathogens are now a major concern for scientists worldwide due to their ability to cause a wide range of illnesses, from mild to severe, which can be fatal if not treated promptly. Their high resistance to antibiotics complicates the treatment process. Therefore, this chapter introduces the morphology, biochemical properties, virulence factors, typical diseases, treatments, and preventive measures for the 10 most significant Gram-negative pathogens currently encountered in clinical settings, including: *Acinetobacter baumannii, Citrobacter spp, Enterobacter spp, Escherichia coli, Klebsiella pneumoniae, Morganella morganii, Proteus mirabilis, Pseudomonas aeruginosa, Serratia marcescens* and *Stenotrophomonas maltophilia.*

II. ACINETOBACTER BAUMANNII

1. **Morphological Description:** Acinetobacter species are Gram-negative aerobic coccobacillary bacteria. They are typically characterized as aerobic, non-lactose fermenting, non-fastidious, nonmotile, catalase-positive, and oxidase-negative (Bonomo, 2012).

2. **Phenotypic and Biochemical Properties:** Hospital medical diagnostic labs frequently use phenotypic methods to identify *A. baumannii* infections. These techniques include various differential biochemical assays, bacteriocin testing, serotyping, and biotyping. Commonly used biochemical tests include oxidase and catalase tests, the oxidative-fermentative (OF) test, the triple sugar iron (TSI) test, the motility test, Simmons' citrate test, the methyl red (MR) test, the Voges-Proskauer (VP) test, and measurements of bacterial growth at 37°C and 44°C. (Falah et al., 2019).

3. **Culture Conditions:** Sheep blood agar and MacConkey agar are the most popular growing media for identification of *A. baumannii.* It is believed that growth on MacConkey agar is facilitated due to the use of various organic carbon and energy sources. Standard laboratory media, such as Brain Heart Infusion (BHI) Agar and Trypticase Soy Agar (TSA), are also utilized for *A. baumannii* culture. Environmental

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strains of *A. baumannii* can withstand a range of temperatures. While thermophilic strains can live at 48°C, psychrophilic strains can withstand 4°C (Aryal, 2022).

4. **Molecular Identification:** Using PCR, it is possible to distinguish *A. baumannii* isolates from one another by recognizing the *blaOXA-like-51* carbapenemase gene. Global research has been done on *OXA*-TYPE genes, especially the *OXA-51* subtype, in *A. baumannii* isolates. Furthermore, a variety of molecular typing approaches are utilized, such as enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR), ribotyping, plasmid profile analysis, and pulsed-field gel electrophoresis (PFGE) (Falah et al., 2019).

5. **Virulence Factors:** The capacity of *A. baumannii* to elude the immune responses of the host, withstand drying out, and thrive in hostile settings all contribute to its pathogenesis. Because of its capsular polysaccharide, *A. baumannii* is resistant to rapid clearance by the innate immune system, which contributes significantly to its pathogenicity. This results in a high bacterial load, which in turn causes lipopolysaccharide (LPS) to interact with Toll-like receptor 4 (TLR4) to cause sepsis. Its survival and spread are further facilitated by antibiotic resistance (Aryal, 2022). Additionally, certain genes equip *A. baumannii* resistance to particular drugs. *Aac(3)* and *Aac(6')* are linked to resistance to aminoglycosides, whereas *blaTEM-92*, *blaSHV*, and *blaGES-11* offer resistance to beta-lactams. Some genes, including *tet(39)* and *tet(A)*, are implicated in tetracycline resistance (Kyriakidis et al., 2021).

6. **Diseases and Treatments:** *Acinetobacter baumannii* may cause infections in wounds as well as in the bloodstream, urinary system, and lungs, which can result in pneumonia. Furthermore, it can continue to colonize the body of patients as a colonizer, thriving there without spreading illness or exhibiting symptoms, especially in open wounds or respiratory secretions like sputum. Pneumonia obtained in hospitals is more common in long-term patients and those who have come into touch with contaminated ventilators or other respiratory equipment (Howard et al., 2012).

   Because of its hydrophobic characteristics, *A. baumannii* can stick to foreign objects like the plastics used in intravascular devices. By neutralizing factor H, outer membrane protein A (*OmpA*) strengthens this adherence and aids the bacteria in avoiding the alternative complement pathway-mediated death. At the site of infection, *A. baumannii* secretes outer membrane vesicles carrying various virulence-related proteins, including catalase, proteases, phospholipases, and superoxide dismutase. These proteins cause tissue damage and expedite the local innate immune response. The polysaccharide capsule plays a critical role in preventing the host's innate immune system from phagocytosing the bacteria. Additionally, lipopolysaccharide (LPS) acts as a chemotactic agent, attracting inflammatory cells and triggering the release of cytotoxic agents (Aryal, 2022).

   The transmission may be through respiratory care supplies, contaminated ventilators, or intra-hospital transmission (Howard et al., 2012). Fever, productive cough, dyspnea, and pleuritic chest discomfort are among the symptoms, which can develop into acute respiratory failure (ARF) very fast (Brigo et al., 2022).

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The prevalence of isolates that are resistant to carbapenems has been steadily rising, even though these medicines have historically been effective in treating A. baumannii infections. Antibiotics that are available to treat infections caused by A. baumannii are limited. Combination therapies like colistin/imipenem or colistin/meropenem have been studied extensively as a therapeutic approach (Aryal, 2022). To treat *A. baumannii* infections, a number of novel treatments have been developed, including radioimmunotherapy, bacteriophage therapy, and bactericidal gene transfer therapy (Howard et al., 2012).

7. **Prevalence and Prevention:** The prevalence of this bacterium varies from reports to reports. In one study, *Acinetobacter baumannii* strains were found in 114 out of the 59,483 clinical samples that were examined (Ahmad et al., 2023). Blood made up the bulk of these samples, with sputum, wound swabs, and bone marrow coming in second and third. With an overall risk ratio of 0.669, *A. baumannii* was found in 52 males (67.53%) and 28 women (75.67%) (Ahmad et al., 2023).

   Preventative measures and vigilant monitoring of the hospital environment for prompt and precise detection of *Acinetobacter* spp. are the best ways to preventing nosocomial infections from spreading, especially among burn patients. Adequate control measures should then be implemented (Falah et al., 2019). Due to the poor immunogenicity and propensity of subunit vaccines for in vivo degradation, none of the potential *A. baumannii* subunit vaccine candidates have advanced to the clinical trial stage as of yet (Yang et al., 2023).

References: Bonomo (2012), Falah et al. (2019), Aryal (2022), Kyriakidis et al. (2021), Brigo et al. (2022), Howard et al. (2012), Ahmad et al. (2023), Yang et al. (2023).

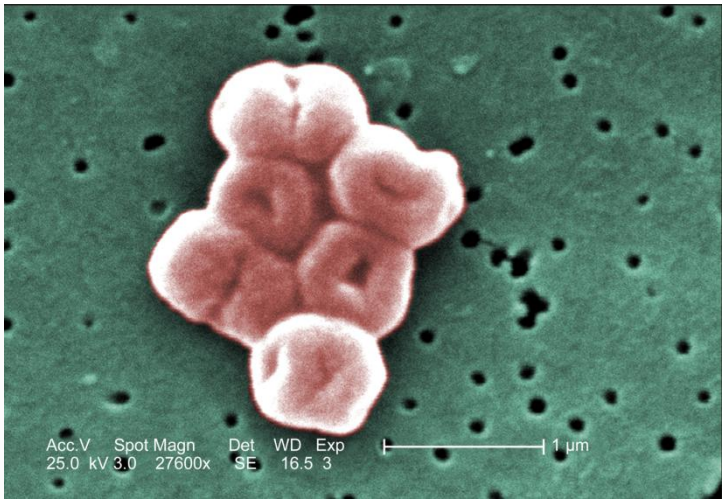

Figure 1: *The digitally colorized scanning electron microscopic (SEM) of Acinetobacter baumannii bacteria.*

Source: Public Health Image Library, Center for Disease Control and Prevention, Janice Haney Carr, 2004.

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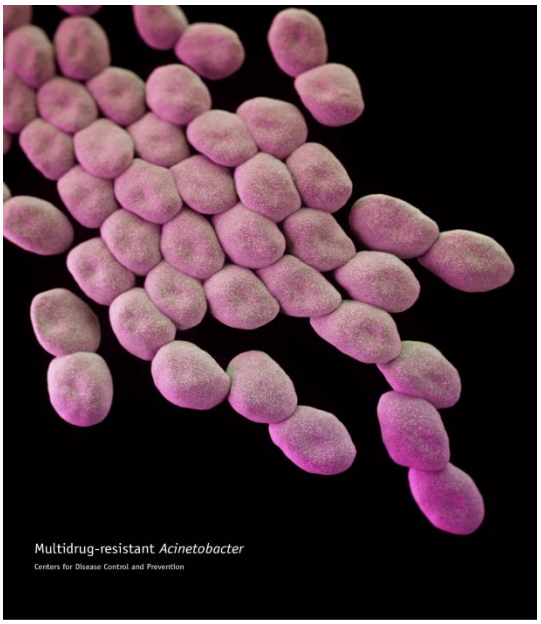

Figure 2: *The computer-generated image of Acinetobacter sp. bacteria.*

Source: Public Health Image Library, Center for Disease Control and Prevention, James Archer, 2013.

III. CITROBACTER SPP

1. **Morphological Descriptions:** Gram-negative facultative anaerobes include species of *Citrobacter*. They appear like bacilli under a microscope, usually with a diameter of 1.0 micrometers and a length of 2.0–6.0 micrometers. Endospores are not produced by *Citrobacter* species, which are usually motile by peritrichous flagella (Kus, 2014).

2. **Phenotypic and Biochemical Properties:** They show signs of catalase positivity, oxidase negativity, and low lysine decarboxylase activity. When D-glucose is fermented by these bacteria, gas, and acid are produced. Their capacity to ferment lactose varies, but almost everyone can produce beta-galactosidase. The Voges-Proskauer test provides negative findings, but the methyl-red test yields positive results (Kus, 2014).

3. **Culture Conditions:** Red Violet Bile agar is suggested to be a selective medium. Together with that, some nutritive culture media is used including TSB, Mossel EE broth, etc (De Jesús Cortés-Sánchez et al., 2023).

4. **Molecular Identification:** DNA sequencing and DNA-DNA hybridization tests were among the techniques used in the past to classify bacteria according to their biochemical, metabolic, and antigenic properties. The recommended technique for categorization is now the *16S* ribosomal RNA gene sequence analysis (Kus, 2014).

5. **Virulence Factors:** Different virulence factors have been gained by *Citrobacter* species, such as the capacity for invasion, colonization, biofilm formation, and toxin generation. Iron absorption and the production of flagellar apparatus are two important virulence factors in *C. koseri*. For the VI capsule polysaccharide, however, *C. freundii* and *C.*

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braakii have the necessary genes. Tad pilus, type IV pilus, and the flagellar apparatus are exclusive to *Citrobacter* species, whereas other secretion systems are present in just some strains. Shiga-like toxins, heat-stable toxins, or virulence islands are present in a number of *C. freundii* strains. Furthermore, their pathogenic potential is further increased by the inclusion of three virulence genes: *hcp*, *msgA*, and *rtx* (Jabeen et al., 2023). Additionally, there have been reports of quinolone resistance genes mediated via plasmids, such as *qnr* and *aac(6')-Ibcr*, in *Citrobacter* species (L. Liu et al., 2017).

6. **Diseases and Treatments:** Lots of *Citrobacter* species. strains are acknowledged as important pathogens, linked to gastrointestinal system infections, meningitis, folliculitis, hives, and other serious illnesses such as urinary tract infections (UTIs). Despite having a broad pharmacological resistance, *Citrobacter* spp. are amenable to cefoperazone/sulbactam, piperacillin/tazobactam, colistin, imipenem, tigecycline, and meropenem therapies. Additionally, the use of silver nanoparticles in medicine has demonstrated promise, and combining bacteriophages with antibiotics has therapeutic potential (Jabeen et al., 2023).

   Person-to-person transmission of *Citrobacter* is more common, although it can also be transmitted by direct contact with medical personnel, mother-to-child transmission, or intake of contaminated environmental sources (Public Health Agency of Canada, 2012).

7. **Prevalence and Prevention:** It has been noted that 2.1% of Indian populations are known to harbor *Citrobacter* spp. With 32.7% of cases affecting females and 67.3% of cases affecting men, *Citrobacter* spp. appear to be an infectious agent that may affect people of different ages (Nayar et al., 2014). The current work used proteome subtraction to assess *Citrobacter freundii*-related proteins for creating a new peptide- and mRNA-based vaccination. The vaccinations that have been developed are nontoxic, nonallergenic, and highly antigenic. Furthermore, predictions were made regarding the secondary and tertiary structures of vaccines (Naveed et al., 2022).

References: Jabeen et al. (2023), Kus (2014), Nayar et al. (2014), De Jesús Cortés-Sánchez et al. (2023), L. Liu et al. (2017), Public Health Agency of Canada (2012), Naveed et al. (2022).

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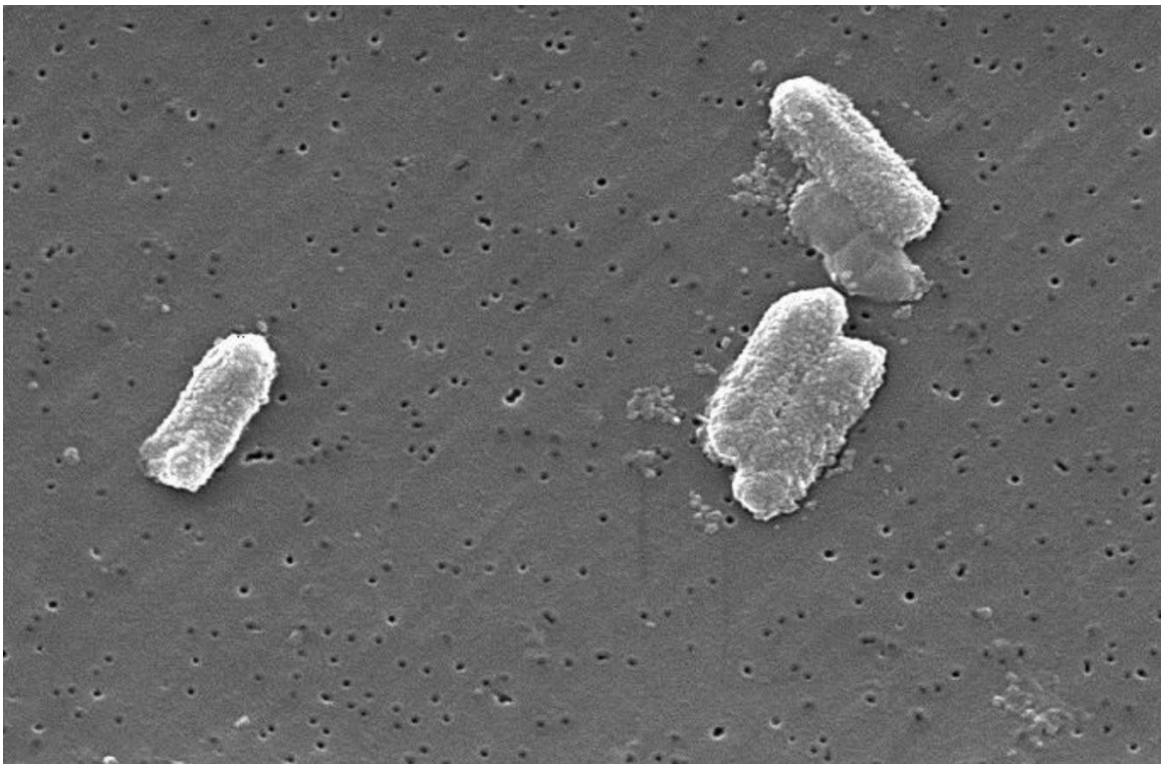

Figure 3: *The scanning electron microscopic (SEM) image of Citrobacter freundii bacteria.*

Source: Public Health Image Library, Center for Disease Control and Prevention, Pete Wardell.

IV. ENTEROBACTER SPP

1. **Morphological Descriptions:** Gram-negative, rod-shaped, facultatively anaerobic bacteria make up the genus *Enterobacter*, which is a member of the *Enterobacteriaceae* family. Their non-spore-forming behavior, flagella, urease positivity, and lactose fermentation capabilities define them (Ramirez & Giron, 2023).

2. **Phenotypic and Biochemical Properties:** *Enterobacter* and the closely related *Klebsiella* bacteria can be distinguished from one another by certain biochemical features such as the creation of the enzyme ornithine decarboxylase and motility (Rogers, 2024). Many biochemical assays, such as lysine and ornithine decarboxylase, citrate consumption, hydrogen sulfide generation, urease activity, tryptophan deaminase, indole synthesis, and others, were used to identify *Enterobacter* spp (Assouma et al., 2023).

3. **Culture Conditions:** The selective media such as Blood agar, MacConkey agar, Tryptic Soy Broth (TSB), nutrient agar, and Eosin methylene blue agar (EMB) medium are used for culturing. Samples can be incubated at 37°C for a period of 24 to 48 hours (Mirzaei et al., 2021).

4. **Molecular Identification:** The analytical methods included light microscopy, *16S rRNA* gene sequencing, and biochemical tests. These biochemical investigations included organic acid measurement in liquid cultures after incubation, antagonistic interaction

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assays, and preliminary evaluations of qualitative P and K solubilization, among other procedures (Roslan et al., 2020). Other suggested methods include internal transcribed spacers (ITS), enterobacterial repetitive intergenic consensus (ERIC) fingerprinting PCR analysis (Pontes et al., 2007).

5. **Virulence Factors:** Numerous virulence genes associated with urinary tract infections (UTIs) are found in *Enterobacteria* species, such as *pap*, *sfa*, *hly*, *cnf1*, and *iucD*. While *hly* and *cnf1* are crucial in intracellular survival and immune system evasion, *pap* and *sfa* genes help bacteria attach to cells (Assouma et al., 2023).

6. **Diseases and Treatments:** A wide range of illnesses, including eye and skin infections, meningitis, bacteremia, pneumonia, and urinary tract infections, can be brought on by pathogenic strains of *Enterobacter*. Because of rising drug resistance, the advent of *Enterobacter* species has challenged treatment efforts. Conventional treatments for infections caused by Enterobacter usually consist of a single antibiotic drug, such as imipenem, aminoglycosides, fluoroquinolones, or cephalosporins. Nevertheless, prolonged use of these drugs could promote the development of *Enterobacter* strains that generate beta-lactamases, which might result in the development of drug resistance, including resistance to carbapenems. There are now more advanced treatment paradigms available, such as the combination of beta-lactam antibiotics with aminoglycosides or fluoroquinolones (Ramirez & Giron, 2023).

   Enterobacter is present in surgical wounds, respiratory sputum, and blood samples from intensive care units (ICUs). *Enterobacter* spp. infections can cause symptoms similar to many other bloodstream infections, including fever, shock, hypotension, systemic inflammatory response (SIRS), and leukocytosis. A chest X-ray may also show signs including consolidations, coughing, and shortness of breath (Ramirez & Giron, 2023).

7. **Prevalence and Prevention:** While community-acquired infections are less prevalent, *Enterobacter* species are known to cause a large number of nosocomial infections. Out of the 2,645 clinical specimens that were examined, 297, accounting for 11.2% were found to be members of the *Enterobacteriaceae* family (Mirzaei et al., 2021). Reducing risk factors, including avoiding pointless medical equipment or overusing antibiotics, can aid in preventing Enterobacter infection colonization or worsening (Ramirez & Giron, 2023).

References: Ramirez and Giron (2023), Roslan et al. (2020), Mirzaei et al. (2021), Rogers (2024), Assouma et al. (2023), Pontes et al. (2007).

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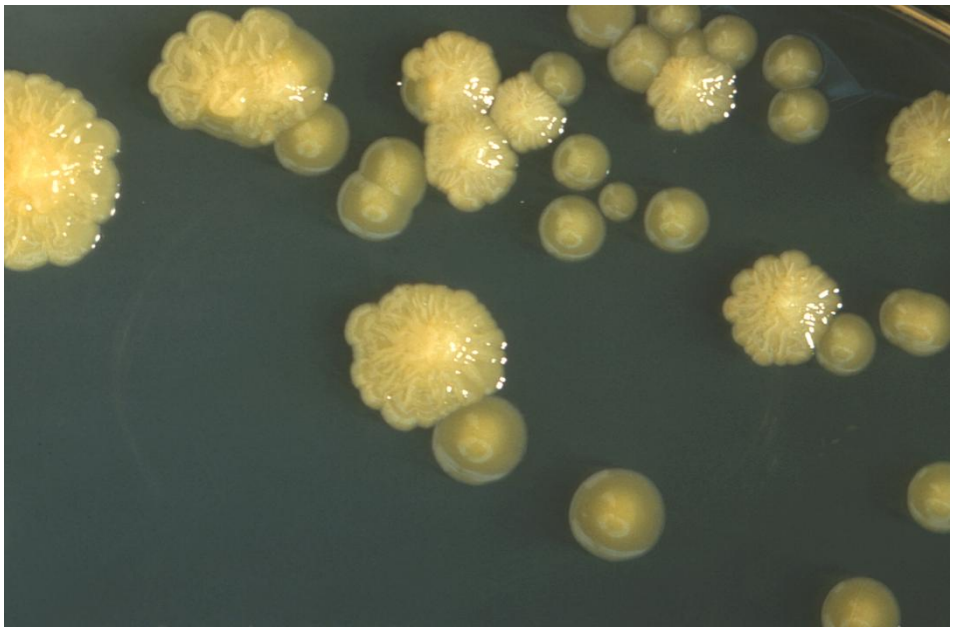

Figure 4: *A petri dish contained a growth medium of trypticase, inoculated with Enterobacter sakazakii bacteria.*

Source: Public Health Image Library, Center for Disease Control and Prevention, Dr. J. J. Farmer, 1978.

V. ESCHERICHIA COLI (E. COLI)

1. **Morphological Description:** *Escherichia coli* is generally a Gram-negative rod-shaped bacteria. Although these bacteria are mostly rod-shaped, they can also have spherical cells or longer, filamentous rods as their real form. Notably, *E. coli* frequently exhibits motility made possible by peritrichous flagella and does not generate spores. They may survive in conditions with lots of or little oxygen since they are facultative anaerobes. Apart from that, *E. coli* may ferment carbohydrates and produce gas as a byproduct in the production of acid and gas from lactose (Percival & Williams, 2014).

2. **Phenotypic and Biochemical Properties:** Both gelatinase and the capacity to hydrolyze urea are absent from *Escherichia coli*. Additionally, it does not deaminate phenylalanine, whereas the majority of strains are able to use sodium acetate and decarboxylate lysine ("Microbiology of Waterborne Diseases," 2014). Tests on *Escherichia* species reveal that they produce indole. They use pH-sensitive markers such as phenol red or methyl red to identify the mixed acids they produce during the fermentation of dextrose (D-glucose). *E. coli* is able to reduce nitrates. It is positive for catalase but negative for oxidase (Naser, 2016).

3. **Culture Conditions:** Because of its simple dietary requirements, *E. coli* can grow on both liquid and solid media in a laboratory setting. Its development is supported by common media such as Nutrient agar, MacConkey agar, and Eosin methylene blue agar (EMB) agar. 10℃ - 40℃ are the temperature ranges in which *E. coli* may grow, some lab strains can even multiply at temperatures as high as 49℃. Although it can live in a pH

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range of 4.5 to 9.5, a neutral pH of 7.0 promotes the best development (Basavaraju & Gunashree, 2023)

4. **Molecular Identification:** Numerous techniques, such as gram-staining, monitoring bacterial growth on different selective mediums, and biochemical testing, can be used to identify the bacterium. *16S rRNA* gene-based PCR and antimicrobial sensitivity can be used for conclusive identification and antibiotic susceptibility determination. Additional approaches that have been suggested are chromatography, fluorescence spectroscopy in conjunction with restriction endonuclease analysis, and different immunological techniques like sandwich or indirect ELISA, etc (Parveen et al., 2021).

5. **Virulence Factors:** Enterotoxigenic (ETEC), enteroinvasive (EIEC), enteropathogenic (EPEC), and Verocytotoxigenic (VTEC) are among the virulence categories shown by *E. coli* (Percival & Williams, 2014). These classes are allowed to cause diseases through some specific virulence features. Fimbrial and fimbrial adhesins, capsules, poisons, iron absorption systems, invasion plasmids, and colonization factors are some of the virulence elements that *E. coli* possesses and which all contribute to its pathogenic potential (Weintraub, 2007). Some virulence genes include papa, *sfa, hlyA, cnf*, etc (Basavaraju & Gunashree, 2023).

6. **Diseases and Treatments:** Numerous *E. coli* infections are prevalent, including bloodstream infections, prostatitis, urinary tract infections (UTIs), gastrointestinal infections, and pelvic inflammatory disease. While many *E. coli* strains cause only minor infections, some, including *Enterohemorrhagic E. coli*, can cause serious diseases, including kidney damage. These strains also produce Shiga toxin. People can get infected by eating infected foods, such as raw ground meats, unpasteurized dairy products, and contaminated fresh products like raw vegetables and sprouts (World Health Organization: WHO, 2018).

   The first sign of gastrointestinal *E. coli* infections is usually watery diarrhea, which is followed by cramps, stomach discomfort, lack of appetite, and low-grade fever. Pelvic discomfort is one of the symptoms of *E. coli*-caused urinary tract infections. The management of *E. coli* infections frequently includes the use of antibiotics such as Nitrofurantoin, Rifaximin, and Ciprofloxacin. The pathophysiology of *Escherichia coli* O157:H7 is attributed to the production of Shiga toxin, which causes hemorrhagic diarrhea by forcing intestinal mucosa cells to shed from the colon (Ameer et al., 2023).

7. **Prevalence and Prevention:** A worldwide incidence of *E. coli* infections estimated at 2.8 million cases per year was determined by analyzing datasets and studies from 10 of the 14 World Health Organization subregions (Ameer et al., 2023). 76.5% of patients with invasive *E. coli* disease were adults, making up the bulk of cases. Noticeably, 52.3% of these patients were infected in the urinary tract. In 77.4%, 65.3%, and 14.1% of patients, respectively, systemic inflammatory response syndrome, sepsis, and septic shock were noted. Furthermore, compared to individuals 60 years of age or younger, those older showed a greater chance of organ malfunction (Doua et al., 2023).

Controlling the spread of illness requires control measures to be put in place at every point of the food chain, from farm-based agricultural production to manufacturing, processing, and

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food preparation in commercial and residential kitchens (World Health Organization: WHO, 2018).

References: Basavaraju and Gunashree (2023), Percival and Williams (2014), Parveen et al. (2021), Ameer et al. (2023), Doua et al. (2023), Weintraub (2007), "Microbiology of Waterborne Diseases" (2014), Naser (2016), World Health Organization: WHO (2018).

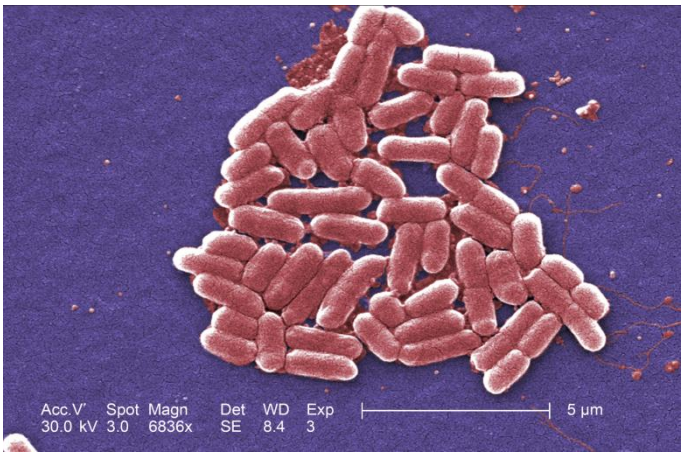

Figure 5: *The digitally colorized, scanning electron microscopic (SEM) image of Escherichia coli bacteria.*

Source: Public Health Image Library, Center for Disease Control and Prevention, Janice Haney Carr, 2006.

VI. KLEBSIELLA PNEUMONIAE

1. **Morphological Description:** *Klebsiella* is a facultative anaerobic gram-negative rod that is non-spore-forming and non-motile. These rods have a capsule and are usually organized singly, in pairs, or in short chains. 37°C is the ideal temperature for growth. These characteristics are also shared by *K. pneumoniae*, a member of the *Enterobacteriaceae* family, which is differentiated by its thin peptidoglycan layer (Vanhooren et al., 1999).

2. **Phenotypic and Biochemical Properties:** Biochemical assays that reveal negative indole, positive urease, variable MR, positive VP, positive Simmons' citrate agar, and nonmotility can be used to identify *Klebsiella pneumoniae* (Moini et al., 2015).

3. **Culture Conditions:** *K. pneumoniae* can be cultured on blood agar or MacConkey agar. *The* colonies on blood agar medium are big, mucoid, dome-shaped, and frequently merge together. On the other hand, colonies on the MacConkey medium have a big, mucoid, dark pink look, which is a sign of lactose fermentation (Moini et al., 2015).

4. **Molecular Identification:** It is advised to use PCR-based identification, which compares the results of *16S rDNA* sequencing to the NCBI database. To improve the identifying procedure, molecular phylogenetic analysis is also suggested (Yan et al., 2024).

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5. **Virulence Factors:** Consists of lipopolysaccharides (LPS), siderophores, capsules, and fimbriae. Significantly, among them, siderophores and tiny iron-binding metabolites are the most important (Abbas et al., 2024). Antibiotic resistance genes *gyrA*, *OqxB*, and *ParC* were shown to be present in the isolated Klebsiella pneumoniae strain using resistance gene studies. Additionally, three drug-resistant proteins were found by proteomic analysis include the modulator of drug activity B, the multi-drug resistant secretion protein, and the multi-drug resistant outer membrane protein *MdtQ* (Yan et al., 2024).

6. **Diseases and Treatments:** Urinary tract infections (UTIs), bloodstream infections, meningitis, intra-abdominal infections, and pyogenic liver abscesses are among the illnesses that are caused by *K. pneumoniae* infections. A *K. pneumoniae* infection is more common in those with underlying medical disorders, together with fever, nausea, bloody or hazy pee, and chest discomfort are possible symptoms. Therapy is difficult because of the thick capsule of the bacteria. Carbapenems, quinolones, and third- and fourth-generation cephalosporins are thought to be the best alternatives (Prince et al., 1997).

   The nosocomial transmission of *Klebsiella* to humans may be considerably influenced by the hospital environment, especially fomites. Furthermore, water and soil contamination also play a role in the spread of *Klebsiella* (Samanta & Bandyopadhyay, 2020). Similar symptoms to community-acquired pneumonia can be found in pneumonia caused by K. pneumoniae. Patients often experience fever, coughing, pleuritic chest pain, and shortness of breath (Ashurst & Dawson, 2023).

7. **Prevalence and Prevention:** In the one-year-period from 2019 to 2020, 152 cases of *K. pneumoniae* were found, 66.4% included adults, compared to 33.6% of pediatric patients. In addition, the infection rates in women were greater than in men (Hafiz et al., 2023). For prevention, it is imperative that visitors and medical professionals wash their hands. and make sure that gadgets are only ever used once (Ashurst & Dawson, 2023).

References: Abbas et al. (2024), Vanhooren et al. (1999), Yan et al. (2024), Moini et al. (2015), Prince et al. (1997), Hafiz et al. (2023), Samanta et al. (2020), Ashurst and Dawson (2023).

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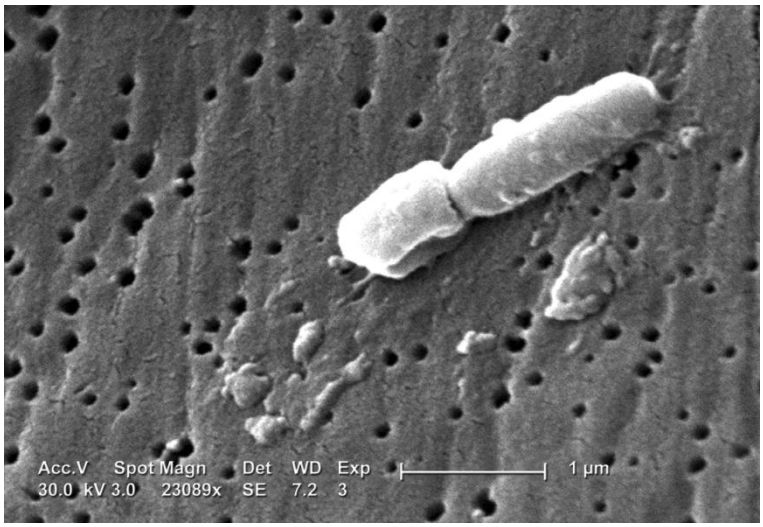

Figure 6: *The scanning electron microscopic (SEM) image at a magnification of 23089X of Klebsiella pneumoniae.*

Source: Public Health Image Library, Center for Disease Control and Prevention, Janice Haney Carr, 2005.

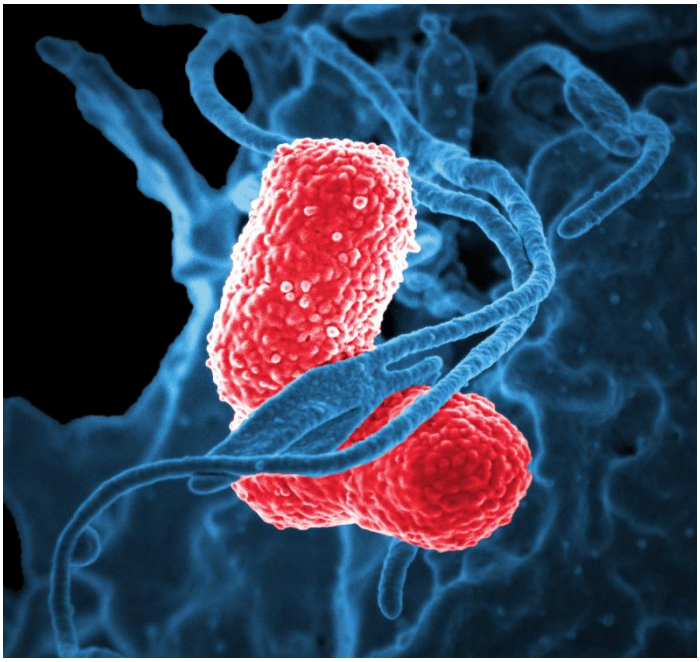

Figure 7: *The digitally colorized, scanning electron microscopic (SEM) image of the interaction between human white blood cells and Klebsiella pneumoniae.*

Source: Public Health Image Library, Center for Disease Control and Prevention, National Institute of Allergy and Infectious Diseases (NIAID), 2014.

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VII. MORGANELLA MORGANII

1. **Morphological Descriptions:** The facultative anaerobic gram-negative bacteria *Morganella morganii* is a rod-shaped, mobile, non-spore-forming, gram-negative bacterium that is frequently found in the oral cavity and intestines of people and animals as well as in a variety of environmental conditions (Holasoo et al., 2022).

2. **Phenotypic and Biochemical Properties:** To identify the bacteria, the isolates underwent a battery of biochemical assays, including the generation of hydrogen sulfide (H_2S), urease, catalase, oxidase, urease, lysine decarboxylase, and the fermentation of glucose, lactose, maltose, mannitol, sucrose, and xylose (Holasoo et al., 2022).

3. **Culture Conditions:** *M. morganii* can be cultivated for 24 hours at 37℃ on blood agar supplemented with 5% sheep blood and MacConkey agar (Holasoo et al., 2022).

4. **Molecular Identification:** DNA-DNA hybridization studies were previously used to classify *M. morganii*. Variations in the total G+C content set *M. morganii* apart from other members of the *Proteeae* tribe, even though they share identical genetic components (Liu et al., 2016). Moreover, the 16S rRNA gene is also used for identification (Holasoo et al., 2022).

5. **Virulence Factors:** *M. morganii* produces lipopolysaccharide, hemolysins, urease, IgA protease, and T3SS, among other virulence factors. Multiple antibiotic-resistant genes are present in certain clinical isolates of *M. morganii*, including aminoglycosides (*aphA6, aadA2*, and *rmtB*) and β-lactamases (*ampC, dha-1, blaNDM-1, blaOXA-48*, and *blaTEM-1*), etc (Alsaadi et al., 2024).

6. **Diseases and Treatments:** Numerous ailments, such as chorioamnionitis, cellulitis, sepsis, urinary tract infections (UTIs), pneumonia, and wound infections, are associated with infections caused by *M. morganii*. Symptoms of the illness include fever, dyspnea, coughing, and vomiting in patients. Carbapenems are often the cornerstone of treatment for bacteremia, with aminoglycosides, ciprofloxacin, and colistin serving as fallbacks. Surgical procedures are frequently employed as therapeutic approaches, in addition to procedures like drainage and tissue or line removal (Alsaadi et al., 2024).

   During birth, this infection can enter babies by vertical transmission from the genitourinary tract of the mother. Furthermore, *M. morganii* can enter human bodies through bites or scratches and can come from the oral bacterial flora of animals (Liu et al., 2016).

7. **Prevalence and Prevention:** Traditionally, *M. morganii* was thought to be an infrequent cause of nosocomial infections and was frequently deemed to be hardly pathogenic. However, as demonstrated by its incidence reaching 1.47% (1.219 out of 82.861 cases) in Changhua Christian Hospital, Taiwan, its significance has increased recently (Liu et al., 2016). Screening for rectal colonization by these organisms, strict handwashing protocols, and contact isolation for sick or colonized patients are some examples of preventive strategies.

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References: Liu et al. (2016), Alsaadi et al. (2024), Holasoo et al. (2022).

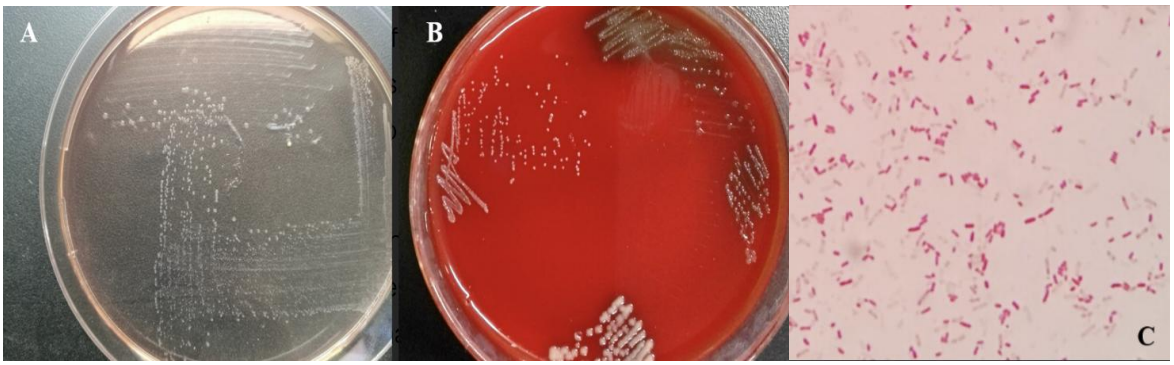

Figure 8: *Morphology of Morganella morganii isolates. (A) Morphology of M. morganii in MacConkey agar. (B) Colony morphology of M. morganii strains in blood agar. (C) Microscopic morphology of M. morganii strains (1000x).*

Source: Tian et al. (2020).

VIII. PROTEUS MIRABILIS

1. **Morphological Descriptions:** The gram-negative facultative anaerobe *Proteus mirabilis* is distinguished by its swarming motility and capacity for self-elongation. It is a member of the *Enterobacteriaceae* family and is capable of fermenting maltose but not lactose (Jamil et al., 2023).

2. **Phenotypic and Biochemical Properties:** *Proteus mirabilis* is a swarming motile bacterium that can cling to surfaces like IV lines, catheters, and medical equipment and release a polysaccharide that facilitates the adhesion and migration (Jamil et al., 2023).

3. **Culture Condition:** For isolating and diagnosing purposes, *P. mirabilis* can be grown on a variety of media, including Blood Agar Base, MacConkey agar, or LB media (Yasseen et al., 2019).

4. **Molecular Identification:** Numerous identification techniques have been used, including as serological testing, biochemical reactions, and the amplification or culture of the *16S rRNA*. Additionally, for the purpose of detecting *P. mirabilis* specifically, PCR-based assays that target certain genes like as *16S rRNA*, *ureC*, and *ureR* have been studied (Zhang et al., 2012).

5. **Virulence Factors:** Ureolytic, proteolytic, and hemolytic activity, swarming motility, and the presence of lipopolysaccharide - the main surface molecule that interacts with the host - are the main pathogenic features of *P. mirabilis* (Stankowska et al., 2008). The most often found gene among the β-lactamase genes was *blaTEM*, which was followed by *blaOXA*, *blaSHV*, *blaFOX*, *blaCIT*, *blaCTX-M1*, and *blaCTX-M9*, etc (Chinnam et al., 2021).

6. **Diseases and Treatments:** Particularly in hospitalized patients, *Proteus mirabilis* can cause wound infections, sepsis, pneumonia, renal failure, and struvite kidney stones.

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About 10 - 20% of *P. mirabilis* strains show resistance to ampicillin and first-generation cephalosporins, despite the fact that they are normally sensitive to most antibiotics with the exception of tetracycline and nitrofurantoin (O'Hara et al., 2000).

The primary method by which the bacterium spreads is through contact with infected materials or ill individuals. Furthermore, infections can enter the body through the digestive system, for example, by being in contaminated food. Because they are so mobile, the germs spread swiftly. They are able to enter the human urogenital system through the stomach or direct touch transfer (L&R Prevent and Protect, 2023).

Urinary urgency, suprapubic or back discomfort, small urine volume, black urine, or hematuria are typical signs of *P. mirabilis* infections. Patients may also have a fever, which may indicate a more serious ailment such as sepsis, bacteremia, or pyelonephritis (Jamil et al., 2023).

7. **Prevalence and Prevention:** Urinary tract infections (UTIs) are a common condition that primarily affects women between the ages of 20 and 50. *Proteus* is accountable for 1% to 2% of all UTIs. However, in hospital-acquired UTIs, their incidence increases to 5%. *Proteus* infection is associated with a higher risk of complicated UTIs, from 20% to 45% (Jamil et al., 2023).

P. mirabilis fimbriae has been the subject of many experimental vaccination trials. Considerable success has been seen in mouse models for both the MR/P fimbriae and the tip adhesin of the fimbria, MrpH. All the experimental vaccinations have not, however, been able to completely defend against infection, which means other targets need to be investigated (Armbruster et al., 2018).

References: Jamil et al. (2023), O'Hara et al. (2000), Chinnam et al. (2021), Zhang et al. (2012), Stankowska et al. (2008), Yasseen et al., (2019), L&R Prevent and Protect (2023), Armbruster et al. (2018).

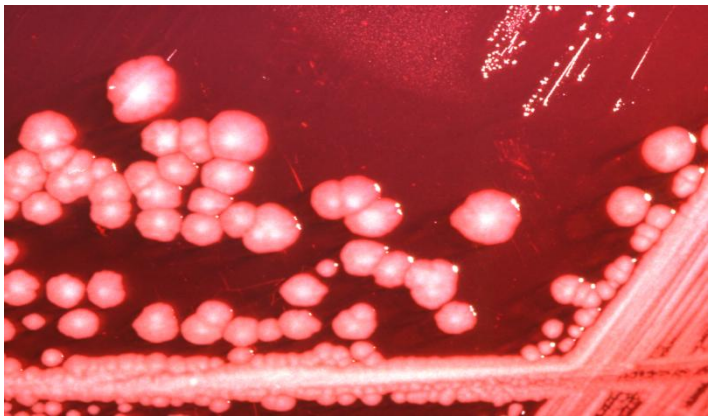

Figure 9: *The colonies of Proteus mirabilis bacteria are grown on a xylose-lysine-deoxycholate (XLD) agar plate.*

Source: Public Health Image Library, Center for Disease Control and Prevention, 1976.

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IX. PSEUDOMONAS AERUGINOSA

1. **Morphological Description:** Gram-negative *P. aeruginosa* cells are often shaped like rods and are commonly observed alone or in pairs. These cells release colors that dissolve in water and seep into the surrounding material. Each strain of *P. aeruginosa* is motile due to the insertion of a single flagellum at the tip of the cell. Certain strains have polar or subpolar flagella. Additionally, polar fimbriae, also known as pili in certain strains, have the ability to retract and function as phage receptors (Sapkota, 2023).

2. **Phenotypic and Biochemical Properties:** The gram-negative morphology, fruity odor, positive oxidase response, inability to digest lactose, and fluoresces under UV light are characteristics that distinguish *P. aeruginosa* (Iglewski, 1996).

3. **Culture Conditions:** *P. aeruginosa* has simple dietary requirements and may grow in a variety of mediums, including Cetrimide Agar, Nutrient Agar, and BHI Broth medium, that provide acetate as a carbon source and ammonium sulfate as a nitrogen source. *P. aeruginosa* colonies usually belong to one of two types. The first kind consists of big, smooth colonies that resemble fried eggs and have flat borders and raised centers. On the other hand, the second kind is made up of convex, tiny, and rough colonies. Large colonies are usually formed by clinical isolates, while smaller colonies are usually produced by isolates from natural sources (Sapkota, 2023).

4. **Molecular Identification:** For the detection and construction of a phylogenetic tree of *16S rRNA*, PCR, and DNA sequencing are advised. It is also recommended to compare local isolates of *P. aeruginosa* with NCBI-Genbank data (Shaebth, 2019).

5. **Virulence Factors:** *P. aeruginosa* is known to harbor three main classes of virulence factors. The first one is those that promote attachment and motility like flagella, pili, and lipopolysaccharides. The second type aids in colonization such as Exotoxin A, elastase, pigments like pyocyanin and pyoverdin, as well as enzymes like alkaline protease. The third type is those that promote chronic infection, including iron acquisition mechanisms, alginates, and biofilm formation (Sapkota, 2023). The proteins *OprI* and *OprL*, which are essential for the interactions between *P. aeruginosa* with its surroundings and innate antibiotic resistance, are present in its outer membrane. Additionally, these proteins found on the outer membrane are connected to efflux transport mechanisms, which affects how permeable cells are (Shaebth, 2019).

6. **Diseases and Treatments:** Numerous illnesses, such as septicemia, cellulitis, pneumonia, ear infections, and urinary tract infections (UTIs), are caused by *P. aeruginosa*. Although *P. aeruginosa* infections are less common in people with healthy immune systems, they can nevertheless be rather serious in immunocompromised people. Cefiderocol, meropenem-vaborbactam, or imipenem-cilastatin-relebactam are possible treatments for *P. aeruginosa* infections (Reynolds & Kollef, 2021).

   Fevers, breathing problems, painful urination, murky or crimson urine, and pelvic discomfort are all signs of *P. aeruginosa* infections (Sapkota, 2023). Contamination of water, fruit, as well as contact with infected surfaces or equipment, or transferred between persons through skin and hands contact can all result in transmission (AaronM, 2023). One distinctive characteristic of *P. aeruginosa* is its ability to instigate severe invasive

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infections while dodging the defenses of immune system, leading to chronic diseases. During the infection phase, P. aeruginosa uses several virulence factors to help cause tissue damage, invasion, and dissemination (Sapkota, 2023).

7. **Prevalence and Prevention:** *P. aeruginosa* is the most common cause of ventilator-associated pneumonia (VAP) worldwide, accounting for 10%-20% of cases, which has death rates ranging from 32% to 42.8%, according to multicenter observational research. One study that looked at 28 different intensive care units (ICUs) in the United States found that *P. aeruginosa* was the cause of 11% of all HAP and VAP cases among ICU patients. Moreover, *P. aeruginosa* is a frequent cause of nosocomial urinary tract infections (UTIs), especially catheter-associated UTIs (CAUTIs), which can account for up to 16% of UTIs in intensive care unit patients and around 10% of all CAUTIs (Reynolds & Kollef, 2021).

To avoid infections, upholding equipment and patient hygiene should be aware. Furthermore, it has been demonstrated that using topical antibacterial medicines for burns dramatically lowers the risk of *P. aeruginosa* infections (Sapkota, 2023).

References: Iglewski (1996), Sapkota (2023), Shaebth (2019), Reynolds and Kollef (2021), AaronM (2023).

Figure 10: *The medical illustration of Pseudomonas aeruginosa.*

Source: Public Health Image Library, Center for Disease Control and Prevention, Jennifer Oosthuizen, 2019.

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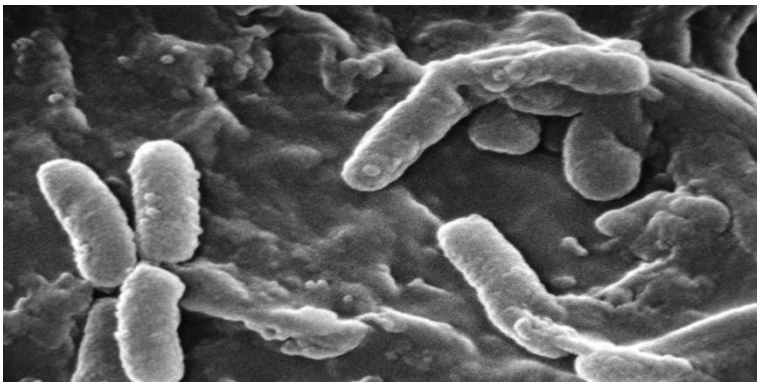

Figure 11: *The scanning electron microscopic (SEM) image of Pseudomonas aeruginosa.*

Source: Public Health Image Library, Center for Disease Control and Prevention, Janice Haney Carr.

X. SERRATIA MARCESCENS

1. **Morphological Descriptions:** These bacteria are rod-shaped and are not able to create endospores or move. Their typical dimensions are 0.5 to 0.8 μm in diameter and 1 to 2 μm in length, however growing circumstances might cause size variations. One characteristic that these creatures frequently exhibit is capsulation (Dahal, 2024).

2. **Phenotypic and Biochemical Properties:** Biochemical assays, such as the IMViC test, oxidase test, catalase test, urease test, triple sugar iron (TSI) test, Sulfur, Indole, Motility (SIM) test, and several carbohydrate fermentation tests, are performed on the isolated colonies (Dahal, 2024).

3. **Culture Conditions:** *Serratia marcescens* is cultivated on a variety of media, including blood agar, cetrimide agar, nutrient agar, tryptic soya agar (TSA), and MacConkey agar. Furthermore, chromogenic and selective media are available, including ChromID Serratia Agar, and Brilliance Serratia Agar, etc (Dahal, 2024).

4. **Molecular Identification:** Numerous techniques can be applied, including gene sequencing, PCR tests, and the evaluation of colony morphology in conjunction with biochemical analysis (Zaric et al., 2023).

5. **Virulence Factors:** Numerous virulence factors are present in *S. marcescens*, such as proteases, DNase, lipase, hemolysin, lipopolysaccharide, and different isozymes of alkaline phosphatase. Additionally, the swarming motility and capacity to build biofilms - both of which are controlled by quorum sensing mechanisms - increase its pathogenicity. (Horinouchi et al., 2010).

6. **Diseases and Treatments:** This microbe can cause a number of hospital-acquired infections, which puts immunocompromised people with underlying illnesses at higher risk. Patients using mechanical ventilation are therefore at risk for pneumonia. Furthermore, it may cause urinary tract infections (UTIs) in those who are catheter users. Additional possible infections include endocarditis, bacteremia or sepsis, and infections

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of the central nervous system, such as abscesses or meningitis. Infections with *S. marcescens* can cause erythema, fever, and edema. When treating *S. marcescens* infections, third-generation cephalosporins, and sometimes even fourth-generation cephalosporins are used in conjunction with carbapenems or aminoglycosides. When treating simple urinary infections, trimoxazole should be taken into consideration (Zaric et al., 2023).

Direct interactions, catheters, droplets, saline irrigation treatments, and other liquids that are thought to be sterile can all spread this microbe. Because of its virulence characteristics, *S. marcescens* may colonize, persist, and cause disease and tissue damage. Its overall pathophysiology may be divided into three stages: adhesion and colonization, biofilm formation, and infection development (Dahal, 2024). A *S. marcescens* infection can cause petechiae, cellulitis, headaches, hypotension, and chest discomfort (Kumar, 2022).

7. **Prevalence and Prevention:** According to data from a recent monitoring project conducted in the US and Europe, *Serratia* spp. is responsible for 3.5% of Gram-negative infections in patients who are not in the intensive care unit and 6.5% of all Gram-negative infections in ICU patients (Sader et al., 2014). *Serratia* is now the 7th most common cause of pneumonia and occurs popularly in the United States, followed by Europe, and Latin America (Jones, 2010).

Good hand hygiene, maintaining a clean environment, contact precautions, sterilization, wearing personal protective equipment, surveillance are all preventative measures against *S. marcescens* infections (Dahal, 2024).

References: Zaric et al. (2023), Jones (2010), Sader et al. (2014), Horinouchi et al. (2010), Dahal (2024), Kumar (2022).

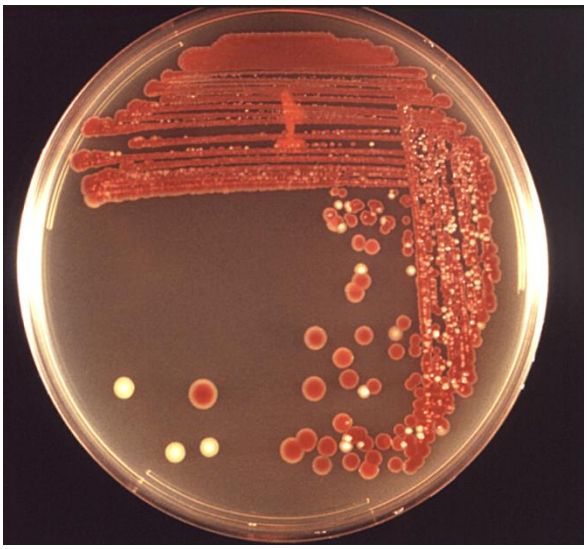

Figure 12: *The blood agar Petri dish with inoculated Serratia marcescens.*

Source: Public Health Image Library, Center for Disease Control and Prevention, Dr. Negut, 1973.

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XI. STENOTROPHOMONAS MALTOPHILIA

1. **Morphological Descriptions:** One gram-negative, rod-shaped pathogen that does not ferment glucose is called *Stenotrophomonas maltophilia*. Its aerobic nature, motility, and absence of spore production are its defining traits (Samonis et al., 2012).

2. **Phenotypic and Biochemical Properties:** These bacteria may be recognized by the presence of one or more polar flagella, which enable them to move. They also frequently form pigmented colonies that are yellow or yellowish-orange in color and show a negative oxidase response. Except for rhamnose and mannitol, *Stenotrophomonas maltophilia* usually acidifies sugars and has a tendency to be proteolytic (Wisplinghoff & Seifert, 2010). Oxidase-negative, catalase-positive, DNase-positive, lysine decarboxylase-positive, indole-negative, hydrogen sulfide-negative, and urease-negative are typical characteristics of this stringent aerobe. Maltophilia gets its name from the fact that it can create acid from maltose, albeit not usually from glucose (Said et al., 2023).

3. **Culture Conditions:** Standard culture mediums facilitate the rapid growth of *Stenotrophomonas maltophilia.* Its colonies are usually yellow-green in color on nutrient agar. The colonies are non-hemolytic, have a little lavender color, and smell like ammonia on blood agar. Because MacConkey agar does not digest lactose, the colonies on it are colorless. Imipenem, vancomycin, amphotericin B, and mannitol/bromothymol blue as an indicator are frequently found in selective medium, which has been demonstrated to be more successful in isolating *Stenotrophomonas maltophilia* from non-sterile samples (Said et al., 2023).

4. **Molecular Identification:** *S. maltophilia* grows well on a widely used medium, making colony monitoring and oxidase response detection easier (Samonis et al., 2012). Furthermore, suggested for precise identification are techniques like species-specific *23S* rRNA-directed PCR, matrix-assisted laser desorption ionization (MALDI-TOF) mass spectrometry, and nucleic-acid amplification testing (Said et al., 2023).

5. **Virulence Factors:** *S. maltophilia* has a number of strong virulence factors. Its ability to build biofilms is what primarily allows for colonization. Furthermore, it frequently exhibits resistance to several antibiotics and uses a variety of virulence exoenzymes, such as proteases, lipases, and elastase, for tissue invasion and host immune evasion. The bacteria may create small-colony variations in chronic infections, which would limit growth and increase stealth, making identification more difficult (Said et al., 2023).

6. **Diseases and Treatments:** Numerous illnesses have been linked to *S. maltophilia*, including wound infections, meningitis, bacteremia, respiratory tract infections, urinary tract infections (UTIs), endocarditis, and severe skin infections. The main treatment for *S. maltophilia* infections is trimethoprim-sulfamethoxazole. However, other choices such as tigecycline, minocycline, some novel fluoroquinolones, and ticarcillin-clavulanic acid are used as backup methods to reduce drug resistance. In cases of severe infections, combination therapy such as piperacillin with tazobactam, ticarcillin with clavulanic acid, or quinolones may be necessary as resistance can arise even after the first monotherapy (Safdar, 2015). In addition to patient-to-patient transmission, transmission can also happen through a variety of separate environmental factors (Said et al., 2023). Patients infected with *S. maltophilia* often suffer shortness of breath, dyspnea, cough, etc (Wang et al., 2020).

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7. **Prevalence and Prevention:** Studies have shown that 11% of people with cystic fibrosis (CF) have temporary colonization with *S. maltophilia*, which affects about 30% of these patients for 6 months (Wisplinghoff & Seifert, 2010). It is advised to maintain good hand hygiene, dispose of possibly contaminated solutions correctly, and handle and disinfect medical equipment carefully in order to prevent transmission. Also maintain water supplies, filter water, and use copper-silver ionization to disinfect plumbing systems (Said et al., 2023).

References: Wisplinghoff and Seifert (2010), Samonis et al. (2012), Safdar (2015), Said et al.(2023), Wang et al. (2020).

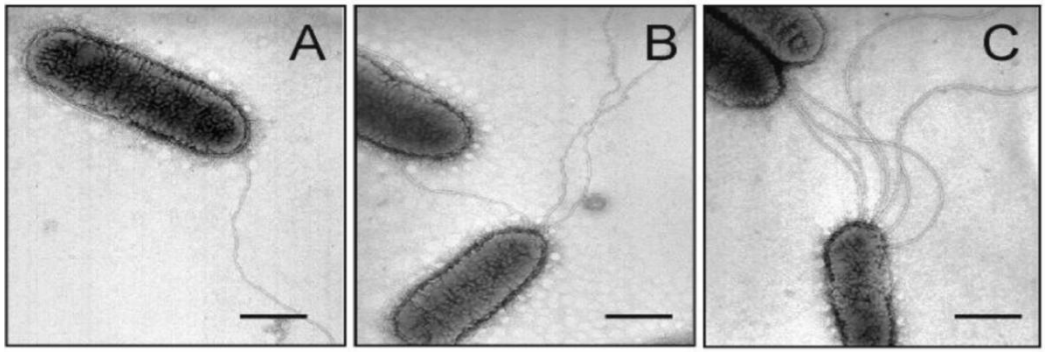

Figure 13: *The electron micrographs of S. maltophilia SMDP92. (A) S. maltophilia has one flagella. (B) S. maltophilia has many flagella.*

Source: De Oliveira-Garcia et al. (2002), Characterization of flagella produced by clinical strains of Stenotrophomonas maltophilia.

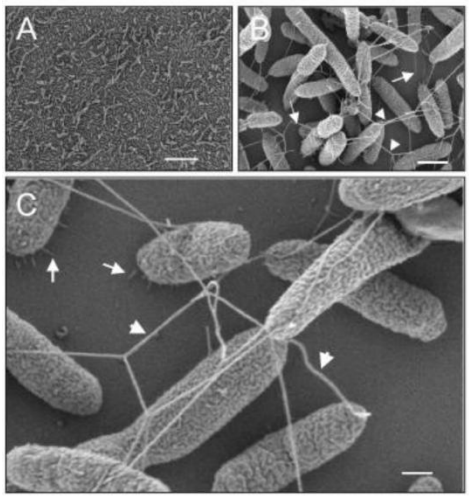

Figure 14: *The ultrastructural analysis of S. maltophilia adhering to plastic. (A) Scanning electron micrographs of the tight adhesion of S. maltophilia SMDP92 to the plastic surface. (B) Arrowheads highlight the structures that resemble flagella that appear to protrude and join bacteria, while arrows indicate the structures that connect the bacteria to the plastic surface. (C) Thin fibrillar structures connect bacteria to the abiotic surface.*

Source: De Oliveira-Garcia et al. (2002), Characterization of flagella produced by clinical strains of Stenotrophomonas maltophilia.

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