A Chapter on:

Understanding Wound Infections, Biofilm Formation, and Methods for Detection and Prevention

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I. ABSTRACT

Wound infection is growing challenge in healthcare settings, considering causes like microorganism resistance, chronic wound complexities, from contamination to systemic infection stages, influenced by wound traits, microbial species and patient factors. Biofilms, microbial communities surrounded by a matrix, are central to infections. Stages of adhesion, co-adhesion, maturation, and detachment define biofilm formation. Detection methods encompass microscopy and indirect assays. Preventive strategies, including disinfection and antimicrobial coatings, are essential. Overall, this work highlights wound infection understanding, biofilm roles, and their detection and prevention for effective healthcare management.

II. INTRODUCTION

Wounds in hospitals often result from accidental injuries, heat exposure, animal bites, and accidents with sharp tools. These wounds can be infected by microorganisms from the outside environment, especially during hospital stays. The rate of infected wounds is increasing as microorganisms become more resistant to anti-microbial agents. The signs of infected wounds include redness, swelling, tenderness, increasing pain, edema, purulent discharge, odor, localized fever or contact bleeding, and secondary signs like lack of healthy granulation, excessive exudate, and degraded wound.

The most common pathogens associated with wound infections include *Staphylococcus aureus* (20-40%) and *Pseudomonas aeruginosa* (5-15%). Other pathogens include *Enterococci* and members of *Enterobacteriaceae*, especially in immune compromised patients and surgery. Up to 80% of wound infections are biofilm-associated, with *Pseudomonas aeruginosa* (12-25%), *Staphylococcus aureus* (50-63%), and *Enterobacteriaceae* bacteria like *Escherichia coli*.

Wounds can be open or closed, and their condition can be interpreted based on wound characteristics such as slough, exudates, maceration, wound edges, infection, complications, and host factors. Wound infections are also related to patient comorbidities, such as diabetes, hypertension, old age, diminished immune function, obesity, and multiple chronic diseases, which can lead to wound chronicity and make treatment difficult.

III. WOUND INFECTION

Robert Koch was a pioneer in isolating pure cultures to identify infection causes. Infections can occur at any time, from community acquired infections within 48 hours of admission, and to hospital acquired infections or nosocomial infections. Infected wounds are localized defects or excavations of skin or soft tissue, with signs like erythema, swelling, pain, heat, and color. Secondary signs, such as lack of healthy granulation tissue, unhealthy color, excessive exudates, degraded wound bed, and a stalled healing trajectory, can also be detected.

Wound infection is a major cause of wound chronicity, which takes more than 4 weeks to heal. Wound infection occurs in three stages: contamination, colonization, local infection, and systemic infection. Contamination occurs when an infectious microorganism is accidentally introduced to a wound, and if the environment, nutrition, and physical conditions are favorable, it will not multiply or cause damage. Colonization occurs when the microorganism grows and divides without causing damage. Local infection occurs when the microorganism only affects the wound area, with signs like erythema, swelling, and pain. Systemic infection occurs when the infection spreads throughout the entire system. Factors influencing wound infection include wound etiology, dimensions, tissues, host factors, structural composition of poly-microbial species, and state of infection, such as planktonic or biofilm. Wound infections can be nosocomial and caused by common or opportunist pathogens.

IV. BIOFILM

Biofilms are microbial cells attached to surfaces and enclosed in a polysaccharide matrix. They are common in microbial infections, with 80% of infections resulting from biofilm formation. Biofilms form when microorganisms adhere to surfaces in a moist environment and reproduce. They can form on metals, plastics, natural materials, medical implants, and more. Biofilms can be formed by single or mixed species of bacteria, fungi, algae, yeasts, and other microorganisms.

Biofilm, produced by microorganisms, contains extracellular polymeric substances like proteins, DNA, polysaccharides, and RNA, and water for nutrient transport. It consists of a water channel and densely packed region.

Biofilm-forming bacteria exchange genes, activating stress genes, leading to resistant phenotypes due to changes in cell density, temperature, pH, and osmolality.

V. BIOFILM FORMATION

Biofilm formation involves three stages: adhesion, co-adhesion, and maturation and detachment. Adhesion occurs when bacteria reach a surface, while co-adhesion involves colonization and matrix synthesis. Maturation and detachment occur when microorganisms grow further, forming a mature biofilm. Potential biofilms include failure of antibiotic treatment, delayed wound healing, increased exudate/moisture, low-level chronic inflammation, erythema, poor granulation, and secondary signs of infection. Biofilms cause chronic infections through genetic changes, surface and excreted molecular messengers, physical barriers, and escape behaviors. Pathogenicity is retained and sometimes increased as bacterial concentration increases, and individuals tend to leave the biofilm. Biofilm formation depends on virulence factors, survival mechanisms, and the host immune response.

Wounds are poly-microbial, with bacteria from both exogenous and endogenous sources. Bacteria that multiply are not considered infectious unless they cause harm. Studies show that 75% of macroscopic wounds contain biofilm when antiseptic wound dressing is not used. In a pediatric hospital in India, 44% Pseudomonas aeruginosa produce biofilm, with half of Acinetobacter isolates producing multi-drug resistant biofilm.

Detection of Biofilm can be done by different methods:-

Detection of biofilms can be accomplished through various methods, both direct and indirect, depending on the desired level of detail and accuracy.

A. Direct Observation Methods:

1. Light Microscopy: This involves using visible light to observe biofilms. It can provide valuable information about biofilm structure and morphology but might not provide very high resolution.

2. Scanning Electron Microscopy (SEM): SEM allows for detailed imaging of the biofilm's surface structure by using focused electron beams. It provides high-resolution images and can reveal the three-dimensional architecture of the biofilm.

3. Transmission Electron Microscopy (TEM): TEM provides even higher resolution than SEM. It involves transmitting electrons through a thin section of the biofilm, enabling visualization of internal structures and details.

4. Confocal Laser Scanning Microscopy (CLSM): CLSM uses laser light to create detailed 3D images of biofilms. It can also be used to visualize specific components of biofilms, like different types of cells or EPS.

B. Indirect Methods:

1. Microtiter Plate Method: This is a quantitative method where biofilms are grown in microtiter plates. After growth, various assays can be performed to assess biofilm formation, including crystal violet staining and biomass quantification.

2. Congo Red Agar Method: This method utilizes Congo red dye to stain and visualize biofilm matrix components. Biofilm-producing colonies on Congo red agar appear as black or dark colonies due to the interaction of the dye with the EPS.

3. Tube Method: In this qualitative method, test tubes are filled with liquid medium, allowing biofilms to form at the air-liquid interface. Biofilm formation can be observed by changes in turbidity, adherence to the tube's sides, or the formation of a pellicle at the air-liquid interface.

Some of the direct methods are very expensive which cannot be used routinely. So indirect methods are preferred over them. Among the indirect methods Congo red agar method and tube methods are qualitative methods while microtiter method is quantitative method. These methods play a crucial role in understanding biofilm formation, structure, and characteristics. Choosing the appropriate method depends on the specific research goals, the nature of the biofilm, and the available resources. Combining multiple methods can provide a comprehensive view of biofilm properties.

VI. PREVENTIONS FOR WOUNDS INFECTIONS

Wound infection prevention involves disinfecting skin with alcohol, chlorhexidine, or iodine, applying antiseptic agents, and applying a protective dressing. In acute traumatic wounds, cleaning and using antiseptic agents reduce infection risk. Tetanus prevention is recommended, and antibiotic prophylaxis is provided for high-risk wounds. Burn wounds should be cleaned and treated with antiseptic agents. In infected or heavily colonized chronic ulcers and wounds, systemic antibiotics and antiseptic agents are used, followed by wound cleaning and treatment of underlying causes.

VII. PREVENTION OF BIOFILM

Biofilm prevention involves preventing microbe growth, using antibiotics for prophylaxis, empirical treatment based on clinical diagnosis, definitive treatment based on clinical diagnosis, and preoperative antibiotics for surgical-related biofilm formation. Antimicrobial coatings, such as biocides and ion coatings, can prevent biofilm formation by interfering with immature biofilm attachment. Silver's antimicrobial property is limitedly used. To prevent microbial surface attachment, antibiotic agents and polymer chains are applied, and ozone is used as an oxidizing agent. Nontoxic agents like imidazole, indole, and sulfide peptides also inhibit biofilm formation.

VIII. CONCLUSION

Understanding wound infections in healthcare is crucial, as biofilms exacerbate them. Innovative detection and prevention strategies are needed, focusing on wound attributes, microbial elements, and patient variables. Effective detection techniques and preventive measures are essential for healthcare providers.

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