Association of Anaerobic Bacteria with Surgical Site Infections: A Review

[*1]Kumurya, A. S., [2]Yusuf, A.A. and [1]Umar, S.G.

[1]Department of Medical Laboratory Sciences, Faculty of Allied Health Sciences, College of Health sciences, Bayero University Kano, Nigeria

[2]Department of Medical Laboratory Services, Federal Medical Centre, Yola, Adamawa State, Nigeria

Abstract: Anaerobes are normally found within certain areas of the body but result in serious infection when they have access to a normally sterile body fluid or deep tissue. This paper reviewed and presents the current information on the relationship of anaerobic bacteria with surgical wound infections. Centre for Disease Control and Prevention (CDC) changed the term surgical wound infections to surgical site infections, classified the anaerobic bacteria on the basis of oxygen requirements, and classified the surgical wounds based on degree of endogenous microbial contamination. The paper also reviewed other risk factors associated with surgical wound infections such as patient factors, surgical factors, preoperative patient care, theatre factors and equipment factors. Laboratory diagnosis of anaerobic bacterial infection, specimen collection techniques for cultivation of the anaerobic bacteria and various anaerobic growth media used in bacteriology have also been reviewed. The use of some antibiotic prophylaxis before surgery, and some ways of preventing the surgical wound infections were covered

Key words: Anaerobiosis, bacteria, surgical, wounds, skin infection

INTRODUCTION

Anaerobic bacteria are microorganisms that do not require oxygen for growth and could possibly react negatively or may even die if oxygen is present (Keneth *et al.,* 2014). Anaerobic bacteria usually do not possess catalase; but some can generate superoxide dismutase which protects them from oxygen (Ryan, 2004). Anaerobic bacteria are widely distributed in nature in oxygen-free habitats (Beilman and Dunn, 2015). Anaerobic infection results from an imbalance between host defenses and infective microorganisms especially bacteria that are either introduced through endogenous or exogenous source (Noor and Khetarpal, 2019). The endogenous contaminations are from surgical wounds while the exogenous contaminations are from external sources; including factors like skin preparation, surgical sterility technique, operating theatre environment and so on (Osakwe *et al.*, 2014). This should be the most important parameter in assessing quality of surgical care in a health institution. Endogenous contamination is usually from the patient. It reflects the dose of anaerobic bacteria in the wound at the time of surgery (Russell *et al.*, 2010).

[1]*Corresponding Author: E-mail: askumurya.med@buk.edu.ng
Phone: +2347032153561

The Centre for Disease Control and Prevention (CDC) term associated with surgical procedures changed from surgical wound infections to surgical site infections in 1992 (Beilman and Dunn, 2015). CDC, 2016 defines surgical wound infection as infection that occurs within 30 days of surgical operative procedure and involves the skin, subcutaneous tissue and deep tissue of the incision site**.** These infections are classified into incisional, organ or other organs and spaces manipulated during surgery.

Anaerobic Infections in Surgical Wound Patient

Although anaerobic infections in the surgical patient are typically associated with procedures that involve the gastrointestinal tract, respiratory tract and other body sites, virtually any anatomic site can harbour anaerobic growth, provided that appropriate host and environmental factors are present (Hentges, 2013). Anaerobic infections, with few exceptions, are derived from the host's own endogenous flora. In a non-diseased state, these organisms represent a significant component of the normal flora that inhabits the mucosal surfaces of the gastrointestinal tract, playing a key role in preventing the colonization of exogenous (pathogenic) microbial populations. In addition, the intestinal anaerobes contribute to the relative homeostasis of the host through vitamin K production, deconjugation of bile acids, and other biotransformation processes (Hentges, 2013). In order for these normally commensal microbial populations to produce disease in the host, there must be some structural or functional alteration of their normal ecological habitat.

This may occur through disruption of mucosal barriers, obstruction of regional vascular supply, organic or mechanical obstruction of gastrointestinal transit, surgical site or other disease processes that compromise the oxidation-reduction potential within the tissues. Once the mechanisms that normally prevent anaerobic bacteria from producing disease within their native environment have been compromised, selected anaerobic populations would be free to express several well-documented virulence factors (Beilman and Dunn, 2015).

Numerous virulence mechanisms facilitate the pathogenesis of anaerobic infections. For, example after a penetrating injury or surgical site to the gastrointestinal tract and others anatomical site of the body, anaerobic populations quickly adhere to the serosal mesothelium lining the peritoneal cavity with such tenacity that multiple mechanic allavages will fail to dislodge them from the surface (Edmiston *et al.*, 2011). A second important virulence mechanism, which has a profound impact on the pathogenesis of intra-abdominal infection, is the ability of the selected encapsulated strains of *Bacteroides fragilis* to resist clearance from the peritoneal cavity, stimulating a series of cellular events, which leads to an influx of polymorphonuclear leukocytes into the site of infection and eventually promotes abscess formation (Gibson *et al.*, 2015). A third virulence mechanism that is often exhibited in an anaerobic infection is the elaboration of toxin or enzymes, which causes widespread tissue damage. Several anaerobic bacteria, including *Bacteroides, Clostridium, Fusobacterium,* and selected strains of *Peptostreptococcus,* have demonstrated the ability to produce toxins or enzymes, influencing the pathogenesis of the infection (Beilman and Dunn, 2015).

Classification of Anaerobic Bacteria on the Basis of Oxygen Requirements

On the basis of oxygen requirements, bacteria can be divided into following different categories:

a.) Obligate anaerobes (Strictly anaerobes): These bacteria need an oxygen free environment to grow and live. They cannot grow in a place with oxygen as it may damage and destroy them (e.g. *Clostridium tetani* and *Clostridium botulinum*).

b.) Facultative anaerobes: They are capable of growth under both aerobic and anaerobic conditions. They use fermentation to grow in places without oxygen, but use aerobic respiration in places with oxygen (e.g. *Staphylococcus aureus*).

c.) Aerotolerant anaerobes (moderate anaerobes): Are anaerobic bacteria that are not killed by exposure to oxygen, do not need oxygen to survive, but can exist in its presence for a period of time (e.g. *Clostridium perfringens*).

Surgical Site Infections

Surgical site infection is an infection that occurs within 30 days of surgical operative procedure, and involves the skin, subcutaneous tissue and deep tissue of the incision site and at least one of the following: purulent discharge from the superficial incision; microorganisms isolated from an aseptically obtained culture of fluid or tissue from the superficial incision; at least one of the following signs or symptoms of infection: pain or tenderness, localized swelling, redness or heat and superficial incision is deliberately opened by a surgeon, unless culture of incision is negative; diagnosis of superficial incisional site infection by the surgeon or clinician (Beilman and Dunn, 2015). Deep incisional surgical site infections must meet three criteria which are: it must occur within 30 days of surgical procedure or one year in the case of implants; must be related to the procedure; must involve deep soft tissues, such as the fascia and muscles plus at least one of the following criteria: purulent drainage from the incision but not from the organ/space of the surgical site; a deep incision spontaneously dehisces or is deliberately opened by a surgeon when the patient has at least one of the following signs or symptoms, fever (>38°C), localized pain or tenderness unless the culture is negative; an abscess or other evidence of infection involving the incision is found on direct examination or by histopathologic or radiological examination; diagnosis of a deep incisional surgical site infection by a surgeon or attending physician (Oliveira *et al.*, 2010).

Classification of Surgical Site Infections

A system of classification for surgical wounds based on degree of endogenous microbial contamination was developed by the United States National Research Council Group in 1964 (Russell *et al.*, 2010). Four wound classes with an increasing risk of surgical wound infections were described as clean, clean-contaminated, contaminated and dirty. According to Bandaru *et al*., 2012, the simplicity of the system of classification has led to its usage to predict the rate of surgery infection.

i.) Clean Wounds: Clean wounds are elective, non-traumatic and primarily closed wounds without acute inflammation and breaks in aseptic techniques, respiratory, gastrointestinal, biliary and genitourinary tracts. Infection rate before and after antibiotic prophylaxis is about 1-2%.

ii.) Clean-Contaminated Wounds: These wounds are usually urgent cases that are otherwise clean, elective opening of respiratory, gastrointestinal, biliary or genitourinary tract with minimal spillage, not encountering infected urine or bile; minor aseptic technique break and infection rate before antibiotic prophylaxis is about 30% for gastric surgeries and 20% for biliary surgeries whereas that after prophylaxis is less than 10%.

iii.) Contaminated Wounds: These wounds have non-purulent inflammation; gross spillage from gastrointestinal tract; entry into biliary or genitourinary tract in the presence of infected bile or urine; major break in aseptic technique; penetrating trauma <4 hours old; chronic open wounds to be grafted or covered and infection rate before antibiotic prophylaxis is up to 60% while it is 15-20% after prophylaxis.

iv.) Dirty Wounds: These wounds have purulent inflammation; preoperative perforation of respiratory, gastrointestinal, biliary or genitourinary tract; penetrating trauma >4 hours old and rates of infection before and after antibiotic prophylaxis are up to 60% and <40% respectively (Russell *et al.*, 2010).

Risk Factors of Surgical Site Infections

Apart from the degree of endogenous microbial contamination of wounds, other risk factors for surgical wound infections are classified secondarily into patient factors, surgical factors, preoperative patient care, theatre factors and equipment factors (Dalhatu *et al.*, 2014).

i.) Patient Factors: The patient factors include age (elderly and neonates), the nature of presenting clinical condition, concurrent disease (for example diabetes mellitus and malignancy), malnutrition and skin diseases (Louie *et al.*, 2013). The presenting clinical condition is usually assessed with the American Society of Anaesthesiologists (ASA) classification system (Dalhatu *et al.*, 2014). The ASA classifies every surgical patient into one of class I-V. ASA score > or = III is high risk. The classes are I – Normally healthy; II – Mild systemic disease; but with no limitation of activity; III – Severe systemic disease that limits activity but not incapacitating; IV – incapacitating systemic disease posing a threat to life; V – moribund, not expected to survive 24 h even with operation (Louie *et al*., 2013).

ii.) Surgical Factors: Surgical factors include operations in infected tissue, transplant surgeries or implant surgeries. They also include poor surgical technique, excessive use of diathermy, long duration of operation, haemorrhage, haematomas and use of drains (Louie *et al*., 2013). The occurrence of surgical wound infections is also affected by the type of suture used. Monofilament sutures like chromic, catgut and vicryl are better than multifilament sutures like silk and nylon in preventing surgical wound infections as the multifilament sutures may harbor bacteria and have wick-like action when used as skin suture (Dalhatu *et al.*, 2014).

iii.) Theatre Factors: Theatre factors include theatre staff with skin infections, unrestricted movement of staff, inappropriate use of theatre clothing, open containers of solutions (for example saline or disinfectants), inadequate operating theatre ventilation and simultaneous operations in the same room (Garibaldi *et al*., 2013).

iv.) Equipment Factors: Equipment factors include inadequate sterilization or disinfection, unclean surgical ward, prolonged preoperative stay; inadequate training of nursing and medical staff, inappropriate wound dressing techniques (Nwankwo *et al.*, 2012).

Some of the Pathogens Isolated From Surgical Site Infections

The primary determinant of bacterial pathogens in surgical wound is the procedure. In clean surgical procedures, *Clostridium tetani,* and *Staphylococcus aureus* are the main agent of infection. The polymicrobial aerobic and anaerobic flora closely resembling the normal endogenous micro flora are the most frequent pathogens isolated from other categories of surgical procedures including clean-contaminated, contaminated and dirty, (Dalhatu *et al.*, 2014).

Anaerobic infections are usually polymicrobial, although B. fragilis is the only bacterium identified to date that can cause abscess formation as the sole infecting organism. Anaerobic gram-positive cocci of clinical importance are found in three common genera *Peptostreptococcus*, *Gemella*, and *Streptococcus*. Enzymes and toxins are produced by certain genus like *Bacteroides*,

Clostridium, and *Fusarium*. The family *Actinomycetaceae* contains three potentially pathogenic genera: *Actinomyces*, *Nocardia*, and *Streptomyces* (Barie, 2017).

Laboratory Diagnosis of Anaerobic Bacteria

Anaerobes are normally found within certain areas of the body but result in serious infection when they have access to a normally sterile body fluid or deep tissue that is poorly oxygenated. Some anaerobes normally live in the crevices of the skin, in the nose, mouth, throat, intestine, and vagina. Injury to these tissues (cuts, puncture wounds, or trauma) especially at or adjacent to the mucous membranes allows anaerobes entry into otherwise sterile areas of the body and is the primary cause of anaerobic infection (Louie *et al.*, 2013). A second source of anaerobic infection occurs from the introduction of spores into a normally sterile site (Anderson *et al.*, 2008). Spore-producing anaerobes live in the soil and water, and spores may be introduced via wounds, especially during surgery or punctures. Anaerobic infections are most likely to be found in persons who are immunosuppressed, those treated recently with broad-spectrum antibiotics, and persons who have a decaying tissue injury on or near a mucous membrane, especially if the site is foul-smelling (Bello, 2012). The identification of anaerobes is highly complex, and laboratories may use different identification systems. Microorganisms are identified by their: colonial and microscopic morphology, growth on selective media, oxygen tolerance, biochemical characteristics which include sugar fermentation, bile solubility, esculin, starch, and gelatin hydrolysis, casein and gelatin digestion, catalase, lipase, lecithinase, and indole production, nitrate reduction, volatile fatty acids as determined by gas chromatography; susceptibility to antibiotics by the microtube broth dilution method (Anderson *et al.*, 2008)**.**

Specimen Collection for the Diagnosis of Anaerobic Infections

The keys to effective anaerobic bacteria cultures include collecting a contamination-free specimen and protecting it from oxygen exposure. Anaerobic bacteria cultures should be obtained from an appropriate site without the laboratory personnel contaminating the sample with bacteria from the adjacent skin, mucus membrane, or tissue. Abscesses or fluids can be aspirated using a sterile syringe that is then tightly capped to prevent entry of air. Tissue samples should be placed into a degassed bag and sealed, or into a gassed out screw top vial that may contain oxygen-free pre-reduced culture medium and tightly capped. The specimens should be plated as rapidly as possible (Wells and Wilkins, 2008).

Identification of Anaerobic Bacteria

i) Gram Stain of Anaerobic Bacteria

a.) Gram-positive anaerobes include the following:

- *Actinomyces* (head, neck, pelvic infections; aspiration pneumonia)
- *Bifidobacterium* (ear infections, abdominal infections)
- *Clostridium* (gas, gangrene, food poisoning, tetanus, pseudomembranous colitis)
- *Peptostreptococcus* (oral, respiratory, and intra-abdominal infections)
- *Propionibacterium* (shunt infections) (Barie, 2017).

b.) Gram-negative anaerobes include the following:

- *Bacteroides* (the most commonly found anaerobes in cultures; intra-abdominal infections, rectal abscesses, soft tissue infections, liver infection)
- *Fusobacterium* (abscesses, wound infections, pulmonary and intracranial infections)
- *Porphyromonas* (aspiration pneumonia, periodontitis)
- *Prevotella* (intra-abdominal infections, soft tissue infections) (Barie, 2017).

ii) Cultivation of Anaerobic Bacteria

An anaerobic bacteria culture is a method used to grow anaerobes from a clinical specimen. Obligate anaerobes are destroyed when exposed to the atmosphere for as briefly as 10 minutes. Some anaerobes are tolerant to small amounts of oxygen. Facultative anaerobes are those organisms that will grow with or without oxygen. The methods of obtaining specimens for anaerobic culture and the culturing procedure are performed to ensure that the organisms are protected from oxygen. It is crucial that the health care provider obtain the sample for culture via aseptic technique. Anaerobes are commonly found on mucous membranes and other sites such as the vagina and oral cavity. Therefore, specimens likely to be contaminated with these organisms should not be submitted for culture (throat or vaginal swab). Some types of specimens should always be cultured for anaerobes if an infection is suspected. These include abscesses, bites, blood, cerebrospinal fluid and exudative body fluids, deep wounds,

and dead tissues. The specimen must be protected from oxygen during collection and Cultures should be placed in an environment that is free of oxygen, at 35°C for at least 48 hours before the plates are examined for growth (Wells and Wilkins, 2008).

Most strict anaerobes require not only the absence of oxygen to initiate growth, but also a redox potential below -300mV, which can be only achieved by the supplementation of media with reducing agents (Wells and Wilkins, 2008). Below are some of the media used to culture anaerobic bacteria.

a.) Thioglycolate broth

Thioglycolate broth is a multi-purpose, enriched differential medium used primarily to determine the oxygen requirements of microorganisms. Sodium thioglycolate in the medium consumes oxygen and permits the growth of obligate anaerobes. This, combined with the diffusion of oxygen from the top of the broth produces a range of oxygen concentrations in the media along its depth. The oxygen concentration at a given level is indicated by a redox sensitive dye like resazurine that turns pink in the presence of oxygen. Reducing media chemically remove molecular oxygen (O_2) that might interfere with the growth of anaerobes. Thioglycolate combines with dissolved O_2 to deplete in media. The primary plating media for inoculating anaerobic specimen includes a nonselective blood agar and one or all of the following mentioned selective media (Hite *et al.*, 2011).

b.) Non-selective media used in anaerobic bacteriology:

1. Anaerobic blood agar: It is a nonselective medium for isolation of anaerobes and facultative anaerobes.
2. Egg-yolk agar (EYA): Nonselective for determination of lecithinase and lipase production by clostridia and fusobacteria.
3. Cooked meat broth: Nonselective for cultivation of anaerobic organisms; with addition of glucose, can be used for gas-liquid chromatography.
4. Peptone-yeast extracts glucose broth (PYG): Non-selective for cultivation of anaerobic bacteria for gas-liquid chromatography (Hite *et al.*, 2011).

c.) Selective and differential media used in anaerobic bacteriology:

1. Bacterioides bile esculin agar (BBE): It is selective and differential for *Bacteriodes fragilis* group and good for presumptive identification.
2. Laked Kanamycin-vancomycin blood agar (LKV): It is selective for isolation of *Prevotella* and *Bacteriodes* spp.
3. Anaerobic phenylethyl alcohol agar (PEA): Selective for inhibition of gram negative rods and swarming by some clostridia.
4. Cycloserine cefoxitin fructose agar (CCFA): selective for *Clostridium difficile*.
5. Thioglycollate broth: Non selective for cultivation of anaerobes; as well as facultative anaerobes and aerobes.

Special culture techniques for anaerobic bacteria

a.) Candle jar: A microaerophile is a microorganism that requires oxygen to survive, but requires environments containing lower levels of oxygen than are present in the atmosphere (20% concentration). Many microphiles are also capnophiles, as they require an elevated concentration of carbon dioxide. In the laboratory they can be easily cultivated in a candle jar. A candle jar is a container into which a lit candle is introduced before sealing the container's airtight lid. The candle's flame burns until extinguished by oxygen deprivation, which creates a carbon dioxide-rich, oxygen-poor atmosphere in the jar. Many labs also have access directly to carbon dioxide and can add the desired carbon dioxide levels directly to incubators where they want to grow microaerophiles. Candle jars are used to grow bacteria requiring an increased CO_2 concentration (capnophiles). Candle jars increase CO_2 concentrations and still leave some O_2 for aerobic capnophiles (Blaser, 2014).

b.) Gas pack: Gas packs can generate CO_2 also are generally used in place of candle jars. The packet consists of a bag containing a Petri plate and CO_2 gas generator. The gas generator is crushed to mix the chemicals it contains and start the reaction that produces CO_2. This gas reduces the oxygen concentration in the bag to about 5% and provides CO_2 concentration of about 10% (Blaser, 2014).

c.) Anaerobic jar: Petri plates can be incubated in an anaerobic jar or anaerobic chamber. Sodium bicarbonate and sodium borohydride are mixed with a small amount of water to produce CO_2 and H^+. A palladium catalyst in the jar combines with the O_2 in the jar and the H^+ to remove O_2 (Blaser, 2014).

d.) Biological method

Biological method can be used to establish anaerobic conditions. One half of the solid medium in the Petri´s dish is inoculated with the Petri dish is sealed with the wax or paraffin and cultured in aerobic environment (Blaser, 2014).

Use of Antibiotic Prophylaxis before Surgery

The use of antibiotic prophylaxis before surgery has evolved greatly in the last 20 years (Etok *et al.*, 2012). The choice of parenteral prophylactic antibiotic agents, timing and route of administration have become standardized on the basis of well-planned prospective clinical studies and it is generally recommended that in elective clean surgical procedures using a foreign body and in clean-contaminated procedures that a single dose of cephalosporin, such as cephazolin, should be administered intravenously by anaesthesia personnel in the operative room just before incision and additional doses are generally recommended only when the operation lasts longer than 2-3 hours (Bello, 2012).

Prevention of Surgical Site Infection

A revised guideline by Health Care Infection Control Practices Advisory Committee in 1999- for the prevention of surgical wound infection provides that the surgical team should be adequately trained on the rudiments of surgical asepsis and that surgical drains should not be used as an alternative to achievement of good haemostasis, with closed system of wound drainage where drainage inevitable (Haley *et al.*, 2014).

The operating theatre staff should be kept to the essential minimum and those staff with boil or septic lesion of the skin or eczema colonized with *Staphylococcus aureus* should not be allowed in the theatre, theatre clothes should not be worn in patient care outside the operating suite, the operating team should wear sterile gowns and gloves and their hands properly decontaminated by scrubbing (Garibaldi *et al*., 2013).

Mechanical ventilation of the operating theatre is recommended. If windows have to be left open, it is advisable to cover them with fly or insect-proof netting. Air conditioning systems should ensure that a minimum of 20 – 24 air changes per hour of filtered air is delivered and with correct design and good control of staff movement, the level of air-borne contamination would then be below 100 colony forming units tested sample, the second half is inoculated with *Serratia marcescens* - aerobic bacteria able to produce anaerobic environment by the consumption of oxygen. (cfu) per cubic meter during operations. Also it is now accepted that ultra clean air (<10cfu/m^3) reduces the risk of infection in implant surgery to achieve this, laminar flow systems (0.5m/s) which deliver about 300 air changes per hour has to be used (Prtak and Ridgway, 2009).

Preoperative stay in the ward should be avoided and if this is necessary for medical reasons, keep the patient in a clean environment to protect them from colonization with bacteria from infected patients while use of prophylactic antibiotic in the ward should be discouraged (Bello, 2012).

Sterilization of surgical instruments is very crucial in the prevention of surgical wound infections. Before sterilization, all equipment must be disinfected and cleaned to remove debris and the methods of sterilization in common use are autoclaving, exposure to dry heat and treatment with chemical antiseptics (Oliveira *et al.*, 2010). Autoclaving should be the main form of sterilization at a district hospital and at the end of the procedure, the outside of the packs of instruments should not have wet spots which may indicate that sterilization has not occurred and dry heat is suitable only for metal instruments and a few natural suture material whereas sharp instruments, other delicate equipment and certain catheters and tubes can be sterilized by exposure to formaldehyde, glutaraldehyde or chlorhexidine (Oliveira *et al.*, 2010).

CONCLUSION

The quality of surgical care is a function of anaerobic bacterial dose at the time of surgery. The expression of virulence by these organisms vary and difficult to culture with standard microbiology technique. Several complications occur due to surgical site infections and include delayed healing or wound dehiscence, systemic infections and their complications including death, increased hospital stay predisposing the patient to further nosocomial infections, increased hospital costs being a financial burden on the patient, increased antibiotic use leading to resistance and negative psychological effects on the patient and his family (Louie *et al.*, 2013).

REFERENCES

Anderson, D.J., Kaye, K.S., Classen, D., Arias, K.M., Podgorny, K., Burstin, H., & Gerding, D.N. (2008). Strategies to prevent surgical site infections in acute care hospitals. *Infection Control*, **29**(1): S51 – S61.

Bandaru, N.R., Ranga, R.A., Vijayananda, P.K. and Rama, M. (2012). A Prospective Study of Postoperative Wound Infections. In A Teaching Hospital of Rural Setup. *Journal of Clinical and Diagnostic Research*, **6**:1266-1271.

Barie, P.S. (2017). Atypical Wound Pathogens. *Surgical Infections*, **18**(4):456-460.

Beilman, G.J. and Dunn, D.L. (2015). Surgical wound infections. In: Schwartz's Principle of Surgery, 10th edition, McGraw Hill, New York, USA.

Bello, E. (2012). *Incidence of surgical site nosocomial infections and antibiotics susceptibility profiles of bacterial isolates in Ahmadu Bello University Teaching Hospital, Zaria, Kaduna State*. Unpublished M.Sc (Pharmaceutical Microbiology) dissertation. Ahmadu Bello University, Zaria, Nigeria.

Blaser, D.Y. (2014). *Principles and Practice of Infectious Diseases*, Elsevier Health Sciences, 8th edition, **1** ISBN 978-1-4557-4801-3. Pp 3904.

CDC (2016). National and State Healthcare-Associated Infections Progress Report. https://www.cdc.gov/HAI/pdfs/progress-report/hai-progress-report.pdf

Dalhatu, A., Olaogun, A., Olayinka, A.T., Ahmed, S., Timothy. G. and Yunusa, U. (2014). Incidence of Surgical Site Infections (SSIs) among Patients Undergoing Major Surgery at General Hospital Funtua, Katsina State, Nigeria. *IOSR Journal of Nursing and Health Science* (*IOSR-JNHS*), **3**(3):16-21.

Edmiston, C.E., Goheen, M.P., Kornhall, S., Jones, F.E. and Condon, R.E. (2011). Fecalperitonitis: microbial adherence to serosal mesothelium and resistanceto peritoneal lavage. *World Journal Surgery*; **14**:176–83.

Etok, C.A., Edem, E.N. and Ochang, E. (2012). Aetiology and antimicrobial studies of surgical wound infections in University of Uyo Teaching Hospital (UUTH), Uyo, Akwa Ibom State, Nigeria. *Open Access Scientific Reports*, **1**, 341 – 352.

Garibaldi, R.A., Dishing, D. and Lerer, T. (2013). Risk factors for post-operative infection. *American Journal Medicine*; **91** (suppl 3B): 158S- 163S.

Gibson, F.C., Onderdonk, A.B., Kasper, D.L. and Tzianabos, A.O. (2015). Cellularmechanism of intra-abdominal abscess formation by *Bacteroides fragilis. Journal of Immunology*; **160**:5000–6.

Haley, R.W., Culver, D.H., Morgan, W.M., White, J.W. and Emori, T.G (2014). The nationwide nosocomial infection rate: A new record for vital statistics. *American Journal Epidemiol*; **121**: 159-167.

Hentges, D.J. (2013). The anaerobic microflora of the human body. *Clinical Infectious Diseases*; **16**(4):S175–80.

Hite, K. E., Locke, M. and Hesseltine, H.C. (2011). Synergism in experimental infections with non-sporulating anaerobic bacteria*. Jounral of Infectious Diseases.***84:**1–9.

Kenneth J., Ryan, C. and Ray, G. (2014). *Clostridium*, *Peptostreptococcus*, *Bacteroides*, and Other Anaerobes. In Sherris Medical Microbiology, 6ed McGraw-Hill Medical ISBN 9780-0-7-181821-6.

Louie, T.J., Bartlett, J.G., Tally, F.P. and Gorbach, S.L. (2013). Aerobic and anaerobic bacteria in diabetic foot ulcers. *Ann Intern Med*; **85**:461–3.

Noor, A. and Khetarpal, S. (2019). Anaerobic Infections. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing.

Nwankwo, E.O., Ibeh, I.N. and Enabulele, O.I. (2012). Incidence and Risk Factors of Surgical Site Infection in a Tertiary Health Institution in Kano, Northwestern Nigeria .*International Journal of Infection Control*, **8**(4): 1 – 6.

Oliveira, A.C., Martans, M.A. and Martinho, G.H. (2010). Comparative study of surgical wound infections diagnosed in-hospital and post discharge. *Revista de saudepublica*. **36**(2):717-722.

Osakwe, J.O., Nnaji, G.A., Osakwe, R.C., Agu, U., & Chineke, H.N. (2014). Role of premorbid status and wound related factors in surgical site infection in a tertiary hospital in sub-saharan Africa. *Family Practice Reports*, **1**(1): 2 – 4.

Prtak, L.E, and Ridgway, E.J. (2009). Prophylactic antibiotics in Surgery.*Am J of Surgery*; **27:** 431-434.

Russell, R.C.G., Norman, S.W. and Bulstrode, C.J.K. (2010). Bailey and Love's Short (**Eds.**), Practice of Surgery: Wound Infection. 24th edition. Oxford: Oxford University press. Pp 118-132.

Ryan, J.R. (2004). Clostridium, *Peptostreptococcus*, Bacteroids, and other Anaerobes. In K. J. Ryan, & C. G. Ray (**Eds.**), *Sherris Medical Microbiology: An Introduction to Infectious Diseases* (4th ed., Pp. 309-326). USA.

Wells, C.L. and Wilkins, T.D. (2008). *Clostridia*: Spore forming Anaerobic Bacilli. In: Baron S, editor. Medical Microbiology.4th edition. Galveston (TX): University of Texas Medical Branch at Galveston.