Journal of Experimental Research

JUNE 2024, Vol 12 No 2

Email: editorinchief.erjournal@gmail.com
editorialsecretary.erjournal@gmail.com

Received: April, 2024
Accepted for Publication: May, 2024

Isolation And Identification Of Bacteria Associating To Necrotizing Fasciitis

Iloputaife, EJ.

Department of Applied Microbiology and Brewing, Faculty of Applied Natural Sciences, Enugu State University of Science and Technology

***Author for Correspondence: emmanuel.iloputaife@esut.edu.ng**

ABSTRACT

Bacterium(a) entrance into a host either through an external injury or by direct spread from a punctured/perforated internal organ (particularly the colon, rectum, or anus) or sexual organ are the means of contacting necrotizing fasciitis. This study was aimed at isolation and identification of bacteria that are associated to necrotizing fasciitis, to determine the spectrum of bacterial organisms responsible for necrotizing fasciitis. A total of twenty (20) samples were collected from patients with sterile swap sticks. Collected samples were cultured on Nutrient, MacConkey and Blood Agar plates and incubated at 37oC for 24 hours. Isolation and identification of bacteria was done using standard microbiological procedures. Results showed that *Staphylococcus aureus* was the commonest isolates accounting for 20(22%) followed by *Escherichia coli* 17(18.47%), *Pseudomonas aeruginosa* 16(17.6%), *Streptococcus* sp., 15(16.5%), *Klebsiella* sp. 13(14.2%) while *Proteus* sp. was the least common isolates accounting for only 10(11%). In conclusion, it is evident from this study that necrotizing fasciitis is largely polymicrobial, deriving significant contributions from both gram-positive and gram-negative organisms. It showed that wound samples used in this study were contaminated with bacteria and are therefore at risk of infection if proper care and treatment modalities were not adhered to. It is therefore a necessity that good hygiene and proper care of wound infection plus the cooperation of antimicrobial drugs during treatment.

INTRODUCTION

Necrotizing fasciitis (NF), also known as flesh-eating disease, is a bacterial infection that results in the death of parts of the body's soft tissue. It is a severe disease of sudden onset that spreads rapidly. Symptoms usually include red or purple skin in the affected area, severe pain, fever, and vomiting (Pasternack *et al.*2015). The most commonly affected areas are the limbs and perineum. Necrotizing means causing the death of tissues. Fasciitis means inflammation of the fascia (the tissue under the skin that surrounds muscles, nerves, fat and blood vessels) (Hakkarainen *et al.* 2014).

Typically, the infection enters the body through a break in the skin such as a cut or burn (Pasternack *et al.*2015). Risk factors include poor immune function such as from diabetes or cancer, obesity, alcoholism, intravenous drug use, and peripheral artery disease (Hakkarainen *et al.*2014). It does not typically spread between people (Pasternack *et al.* 2015). The disease is classified into four types, depending on the infecting organism. Between 55 and 80% of cases involve more than one type of bacteria. Methicillin-resistant *Staphylococcus aureus* (MRSA) is involved in up to a third of cases. Medical imaging is often helpful to confirm the diagnosis (Maya *et al.*2014).

Necrotizing fasciitis may be prevented with proper wound care and hand washing. It is usually treated with surgery to remove the infected tissue and intravenous antibiotics (Hakkarainen *et al.* 2016; Pasternack *et al.*2015). Often, a combination of antibiotics is used, such as penicillin G, clindamycin, IV vancomycin, and gentamicin (Hakkarainen *et al.* 2016). Delays in surgery are associated with a much higher risk of death (Maya *et al.*2014). Despite high-quality treatment, the risk of death is between 25 and 35% (Hakkarainen *et al.* 2014).

Necrotizing fasciitis occurs in about 0.4

people per 100,000 per year in the U.S. and about 1 per 100,000 in Western Europe (Maya *et al.*2014). Both sexes are affected equally (Hakkarainen *et al.* 2014). It becomes more common among older people and is rare in children (Maya *et al.*2014). It has been described at least since the time of Hippocrates (Hakkarainen *et al.*2014). The term "necrotizing fasciitis" first came into use in 1952 (Maya *et al.*2014).

Symptoms may include fever, swelling, and complaints of excessive pain. The initial skin changes are similar to cellulitis or abscess, thus making the diagnosis at early stages difficult. Hardening of the skin and soft tissue and swelling beyond the area of skin changes are commonly present in those with early necrotizing changes (Hakkarainen *et al.*2014). The redness and swelling usually blend into surrounding normal tissues. The overlying skin may appear shiny and tense (Loudon, 1994). Other signs which are more suggestive of necrotizing changes (but present in later stages in 7 to 44% of the cases) are: formation of bullae, bleeding into the skin which is present before skin necrosis (Hakkarainen *et al.* 2014) (skin turning from red to purple and black due to thrombosis of blood vessels)presence of gas in tissues, and reduced or absent sensation over the skin (due to the necrosis of the underlying nerves) (Loudon, 1994). Rapid progression to shock despite antibiotic therapy is another indication of necrotizing fasciitis. Necrotizing changes affecting the groin are known as Fournier gangrene (Hakkarainen et al.2014).

However, those who are immunocompromised (have cancer, use corticosteroid, on radiotherapy, chemotherapy, HIV/AIDS, or prior organ or bone marrow transplantation) may not show typical symptoms. Immunocompromised persons also have twice the risk of death from necrotizing infections, so higher suspicion should be maintained in this group (Hakkarainen *et al.*2014).

More than 70% of cases are recorded in people with at least one of these clinical situations: immunosuppression, diabetes, alcoholism/drug abuse/smoking, malignancies, and chronic systemic diseases. For reasons that are unclear, it occasionally occurs in people with an apparently normal general condition (Pricop, 2011).

Necrotizing fasciitis can occur at any part of the body, but it is more commonly seen at the extremities, perineum, and genitals. Only a few of such cases arise from the chest and abdomen. Trauma is the usual cause of the infection, such as from intravenous drug injection, insulin injection, animal and insect bites, catheter insertion over the skin, or a fistula connecting skin to the internal body organs. Skin infections such as abscess and ulcers can also complicate necrotizing fasciitis. Spreading of infection through blood has been suggested for those with streptococcal pharyngitis. For infection of the perineum and genitals (Fournier gangrene), trauma, surgery, urinary tract infection, stones, and Bartholin gland abscess are the usual causes (Hakkarainen *et al.* 2014). The risk of developing necrotizing fasciitis from a wound can be reduced by good wound care and hand washing (Pasternack *et al.* 2015).

Necrotizing fasciitis (NF) is a progressive, polymicrobial, potentially fatal soft tissue infection that can affect both sexes, all age groups and any anatomical region of the body. Many organisms are known to be associated with necrotizing fasciitis. Identification of the offending microorganisms is important, since the eventual outcome of treatment is dependent on aggressive surgical, chemotherapeutic and supportive therapy.

Aim of the Study

The aim of this study is to isolate and identify bacteria associated with necrotizing fasciitis. The objectives of the study are to determine the spectrum of bacterial organisms responsible for necrotizing fasciitis in orthopaedic hospitals Enugu Nigeria and to establish a baseline for which further studies can be conducted.

MATERIALS AND METHODS

Materials

The materials used for the practical were Petri dishes, test tubes, inoculating loop, autoclaves, incubator, weighing balance, swab sticks, gloves, distilled water and ethanol media used included Blood agar (blood from sheep), Nutrient Agar and MacConkey agar.

Source of Material

Specimens were gotten from 20 patients at National Orthopaedic Hospital Enugu with cuts, scrapes, burns, insect bites, puncture wounds (including those due to intravenous drug use) and

surgical wounds with swap sticks accurately labelled and accompanied by a properly completed requisition form, indicating the nature of the specimen, the date of sample collection, relevant clinical information, the investigations required, and details of antibiotic therapy and brought to the Laboratory section of the Department of Applied Microbiology and Brewing in Enugu State University of Science and Technology for bacterial enumeration. All other reagents and materials used were supplied by the laboratory of the Department of Applied Microbiology and Brewing, ESUT.

Sterilization of Materials

Materials were washed and cleaned with absolute alcohol, thereafter wrapped with using an aluminium foil and further sterilized in a preheated hot air oven at 1000°C for 1hour.

Isolation Methods

All the media used were produced according to their manufacturer's instruction. All specimens collected were immediately applied to freshly prepared blood agar, MacConkey agar and Nutrient agar, streaked and incubated overnight at 37°C for 24 hrs. After incubation, the bacterial colonies were observed and discrete colonies were picked and purified by sub- culturing onto freshly prepared Nutrient agar, MacConkey agar and blood agar using a streak plate technique. Isolated colonies that grew on the plates were then transferred onto Nutrient agar slants with a proper label. These agar slants were stored in the refrigerator at 4°C and were used for further characterization.

Identification of Bacterial Isolates

Characterization and identification of bacterial isolates were based on standard microbiological methods including gram staining, morphological and cultural characteristics on Nutrient agar media. Biochemical tests such as catalase, coagulase, indole, oxidase, citrate and urease tests were also carried out to determine their biochemical properties.

RESULTS

Bacteria isolates showing varying colonial and morphological characteristics on different media (MacConkey agar, Blood agar and Nutrient agar) were isolated. These bacteria included *Staphylococcus aureus*, *Pseudomonas aeruginosa, Escherichia coli, Proteus, Streptococcus*, *Klebsiella* species (table 1).

In Table 2, the biochemical characteristics of bacteria isolates confirmed the presence of *Staphylococcus aureus*, *Pseudomonas aeruginosa, Escherichia coli, Proteus, Streptococcus*, *Klebsiella* species in the wound sepsis.

Table 3 shows the occurrence of bacterial isolates. Out of the 20 samples cultured, *Staphylococcus aureus* has the highest number of occurrence 20(100%), followed by *Escherichia coli* 17(85%) while *Proteussp.* has the least occurrence10(50%).

Table1: Colonial Characteristics of Bacterial Isolates

Organisms suspected	MacConkey Agar	Blood agar	Nutrient agar	Colony morphology
Staphylococ cusaureus	Golden yellow	Golden yellow	White	Round
Pseudomon as aeruginosa	White	White	Green	Round and irregular
Proteussp.	Pink	Pink	White	Swampy and flooded
Streptococc ussp.	Pink	Pink	White	Round
Klebsiellasp.	White	White	White	Raised and round
Escherichia coli	Pink	White	White	Convex and round

Table 2: Biochemical Characteristics of Bacterial Isolates

Suspected organisms	Gram reaction	Shapes	Catalase	Urease	Oxidase	Citrate	Indole	Coagulase
Staphylococcusaureus	Gram positive	Singly, clusters	+	-	-	-	-	+
Pseudomonasaeruginosa	Gram negative	Singly, rods	+	-	+	-	-	-
Proteussp.	Gram negative	Rods	+	+	-	-	-	-
Streptococcussp.	Gram positive	Cocci in chains	-	-	-	-	-	-
Klebsiellasp.	Gram negative	Singly stout	+	-	-	+	-	-
Escherichiacoli	Gram negative	Singly, rods in clusters	+	-	-	-	+	-

Key: +: positive and -: Negative

Table 3: Occurrence of Bacterial Isolates

Organisms isolated	Occurrence (%, n=20)	% Frequency of Occurrence (n=91)
Staphylococcus aureus	20(100)	22
Pseudomonas aeruginosa	16 (80)	17.6
Proteussp.	10 (50)	11
Streptococcus sp.	15 (75)	16.5
Klebsiellasp.	13 (65)	14.2
Escherichiacoli	17 (85)	18.7
Total	**91**	**100**

DISCUSSION

This study was carried out to isolate and characterize the bacterial pathogens associated with wound sepsis. The findings show that *Staphylococcus aureus* was the commonest isolates accounting for 20(22%) followed by *Escherichia coli* 17(18.47%), *Pseudomonas aeruginosa* 16(17.6%), *Streptococcus* sp., 15(16.5%), *Klebsiella* sp. 13(14.2%) while *Proteus* sp. is the least common isolates accounting for only 10(11%) (Table 3). These results were in agreement with previous studies carried out globally and in different parts of the country such as in Umuahia by Anthony *et al.*(2010); Wukari by Athanasopoulos *et al.* (2006); Ibadan by Isibor *et al.* (2008); Benin Cityby Janet *et al.*(2005) and Ekpoma by Jones *et al.* (2004). Findings from the study carried out at a University hospital in Nigeria showed that the commonly isolated bacteria were *Staphylococcus aureus* (25%) and *Pseudomonas aeruginosa* (20%) (Akinjogunla *et al.* 2009). A similar study carried out at a University teaching hospital in Iran, also reported *Staphylococcus aureus* to be the commonest bacteria isolated (43%) (Kenneth, 2017). The high prevalence of *Staphylococcus aureus* infection maybe because it's an endogenous infection source.

Contamination from the environment, such as contamination of surgical instruments, can also cause infection with this organism. *Staphylococcus aureus*, the prevalent bacteria on surfaces such as

human skin, can easily enter wounds due to the disruption of the natural skin barrier. Also, Anthanasopoulos *et al.* (2008) proposed that the extracellular adherence protein (Eap) of *Staphylococcus aureus* played a key role in delayed wound healing by blocking angiogenesis in the proliferative state and slowing the inflammatory response. Inflammation plays a crucial role in wound healing and is responsible for the elimination of germs Okesola *et al.* (2008). The presence of bacterial components in chronic wounds, on the other hand, may trigger an excessive inflammatory response and chronic wounds may not heal unless the excessive inflammation is decreased (Potter *et al.* 2019).

The results show that most of the wounds used in this study were contaminated with these bacteria and are therefore at risk of infection if proper care and treatment modalities are not adhered to.

In agreement with some studies (Akinjogunla *et al.* 2009; Anthony *et al.* 2010; Isibor *et al.* 2008), gram-positive bacteria were the most frequently isolated organisms. The predominant bacterial cause of necrotizing fasciitis has been the subject of debate. While some report gram-positives as the commonest (Anthony *et al.* 2010), other reports favour gram-negatives (Isibor *et al.* 2008); although recent studies (Yu *et al.*2017) report an increasing aetiological contribution by the latter. However, it is well established that in necrotizing fasciitis, there is a synergistic relationship between gram-positive and gram-negative bacteria (Yu *et al.*2017). Consequently, the resultant effect is usually far more fulminant than the regular effect attributable to the individual pathogen.

Wound infection is an ongoing burden. The presence of these bacteria in the various wound samples investigated could have been as a result of exposure to the dirty environment, contaminated water or materials used for treatment and even from the hospital (nosocomial infections). To avoid wound infection, good personal hygiene should be maintained and the wound should regularly be cleaned with antibiotics to prevent bacteria colonization around the wound. Finally, the presence and multiplication of the above bacterial in the wound may delay the healing process of the wound. Therefore, whenever there is a wound, especially one which delays healing, routine culture should be carried out to determine bacterial associated with such wound and its susceptibility to various antibiotics should also be carried out to determine the choice of antibiotic for treatment. Hence good hygiene and proper care of wound infection plus the cooperation of antimicrobial drugs during treatment is advised.

CONCLUSION

It is concluded from this study that necrotizing fasciitis is largely polymicrobial, deriving significant contributions from both gram-positive and gram-negative organisms. Bacteria species isolated from this study included *Staphylococcus aureus*, *Pseudomonas aeruginosa, Escherichia coli, Proteus, Streptococcus*, *Klebsiella* species. *Staphylococcus aureus* was confirmed to be the most prevalent bacteria in wounds accounting for 20(22%) of the 91 isolates from this study.

It has been observed that surface swab cultures may remain negative while organisms continue to invade the deeper tissues. This understanding necessitated the use of tissues and/or tissue fluid, in addition to surface swabs for culture in our patients. The results of this study would enable a more objective initial empirical antibiotic treatment of necrotizing fasciitis. Future studies to obtain fungal, viral or anaerobic bacterial cultures with necrotizing fasciitis should be carried out.

REFERENCES

Akinjogunla OJ, Adegoke AA, Mboto CI, Chukwudebelu IC, Udokang IP. (2009). Bacteriology of automobile accident wounds infection. *Medical Science.1:* 23-27.

Anthony AA, Okoh TM, Anthony I, Steve J. (2010). Studies on multiple antibiotic resistant bacterial isolated from surgical site infection. *Science.5:* 3876-3881.

Athanasopoulos AN. (2006). The extracellular adherence protein of *Staphylococcus aureus* inhibits wound healing by interfering with host defence and repair mechanisms. *Blood.107:* 2720-2727.

Hakkarainen Timo W, Kopari Nicole M, Pham Tam N, Evans Heather, L. (2014). Necrotizing soft tissue infections: Review and current concepts in treatment, systems of care, and outcomes. *European Journal of Emergency Medicine.51*(8): 344–362.

Isibor JO, Oseni A, Eyaufe A, Osagie R, Turay A. (2008).

Incidence of aerobic bacteria and *Candida albicans* in postoperative wound infections. *African Journal of Microbiology Researches.2:* 288-291.

Janet M, Torpy MD, Alison, MA, Richard M, Glass MD. (2005). Wound infections. *Medical Association.294:* 21-22.

Jones SG, Edwards R, Thomas DW. (2004). Inflammation and wound healing: The role of bacteria in the immuno-regulation of wound healing. *International Journal of Lower Extremity Wounds.3*: 201-208.

Kenneth IEP. (2017). Identification of bacteria associated with wounds in Wukari and environs, North-East, Nigeria. *Journal of Health.4:*63-67.

Loudon I. (1994). Necrotising fasciitis, hospital gangrene, and phagedena. *The Lancet.344*(4): 1416-1419.

Maya SD, Beltrán D, Lemercier P, Leiva-Salinas C. (2014). Necrotizing fasciitis: an urgent diagnosis. *Skeletal Radiology.43(5):* 577–589.

Okesola AO, Kehinde AO. (2008). Bacteriology of non-surgical wound infections in Ibadan, Nigeria. *African Journal of Medical Sciences.37:* 261-264.

Pasternack MS, Swartz MN, Mandell GL. Bennett JE, Dolin R. (2015). Cellulitis, necrotizing fasciitis, and subcutaneous tissue infections. Principles and Practice of Infectious Diseases. Philadelphia (PA): Churchill Livingstone Elsevier volume 1. Washington. pp: 1195–1216.

Potter PA, Perry AGM, Hall A, Stockert PA. (2009). Necrotizing fasciitis: microbiological characteristics and predictors of postoperative outcome. *Fundamentals of Nursing.7*: 1476-1483.

Pricop M, Urechescu H, Sîrbu A, Urtilă E. (2011). Necrotizing cervical fasciitis: clinical case and review of literature. *Journal of Infectious Diseases.2(1):* 1-6.

Yu JC, Hsieh PH, Tsai HW. (2017). Rapid identification of pathogens responsible for necrotizing fasciitis on an integrated microfluidic system. *Biomicrofluidics. 11 (6)*: 064108.