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Isolation of Pathogenic Bacteria on injury site of the Skin and the assessment of antimicrobial resistance pattern among patients living in Birnin Kebbi city, Kebbi State Nigeria

¹Jamilu Garba, ²Asime Oba, ³Barka John, ⁴Sanusi Muhammad Mairiga and ⁵Kwata Veronica John

¹jamiluhaji192@gmail.com,
²asimejamesoba@gmail.com,
³barkajohngagava26@gmail.com,
⁴sanusimuhammadmairiga@gmail.com,
⁵kwatajohn1@gmail.com

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Abstract:- This study was carried out with the aim of isolating bacterial pathogens from wound infection as well as assessing the antimicrobial resistance pattern, with emphasis of assessing multiple drugs resistance pathogenic bacteria. Random sampling techniques was used to generate Data of detecting pathogens and their antimicrobial resistivity of the bacterial pathogens species in infected wounds specimens including buruli ulcer, watery substances derided tissues, aspirates and swabs received from people living with wounds around Birnin kebbi city, from September 2021 to January 2022). Out of sixty two (62) samples of wound swabs that was investigated through Microscopy, Culturing, and Sensitivity for Microbiological assessment of Bacterial pathogens from wound infection. 46(75.85%) shows bacterial growth and 16(24.1%) of the samples turns out to be negative (no growth of bacterial pathogens was observed). Bacterial pathogens isolated from specimens, 17 (27.4%) indicates the growth of S. aureus, 10(16.1%) indicates E. coli growth, 8(12.9%) indicates growth of pseudomonas spp, 3(4.8%) for Klebsiella spp, 3(4.8%) for Proteus 1(1.6%) for Stresptococcus spp, 1(1.6%) for Enterobacter spp, and 3(4.8%) for Citrobacter spp. Wound infection according to gender, out of the sixty two (62) samples, Male patients recorded the highest with 36(58.0%) of bacterial growth followed by 26(41.9%) indicating the bacterial growth for the female patients. The bacterial isolated from wound include Staphylococcus aureus, Streptococcus, pseudomonas, Enterobacter are resistance to Amoxicillin while Proteus, Citrobacter, E. coli and Enterobacter were highly resistant to Pefloxacin and Cefotaxime. The treatment of wound infection with antibiotics without completing the dosage results to the transformation of certain bacterial to secret enzymes that will be inactivating antimicrobial substance enabling the growth certain organisms.

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1.0 Introduction

1.1 Background

The rate of Bacterial infection has increased due to the percentage of people that are living with injuries on the skin or any other part of the body. Infection on the injury exposed part of the body remains a major problem in the world today (Giacometti et al., 2000). The exposed injury on the skin or other body are predisposed to pathogenic bacteria which grow on the affected part, mechanical injury on the skin, the tissue that protect the internal body are damaged and the tissue damage body part becomes suitable for pathogenic bacteria live and multiply (Siddiqui et al., 2010). The site of tissue damage on the skin remain a major concern as it is prone to bacterial infection (Siddiqui et al., 2010). Many organisms that grows on the injury site result to infection of the wound (Melling et al., 2001). Infection of the wound which is as a result of external skin damage or other complications such as the use of unsterilized sharp objects like needles and syringes after surgerical procedures undertaken in health Centres for managing patients with Skin or tissue damage which is known as the process for wound healing procedures. The process of injury management is consider as a process of healing the wound to reduce the growth of pathogens and restoration of tissues at the site of tissue damaged and encouraging the generation of new tissues (Nguyen et al., 2009). The infection of the Wound happens when pathogenic organism enters the site of tissue damage and multiplies (Rajan, 2012). Upon exposing the ulcerate surface of the wound, Bacteria that are considered to Skin transient and residential floras start to increase in number following adherence to the injury site, and do not causeharm. The treatment procedures of healing the surface of wound is not been slowed by bacteria that colonised the injury site, but in some certain instances the pathogenic bacteria growth may encourage the process of healing the injury site. (Rajan, 2012). When pathogens enter the injury site, they spread to different part of the internal surface and get localised following which infection of the wound occurs as the bacteria pathogens over power the immune the system of the patient and start to damage the tissues on the site (Rajan, 2012). Wound exposure is one of the major factors that predisposed the injury or site of tissue damage to bacterial infection (Bergin et al., 2012). The infection move across body through blood resulting to symptoms of blood poisons when circulate around the body system which include, fever, shock like syndrome, and chills leading ulcerated lesion of the damaged site and external body surface (Rajan, 2012). In

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most complicated cases with people having other clinical issues such has diabetes and impaired immune system are attributed to infection of the wound specifically in an asymptomatic condition (Bergin et al.,2012). The clinical manifestation of certain infection of the wound with short time duration may not be present in patient with ulcerated lesion. Infection sign will suggest whether is of long time issues attributed to bacterial infection including colour changes and pus formation on the site of tissue damage and inhibit regeneration process (Reddy et al., 2012).

The activities of both normal residential and transient flora on the skin are mostly colonised by Streptococcus spp., Pseudomonas spp, Enterococcus spp. and enterococcus spp (Bowler et al., 2001). The flora of wounds is predominated more than other pathogens found on the tissue damage site and infections of this nature in wounds are as of Staphylococcus aureus (Healy and Freediman, 2006). Some characteristics bacterial infection on the ulcerated surface of the skin, for instance, localized damage of the skin don't actually result to infection, expect if infection does occur especially when the bacteria colonise the site of tissue damage. Pathogens that are found on freshly exposed wound according to previous studies is S. aureus because it is mostly isolated from wound or burned site of the Skin. Both gram-positive and negative Organism are found to be colonising the Skin (Posluszruy et al., 2011).

Diagnosis is often use for inhibiting the progress of infection of the wound that is significant for Skin care. The Skin serves as a protection to the internal system of the body and it is been colonised by bacteria that inhabited the skin, this activity of normal flora that does cause harm. When there is a sudden cut on the skin, the normal flora penetrate and get adhered to injury surface, enter andmove across through the system and cause further damage (Reddy et al.,2012). Swab is often used as a method of taking samples from the site of skin damage. However tissue microscopy is one among the techniques of gold standard. The use of sterile swabs on the injury or ulcerated lesion of the skin to take samples which will further undergo microscopy, culturing and sensitivity are commonly practiced in most primary health care centres. As the injury surface becomes watery and pus formation, the use of sterile swab should be use to take sample of the pus portion after damping with clean clothes and most times the samples are taken even without properly cleaning the surface. If sanitary measure is not instituted when managing the injury site on the skin, it normally result to isolation of different organisms that is not important instead of

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producing the desired and targeted bacterial species (Starr et al.,2003). Washing of freshly exposed injury site on the skin leads to the reduction of microorganisms that are found on the wound surface, receive treatment to slow down the growth of organisms that will cause further damage and encourage the isolation of desired colonies. Sterile swabs are to be use for washing the wound surface with sterile saline damped with cotton wool and iodine or 70% of alcohol (Siddiqui and Bernstein, 2010). Normally, people with tissue damage are supposed to have received treatment by antibiotic before taking sample from their injury site as it destroy the growth of organisms leading inaccurate result (Siddiqui and Bernstein, 2010). Antimicrobial substance use for the treatment of infection are mostly pharmaceutical products, which are the broad spectrum and narrow spectrum. The antibiotics are used for testing the effectiveness on the isolated organism to know the right drug of choice to be prescribe for the patient. This is carried out in the laboratory through Microscopy, culturing and sensitivity. Misuse of antibiotics enable Pathogens that are mostly found on injury site of the skin to get resistance to antibiotics which will result to multi drug resistance (Atiyeh et al., 2007).

2.0 Methodology

2.1 Study Area

This study was carried out around Birnin Kebbi city in Kebbi State, positioned at the north-western part of Nigeria. Predominated by the Gwandu Emirate, With coordinates of 12o 27'13''N4o 12'01''E (TSN,2010).

2.2 Population

Samples of this study were taken at random from the population which include people living in areas such as bayan Oando, baderiaya, Gesse pase 1 and Gwadangaji of Birnin Kebbi local Government were swabs collection of pus from injury site of patients recruited into study. Patients whose decline consent were excluded from the research and those that consent were included.

2.3. Sample Collection

A total of (62) samples were collected from patients that agree to participate in the research, sample collection was observed using Levine's technique, as exposed injury surface with infection were properly washed with a wet sterile piece of clothing damped with normal saline.

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Aseptically the stick swab was moved over 1 cm2 area with few seconds and adequate pressure to express fluid and bacteria to surface around tissue damage site (Gardner et al., 2007). Samples collected from the patients were sent to Laboratory with 0.5 ml sterile normal saline including bacterial growth. The 62 samples taken from people living around Birnin kebbi were processed and transported to the laboratory and the study period was from September (2021) to January (2022). Samples collection was done using sterile cotton swabs from patients clinically diagnosed with exposed site of tissue damage colonised by bacterial growth resulting to infections.

2.4 Observation of isolated colonies of bacteria

Isolated bacterial was identified using culture methods and biochemical tests. The samples were the first culture on Blood agar and MacConkey agar then incubated for (24h. At 37oc) after which the bacterial growth was diagnosed by using specific culture and biochemical tests.

3.0 Result

3.1 Intensity of disease causing bacterial isolated from wound

The degree intensity of different types of bacterial isolates producing virulence factors was examined and the results are shown in Table (3.1)

3.1 Table. Biochemical test of bacterial isolates.

S/N O	Bacterial species	Caps ule	Hemol ysin	prote ase	CF AI	CFA III
1	Pseudomo nas spp	+	+	+	-	+
2	klebsiella	+	-	++	+	+
3	E. coli	-	+	+	+	+
4	Proteus	-	+	+	-	+
5	Streptoco ccus	+	+	+	-	+
6	Enterobac ter	+	+	-	+	+
7	Citrobact er	-	+	+	+	+
8	Staphyloc occus	+	+	+	-	+

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Isolated pathogens of Bacteria observed from this study were predominated by Staphylococcus aureus. Other include Pseudomonas spp, klebsiella spp, E. coli, Proteus spp, Streptococcus, Enterobacter, and Citrobacter spp. Organisms isolated. From the injury site of the skin, some intensity of disease causing factors produce by the organism was assessed and the outcomes are displayed in table (3.1). Only specie of S. aureus isolates show possession of capsule polysaccharide indicating the significant component of pathogenicity that enhances bacterial virulence by modifying S. aureus adherence to internal tissue surface in vitro, the finding is similar to the outcomes stated by (Nair et al., 2000).

From this findings, S. aureus was observed to have produced the hemolysin as Beta-hemolysis creating clear zone of hemolysis, klebsiella spp, were negative during biochemical test assessment, Proteus and E. coli do not produce capsule, Streptococcus spp w reacted to capsule, hemolysin and protease production which is similar to outcomes stated by Dinges et al., (2000). S. aureus reacted to protease production as a significant disease causing factor as shown in table (3.1), that is similar to (Karlsson and Arvidson, 2002). Who pointed to the production of four major extracellular proteases by S. aureus (serine protease, cysteine protease, metalloprotease, 39 and staphopain) which are important virulence factors and controlling the gene expression for this enzyme by regulator factors. The ability of Staphylococcus aureus to produce colonization factor antigen was tested and it shows that (100%) of bacterial isolates are capable produce colonization factor antigen III (CFA III) (Karlsson and Arvidson, 2002).

E. coli reacted to capsule production which was observed as (100%) from these isolates could produce the polysaccharide capsule. Silver and Vimr, (1990) showing that some capsules have been correlated with pathogenic strains of E. coli. Hemolysin production by E. coli was assessed, and it was found that (100%) of the isolates was able to produce -hemolysin on a blood agar plate. These results agree with Herlax et al., (2010). The erythrocytes are not affected by hemolysin, but also leucocytes (white blood cells). Concerning protease, the ability of E. coli to produce extracellular protease in M9 media was assess and examined, and it was discovered that all these isolates (100%) have the potential to produce extracellular protease after 24 hours of incubation table (3.1). Extracellular have a significant role in cell survival and cell to cell communication (Abhrajyoti et al., 2009). The ability of Klebsiella spp. isolates to produce disease causing factors were assessed and displayed in table (3.1).

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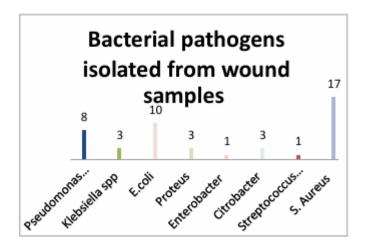
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Also Pseudomonas spp. reacted to capsule and protease production. Surface structures, including pili and the polysaccharide capsule or glycocalyx, appear to mediate the initial attachment of P. aeruginosa to its prospective host, thus permitting colonization (Umar et all.,2016). Extracellular enzymes such as alkaline protease, elastase, phospholipase C, and exotoxin. A degrade infected tissues and promote bacterial invasion (Pollack, 1984).

Citrobacter spp are often isolated from different people having tissue damage and exposed exudates. Progressing of wound infection depends upon interplay of many factors and spread of MDR pathogens is adding new dimension to the problem in poor- resource countries.



3.2 Bacterial Pathogens isolated from Patient's wound Examined

62 samples of wound swab that was examined through Microscopy, Culturing, and Sensitivity for Microbiological assessment of Bacterial pathogen from wound infection. 46 (75.85%) was positive of bacterial growth and 16 (24.1%) of the samples turns out to be negative. Bacterial pathogens that was isolated from each samples, 17 (27.4%) were for the growth of S. Aureus, 10(16.1%) for E. coli, 8(12.9%) for pseudomonas spp, 3(4.8%) for Klebsiella spp, 3(4.8%) for Proteus 1(1.6%) for Stresptococcus spp, 1(1.6%) for Enterobacter spp, and 3(4.8%) for Citrobacter spp. Wound infection according to gender, out of the sixty two (62) samples, 36(58.0%) indicates the presence of bacterial growth in Male patients and 26(41.9%) indicates the bacterial growth for the female patients.

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3.3. isolates Antibiotic Susceptibility pattern of bacterial

The pattern of susceptibility of bacterial isolates to antibiotics was assessed using Oxiod AST Discs assay Kirby-Bauer method. The finding indicated that the organisms are different in their susceptibility to all the antimicrobials used. Many of them are resistance to multiple drugs which were used for assessing their resistivity pattern.

Table 3.3 Antimicrobial Susceptibility pattern of bacterial isolates

Bacterial isolates	CN	PEF	AMC	AU	CEP	OFX	СТХ	С	NA	s	CPX	PN
Pseudomonas spp	s	R	R	s	R	R	s	R	s	s	s	s
klebsiella	s	s	R	s	R	s	s	R	s	s	s	s
E.coli	s	R	s	s	R	R	s	s	R	R	s	s
Proteus	s	R	s	R	R	R	s	s	R	R	s	s
Streptococcus	s	R	R	s	R	s	R	s	R	s	s	R
Enterobacter	s	s	R	R	s	s	s	s	R	s	R	s
Citrobacter	s	R	s	s	R	s	R	R	s	s	s	s
S. aureus	R	R	R	R	R	R	R	s	R	R	R	s

PEF=Pefloxacin, CPX= Ciprofloxacin, CTX= Cefotaxime,
OFX= Cefoxitin, C= Chloramphenicol, AMC=
Amoxicillin

$$\begin{split} CEP&= Cephalexin \ , \ CN= \ Gentamycin, \quad AU&= Augumentin \ , \\ NA&= Nalidixic \ acid \ , \ S= Streptomycin, \quad PN= Ampicillin, \end{split}$$

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3.4 Pattern of antibiotics Resistance of bacterial isolated from the Wound of Patient

S/N _	Antibiotics	No. Specimen	Percentage (%)
1	Augumentin	3	(25.0%)
2	Cefotaxime	3	(25.0%)
3	Nalidixic Acid	5	(41.6%)
4	Cephalexin	7	(58.3%)

5	Pefloxacin	6	(50.0%)
6	Amoxicillin	5	(41.6%)
7	Cefoxitin	4	(33.3%)
8	Streptomycin	3	(25.0%)
9	Gentamycin	1	(8.33%)
10	Ampicilin	1	(8.33%)
11	Ciprofloxacin	2	(16.0%)
12	Chloramphenicol	3	(25.0%)

The antibiotics that has non inhibitory effect on bacterial isolates (Resistance) indicate the number of specimens of which zone of inhibition was not observed from the discs plates. Out of twelve (12) antibiotics that was used for the antibiotics susceptibility test, Augumentin and Cefotaxime has no effect on 3(25.0%) wound specimens, 5(41.3%) wound specimens indicates no zone of inhibition to Nalidxics Acids, 7(58.3%) specimens indicates no inhibitory zone to Cephalexin, 6(50.0%), indicates resistance to Pefloxaxin, 5(41.6%) are resistance to Amoxicillin,

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4(33.3) indicates resistance to Cefoxitin, 3(25.0%) indicates resistance to Streptomycin, 1(8.33%) are resistance to both Gentamycin and Ampicilin, 2(16.0%) indicates resistance to Ciprofloxacin, and 3(25.0%) indicates resistance to Chloramphenicol. The isolated organisms has shown that Staphylococcus aureus, Streptococcus, pseudomonas, Enterobacter are highly resistance to Amoxicillin and Ciprofloxacin while Proteus, Citrobacter, E. coli and Enterobacter were highly resistant to Pefloxacin and Cefotaxime.

4. Discussion

62 samples of wound swab was investigated through Microscopy, Culturing, and Sensitivity for Microbiological assessment of Bacterial pathogen from wound specimen. 46 (75.85%) was positive of bacterial growth and 16 (24.1%) of the samples turns out to be negative. Bacterial pathogen that was isolated from each samples, 17 (27.4%) were for the growth of S. Aureus, 10(16.1%) for E. coli, 8(12.9%) for pseudomonas spp, 3(4.8%) for Klebsiella spp, 3(4.8%) for Proteus 1(1.6%) for Stresptococcus spp, 1(1.6%) for Enterobacter spp, and 3(4.8%) for Citrobacter spp. Wound infection according to gender, out of the sixty two (62) samples, 36(58.0%) indicates the presence of bacterial growth in Male patients and 26(41.9%) indicates the bacterial growth for the female patients. It was observed that the rate of infection was most pronounced among male patients that are within the third decade of life. This is similar to the findings of (Saleh et al., 2013), (Tom et al., 2018), and (Isyaka et al., 2019). WHO asserted that the predominance among patients in this category is most likely due to the fact that male exposure to a possible wound greater as they represent the majority of the workforce responsible for hard/risky Labour. Others suggested that age significantly have public health implication the prevalence of wound infections, since adolescent and active-age adults are usually the ones involved in activities such as sports and farming which may expose them more to injuries and infections (Omole and Stephen, 2014).

Staphylococcus aureus was the most predominant bacteria spp observed in this study. This is similar to the finding of (Mohammed et al., 2013) and (Isyaka et al., 2019). But concurred with reports of similar studies conducted from different parts of Nigeria (Giacometti et al., 2000), (Thanni et al., 2003), (Surucuoglu et al.2005), (Motayo et al., 2013), (Omole and Stephen, 2014), and (Isyaka et al., 2019). Some suggested that the sources of most wound infections are endogenous flora of the patient's skin or mucous membrane. Staphylococcus aureus, E. coli and

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Pseudomonas spp. are among major bacterial speciesincriminated in nosocomial wound infection and are associated with bacteraemia, septicaemia, shock and prolonged hospital stay (Sahu et al.,2011). S. aureus is the major causative agent of surgical wound infections and epidermal skin diseases in newborn infants (Diekema et al.,2001), and (Isyaka et al., 2019). Virulence in S. aureus is mediated by the release of several virulence factors like invasins, hyaluronidase, catalase, coagulase, hemolysins, leukotoxin, and leukocidin (Bessa et al., 2015) and (Isyaka et al., 2019). These enzymes have invasive and degredative abilities in tissues and can enhance the progression of wound disease (Nita et al., 2018).

E. coli has also been isolated in significant numbers, together with Pseudomonas aeruginosa. E. coli, Enterobacter and Citrobacter naturally inhabit the gastrointestinal tract and are associated with skin infections in regions of close proximity to the rectum, particularly with incontinent individuals. Individuals undergoing surgical procedures associated with the gastrointestinal tract and lower regions of the spine are also at risk of contracting infection (Dryden et al., 2010) and (Isyaka et al., 2019). Pseudomonas spp. has been implicated in diverse nosocomial infection likes nosocomial pneumonia, urinary tract infection, surgical site infection, severe burns and infections of patients undergoing either chemotherapy for neoplastic disease or those on antibiotic therapy from the previous study written by (Isyaka et al., 2019). Organisms like Enterobacter and Citrobacter were not isolated, but from this study both Enterobacter and Citrobacter were isolated from the previous study, having a Significant public Health implication and the unique feature of P. aeruginosa is the resistance to a variety of antibiotics, primarily attributed to low permeability of the cell wall, production of inducible cephalosporinase, active efflux and poor affinity for the target (DNA gyrase) (Umoru et al., 2018) and (Isyaka et al., 2019).

==Infection is a major complication in burn wounds, and is estimated to cause 75% of deaths. Burned tissue is susceptible to contamination by microorganisms from the gastrointestinal and upper respiratory tracts and many studies have indicated the contamination of wounds by aerobes such as P. aeruginosa, S. aureus, E. coli, Klebsiella spp., Enterococcus spp., and Candida spp. (Revathi et al., 1998), (Bowler et al., 2001), and (Isyaka et al., 2019).

The sign and symptoms of an infected wound is progressive especially when it involves with exudates patients such as diabetic patients, because they are more susceptible to infections due to

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increased glucose levels and suppressed immune response as well as the neuropathy associated with a decreased blood flow to extremities that lead to slow-healing of the wounds (Enoch et al., 2003) and (Isyaka et al., 2019). Infected wounds after surgery rocedures resulting skin damage sites reactions, bacterial fluid lesions and subcutaneous nodules leading to metastasis, when not properly addressed. The risk of infection is generally based on the degree of susceptibility of a surgical wound to microbial contamination (Bowler et al., 2001)s and (Isyaka et al., 2019). Clean surgery carries a 1% to 5% risk of postoperative wound infection, and in dirty procedures that are significantly more susceptible to endogenous contamination, a 27% risk of infection has been estimated (Bowler et al.,2001) (Nichol and Smith 1994). The damage in Gunshot wounds in most instances, extends beyond the subcutaneous fat layer of the skin affecting both bone and muscle as well as supporting structures with extensive drainage and tends to be necrotic(ICU, 2016) and (Isyaka et al., 2019). The condition may become worse if microbial invasion is involved, the resultant consequences may include prolonged hospital stay associated with difficulties in therapy due to drug resistance, bacteraemia, septicaemia, immune-suppression, shock and even death.

The frequency at which bacterial pathogens acquiring resistance factors remain a major challenge and patients stand the risk of developing multiple resistant wound infections. Prevalence of a resistant strain in an area that is related to the frequency of antibiotic usage, and the domination of multiple-resistant strain may be as a result sporadic spread of any of the antibiotics of which it is resistant (Isyaka et al., 2019). Amoxcillin recorded the most resistance antibiotic indicating 7(58.3%) and is similar to previous finding by (Isyaka et al., 2019).

5. Conclusion

A total of 62 samples were collected from patients consented to participate in the research, sample collection was observed using Levine's technique, as exposed injury surface with infection were properly washed with a wet sterile piece of clothing damped with normal saline. Aseptically the stick swab was moved over 1 cm2 area with few seconds and adequate pressure to express fluid and bacteria to surface around tissue damage site found a high infection rate of wounds by potential bacterial pathogens among which were Staphylococcus aureus, Streptococcus spp, E. coli and Pseudomonas aeruginosa, Proteus, Enterobacter, and Citrobacter. Infection was highest among Males. Which also reveal that exudates and tissue sites were often

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got infected with microbial growth, and are isolated, at the end of the assessmen it was shown that resistivity include, Amoxicillin, Chloramphenicol, Streptomycin, Gentamycin, Ciprofloxacin, Cefotaxime, Cefoxitin, and Pefloxacin.

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