American Journal of Biomedical and Life Sciences
2020; 8(2): 25-32
http://www.sciencepublishinggroup.com/j/ajbls
doi: 10.11648/j.ajbls.20200802.11
ISSN: 2330-8818 (Print); ISSN: 2330-880X (Online)

Conventional and Molecular Diagnostic Tests for the Detection of Bacterial Pathogen in Burn Wound and Antimicrobial Properties of Some Medicinal Plants

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To cite this article:

Nasir Hassan Wagini, Amina Lema Rafukka, Aliyu Musa Yusuf, Sani Muhd Gidado, Samaila Abubakar, Mudassiru Badamasi, Abubakar Mannir Darma, Taofiq Ademola Babatunde, Abubakar Bello, Murtala Yusuf, Jibrin Naka Keta, Lawan Buba Amshi, Hussaina Usman Babba. Conventional and Molecular Diagnostic Tests for the Detection of Bacterial Pathogen in Burn Wound and Antimicrobial Properties of Some Medicinal Plants. *American Journal of Biomedical and Life Sciences*. Vol. 8, No. 2, 2020, pp. 25-32. doi: 10.11648/j.ajbls.20200802.11

Received: January 5, 2020; **Accepted**: January 15, 2020; **Published**: March 24, 2020

Abstract: This research aims to compare bacterial pathogens in different categories of burn wounds and evaluate the specificity and sensitivity of conventional and molecular diagnostics techniques in the detection of bacterial pathogens in burn wounds. This research project also tends to evaluate the potential antimicrobial activity of natural product by using *Vachellia nilotica* and *Prosopis africana* plant extracts. Burn wounds synovial fluid was collected from 50 patients each from three categories of burn wound over a period of 14 months. Samples were subjected to conventional and molecular diagnosis for the screening of the bacterial pathogens. Antibacterial properties of the plants extracts were tested using disc diffusion technique. Of the 50 samples, *P. aeruginosa* were isolated from 7 (14%), 12 (24%) and 17 (34%) samples of first, second and third degree of burn wounds respectively and were considered both positive for *P. aeruginosa* infection. However, *Staphylococcus aureus* was isolated only in the third degree from 4 out of 50 samples and was considered to be negative from first and second degree. Coliform (except *E. coli*) were absent from first degree but present in both second and third degrees (4 and 6) respectively. Gram stain can be considered as a rapid test but solely depend on the microbiological culture test, likewise majority of the biochemical test such as oxidase and API 20E tests. It was discovered that there is highest sensitivity of PCR over culture and or biochemical tests in the detection of P. aeruginosa from burn wound patients while some found no difference or even lower sensitivity in PCR assay. The result shows relatively antibacterial properties of both plant extracts against *P. aeruginosa*. It is concluded that *P. aeruginosa* is the most prevailing bacterial pathogen in burn wound and these plants extracts are active against the pathogen. Finally, a research to isolate and test individual chemical compounds responsible for the antibacterial properties from these plants is highly recommended.

Keywords: Conventional, Molecular, Antibacteria and Wound

1. Introduction

Traditional diagnostics method of detection and identification of bacterial pathogen in a burn wound are based on the ability of the microorganisms to grow under artificial conditions [1]. These conditions include: ability to grow in a selective or differential microbiological culture medium, microscopy, Gram-staining and biochemical tests [2].

Conventional methods are in expensive, highly sensitive, reliable and provide both qualitative and quantitative results on the bacterial populations present in the clinical sample. Clinical samples in these regards relate directly to the symptoms and site of the infection and of course if there is an outbreaks of the infections. In burnt patients ample is taken from form the burn wound [3]. However, these samples are commonly mixed cultures including diverse pathogens and also microorganisms of the normal body flora. Typically, the isolation of pure cultures applying several growth steps is necessary to characterize the bacteria in more detail. To finally identify the bacteria we use a dichotomous key. Note that, the traditional methods are labour-intensive and time-consuming, since the results are not available until at least 1-3 days. Moreover, the culture-based methods reach their limits with specific microorganisms that do not grow on or in artificial media [1].

Since the human genome was first mapped, how our genetic makeup's from one person to another has been the subject of complex study. One important result of these efforts is the field of molecular diagnostics with the capacity to identify specific genetic variations in patients. Molecular diagnostics represent an invaluable tool kit for today's health professionals who can now chart highly individualized plans for their patients. Molecular diagnostics can be used to detect and manage the disease, to detect the risk for disease and to help healthcare professionals determine the best form of therapy for their patients. The power of molecular diagnostics extends beyond cancer monitoring and detection butexplain how molecular diagnostics can be used to manage healthcare associated infections such as methicillin resistant *Staphylococcus aurious* (MRSA). The identification of patients with MRSA is crucial to limiting the transmission of infection through healthcare workers [1, 3].

Molecular diagnostics is growing rapidly as tools to help analyze biological markers become more advanced. Molecular diagnostic testing is relevant to both identifying markers associated with disease, and clinical applications such as infectious disease detection, oncology, pharmacogenomics, or genetic disease screening [4]. Techniques can be used to diagnose and monitor disease, detect risk, and determine therapies for individual patients. By analyzing markers unique to a patient and their disease, molecular diagnostics looks toward the future of personalized medicine [4].

Environmental Gram negative bacteria have become the most abundant etiological agent of incursive infections in wounds due to their characteristics of virulence factors and antibiotic resistance. *P. aeruginosa* is a protean Gram-negative rod-shaped bacterium that lives in soil, swamp habitats, and animal tissues. It is one of the pervasive, opportunistic environmental pathogen responsible for causing infections in human, mainly in patients with compromised host defense mechanisms [5]. It is the most abundant isolated bacterium pathogen from patients hospitalized for more than seven days, and it is a persistent cause of hospital originating infection (nosocomial infections) such as urinary, bacteraemia, pneumonia, and wound infections [6].

In view of the above, *P. aeruginosa* was known in its ability to develop resistance to most widely used antibiotics thereby causing complications as well as difficulties in the choice of therapeutic antibiotics, increased lengths of hospital stays, and can be life-threatening to infected patients [7]. It was reported by [3] that *P. aeruginosa* is the most prevalent causative agent of skin and soft tissue infection (SSTI) in injuries caused by blasts, and the SSTI leads to bacterial colonisation of viable tissues, Bacteraemia (bacterial infection of the blood) which are the hallmark of an incursive blast injuries infection and responsible for substantial volume of mortality rate on infected candidates if the infection is not treated on time or treatment is delayed. Also, the damage caused by the blast to the skin barrier is associated with alteration of host defences and immune system responses which are significant factors aiding to infectious complications and difficulties in hospitalized patients with burn injuries [7]. The affected area of the burn wound is rich in the protein comprising an eschar that yields a favourable environment for opportunist microbial growth. Therefore, Appropriate antibiotic therapeutic treatment and on timely manner could yield a significant number of patients survival [6] and [8]. This lab report attends to a case of a healthy male who sustained blast injuries from an improvised explosive device (IED) out in the field where *P. aeruginosa* infection was suspected after 10 days of hospitalization when an appropriate diagnostic assessment and therapeutic regimen has been made and reported.

Traditional medicine refers to the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, used in the maintenance of health and in the prevention, diagnosis, improvement or treatment of physical and mental illness [9].

A burn is a type of injury to skin, or other tissues, caused by heat, cold, electricity, chemicals, friction, or radiation [1]. Most burns are due to heat from hot liquids, solids, or fire [10]. While rates are similar for males and females the underlying causes often differ. Among women in some areas, risk is related to use of open cooking fires or unsafe cook stoves among men, risk is related to the work environments. Alcoholism and smoking are other risk factors Burns can also occur as a result of self harm or violence between people [10].

Traditional medicine referred to as herbal medicine, herbalism or botanic medicine involves the use of herbs in the treatment of illness due to their therapeutic or medicinal properties, herbal plants synthesized and contain a large number of chemical substances that act on pathogens [11]. These chemical are the basis for a large number of commercial drugs used today for the treatment of diseases

and therefore, plants represent a large storehouse of drugs [12]. The use of herbs as alternatives to orthodox medicines is increasingly gaining acceptance among Africans and the world over. This perhaps is unconnected to the fact that many of the orthodox medicines are fast becoming unreliable as far as the treatment of some diseases are concerned as a result of drug resistance, drug abuse and rapid mutation of the target pathogens [13]. Man has relied on the efficacy of herbs as antimicrobial, anti protozoan, anti helminthic, muscles relaxant and vasolidiatory agents since earliest times. Medicinal properties of plants are normally Dependant on the presence of certain phytochemicals bases such as alkaloids, tannins, anthraquinones, etc. Some of these agents are useful in the treatment of diseases, for example Quinine from cinchona bark tree are use treat malaria, pica root for amoebic dysentery, *Azadiractaindica* (dogonyaro or neem tree) is a commonly used medicinal plant in Nigeria for the treatment of various forms of feverish disease's [13]. In view of the above facts therefore, researches into the ethno-medicinal properties of plants become imperative.

2. Methodology

2.1. Study Areas

This study was conducted in Katsina state, which covers an area of 23,938 sq km, is located between latitudes 11°08′N and 13°22′N and longitudes 6°52′E and 9°20′E. The state is bordered to Niger Republic to the north, Jigawa and Kano states to the east, Kaduna state to the south and Zamfara state to the west. The state has 34 Local Government Areas. For the purpose of this study, two local areas were randomly selected as the case study, Batsari and Jibia local governments. Most of the people in Katsina state were engaged in farming, fishing, black smith, trades, transportation, welding/fabrication, plumbing, furniture and so many other activities. The majority of the people are Muslims Hausa Fulani, the rainfall is between months of May to November annually but reduced progressibly from the Southern part of the state to the Northern parts.

2.2. Conventional Diagnosis of Bacterial Pathogens from Burn Wound

Table 1. *Culture-based, biochemical, morphological and molecular testing techniques.*

Test	Type
Gram stain	Morphological
Culture on (selective) agar - nutrient agar cetrimide agar acetamide agar	Culture
Oxidase reaction	Biochemical
API 20e	Biochemical
Species specific gene RT-PCR amplification (Gyrase and Endotoxin encoding genes)	Molecular
16S rRNA amplification and sequencing	Molecular
Antibiogram	Culture
Phytochemical Screening by TLC and Column chromatography	molecular

It Shows a plan for identification of etiological agent of the infection in burned patient. The plan involves Gram-stain for morphological identification, culture for colony growth, biochemical test (Oxidase test and API 20 E) for species identification, molecular test to determine the presence of the bacterial gene and antibiogram to find antibiotic sensitivity and resistance. The methode was that of [4, 9].

Table 2. *Antibiotic-containing discs as positive controls.*

S/NO	ANTIBIOTIC
1	Ampicillin
2	Ciprofloxacin
3	Gentamicin
4	Sulphamethoxazole Trimethoprim
5	Imipenem

2.3. Molecular Diagnosis of Bacterial Pathogens from Burn Wound (Rt-pcr Amplification of Target Genes)

The PCR amplification of target genes was carried out as described by [14]. 0.2 ml PCR reaction tubes were labelled for each gene to be probe for Positive Control, Negative Control and PCR product. 1.5 ml reaction tube (or microfuge tube) was prepared and marked as "reaction mixture" by adding 4 µl each of relevant Forward and Reverse primers (table 3), 100 µl SYBR green master mixed and 52 µl PCR water and vortex well. (This was enough for 4x reactions to allow for pipetting). 10 µl each of sterile water was added to 3 different tubes and labelled Negative Control, control DNA from a known species to the tube labelled Positive Control and the unknown DNA to the tube labelled PCR product respectively.

Aliquot 40 µl of 'master mix' was added into each PCR tube and mixed well by Vortexing then micro-centrifuge for 5 seconds to collect the solution to the bottom of the tube. Using a thermocycler one step RT-PCR block, the PCR reaction tubes was subjected to denaturing step at 94°C for 2 mins, Amplification step 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min. The steps was then repeated for 40 cycles, and a final elongation of 72°C for 3 minutes was given to the reactions.

The RT-PCR protocol was carried out as mentioned above for the 3 different target genes using relevant primers (short neucleotides). After 40 cycles, 15 µl fromeach PCR reactions of neat, 1/10, 1/100 DNA dilutions and water wewe considered and analysed using 2% agarose gel electrophoresis and were also be stained with ethidium bromide and run for 45 mins at 80 volts in 1 X TAE buffer. The visualization and image capture was made using the gel documentation system.

Table 3. *PCR primers for pathogen target genes.*

Pseudomonas aeruginosa gyrasegene:
Forward primer - gyrPA-398, reverse primer – gyrPA-620
Pseudomonas aeruginosa endotoxin-encoding gene:
Forward primer - ETA-1, reverse primer – ETA-2
16S rRNA:
Forward primer – 16S1, reverse primer -16S2

2.4. Plant Sample Collection, Preparation and Extraction

The medicinal plants *Prosopisafricana*were first identified in Barawa village, Batagarawa local government area while the *Vachellianilotica*in Wagini village of Batsari local government allin Katsina state with the help of a traditional medicinal practitioner. The Stem bark for *Prosopisafricana* and pods of *Acacia nilotica*were collected from plant species in the different locations and the method of collection was that of [9, 15].

The collected samples of the two medicinal plants were allowed to dry at room temperature and crushed into powdered using mortar and pestle. The powdered sample were placed in a separate polythene bags and stored at room temperature before the extraction of the samples for phytochemicals Screening [15]. The powdered sample was soak in triply distilled water in a beaker and allowed to stand for forty eight (48) hours. The resulting solutions were then filtered and the filtrate (extracts) was carried on in the phytochemical screening [15].

2.5. Antimicrobial Susceptibility Testing

Agar diffusion test well variant method was used for the test for anti-bacterial activity. The bacteterialinoculums was uniformly spread using sterile cotton swab on a sterile petri dish MH agar. Nine (9) serial dilutions yielded concentrations of 100, 80, 60. 40, 20, 10, 5, 2.5, and 1.25 mg/mL for extracts and fractions and four (4) serial dilutions yielded concentrations of 20, 15, 10, 5 mg/Ml for pure substances. 50 µ/L of the two plants Species were added to each of the 5 wells (7 mm diameter holes cut in the agar gel, 20 mm apart from one another). The systems were incubated for 24 h at 36°C ± 1°C, under aerobic conditions. After incubation, confluent bacterial growth was observed. Inhibition of the bacterial growth was measured in mm. Reference commercial discs were used. Tests were performed in duplicate [16].

2.6. Data Presentation and Statistical Analyses

Variability between assays and significance between replicates in the susceptibility, culture growth rate of before and after exposure to antimicrobials and pathogenicity assays was carried out and expressed as mean and SD which we were able to be calculated using GraphPad PRISM software. Also, one-way ANOVA and Dunnett multiple comparison with a control test will be used to determine the significance difference of $p<0.05$ at 95% confidence level. The data would be presented in histograms, scatter line graphs, tables, and images.

3. Results and Discussion

In the present study, a total of 50 samples each of the three categories of burn wound were collected (Table 4). Of the 50 samples, *P. aeruginosa* were isolated from 7 (14%), 12 (24%) and 17 (34%) samples of first, second and third degree of burn wounds respectively and were considered both positive for *P. aeruginosa* infection. However, *Staphylococcus aureus* was isolated only in the third degree from 4 out of 50 samples and was considered to be negative from first and second degree. Coliform (except *E. coli*) were absent from first degree but present in both second and third degrees (4 and 6) respectively. The distribution of the bacterial isolates among the categories of burn wound is listed out in Table 4. In this study the most prevailing bacterial pathogens from all the categories of burn wound is *P. aeruginosa*.[14] reported that burn wound sepsis is presently the main cause of morbidity and mortality after burn trauma. Infections by notorious pathogens such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Acinetobacter baumannii* impair patient recovery and can even lead to fatality. Severe burns are very devastating forms of trauma which require immediate and specialized medical care. The immunosuppression state, triggered by the burn trauma and the wound local micro environment are favorable elements for microbial colonization and proliferation. Therefore extra care should be given to burn wound patients to avoid microbial contaminations throughout the period of medication and diagnosis. Prior to the late 1950s burn deaths related to infection were caused by the gram-positive bacteria *Staphylococcus aureus* and *Streptococcus pneumonia* [17]. In another research by [15] it was reveals that variety of aerobic bacteria were responsible for colonization and as well as infection of burn wounds. In their study, It was observed that more or less 30% infections of burn wound caused by Gram positive (including Coagulase positive, Coagulase negative Staphylococci and Enterococci) bacteria.

Table 4. *Frequency of occurrence and distribution of different bacterial species according to category of burn.*

S/N	Bacterial pathogen	1st degree	2nd degree	3rd degree	Gram staining
1	*P. aeruginosa*	7	12	17	-
2	Staphylococcus aureus	0	0	4	+
3	Coliform (except *E. coli*)	0	2	6	-

A prepared cultured bacterial plate sample was provided where Gram negative bacilli bacteria was identified by using Gram's stain technique and pinkish coloration, rod-like, mostly single cells were present (Figure 1). And for the culture test, a circular raised with smooth margin green pigment colonies on cetrimide agar as a selective for the isolation of *P. spp* was isolated. On acetamide agar, alkalinisation had occurred which is characterized by changes in colour from phenol red to purple red indicating that non-fermenting Gram negative bacteria has deaminated the acetamide resulting in the production of ammonia which is as a result of raising the medium pH (Figure 1).

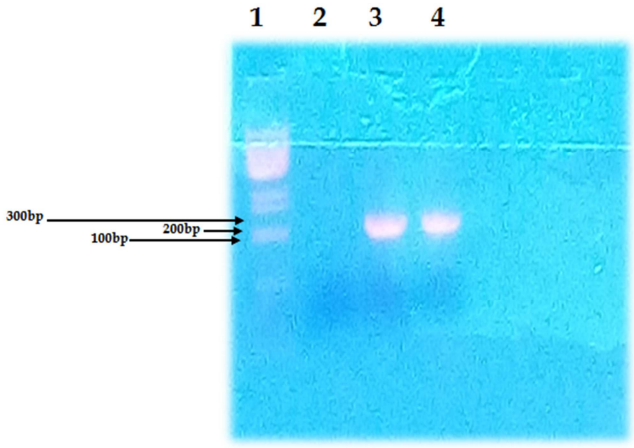

Figure 1. *Gel electrophoresis amplification of 16S rRNA gene, lane 1 BstEII marker, lane 2 negative control, lane 3 positve control, and lane 4 case study sample. The gene cut size is 300bp.*

Figure 1, represents a RT-PCR amplification of 16S rRNA target gene, the case study sample on lane 3 marches with the positive sample on lane 4 (have the same band intensity), both samples cuts at 300bp confirming the presence of *P. aeruginosa* fragment in the case study sample and no amplification was detected from the negative control. The identification was completed by copying the sequence for 16S rRNA gene and pasted into NCBI BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi#alnHdr_1016892938) database and an identity corresponding to Pseudomonas aeruginosastrain ATCC 27853, complete genome was obtained at 100% exactness (Table 5). Also, Figure 2 is the melt curve for endotoxin encoding gene of *P. aeruginosa*, the amplified sample curves matches with that of the positive samples indicating that the gene was detected from the case study sample. Also, in Figure 3 shows the expression of gyrase gene in both the case study and positive samples suggesting that the gene was present in both samples while no peak curve was detected in all the negative controls for both endotoxin and gyrase melt curves.

Table 5. *16S rRNA gene sequence information for P. aeruginosa strain ATCC 27853 from NCBI BLAST database.*

Hints	Score	Total score	E. value	Identities	Gaps	Strand	Accession No.
P. aeruginosa strain ATCC 27853, complete genome	2279 bits (1234)	9119	0.0	1234/1234 (100%)	0/1234 (0%)	Plus/minus	CP015117.1

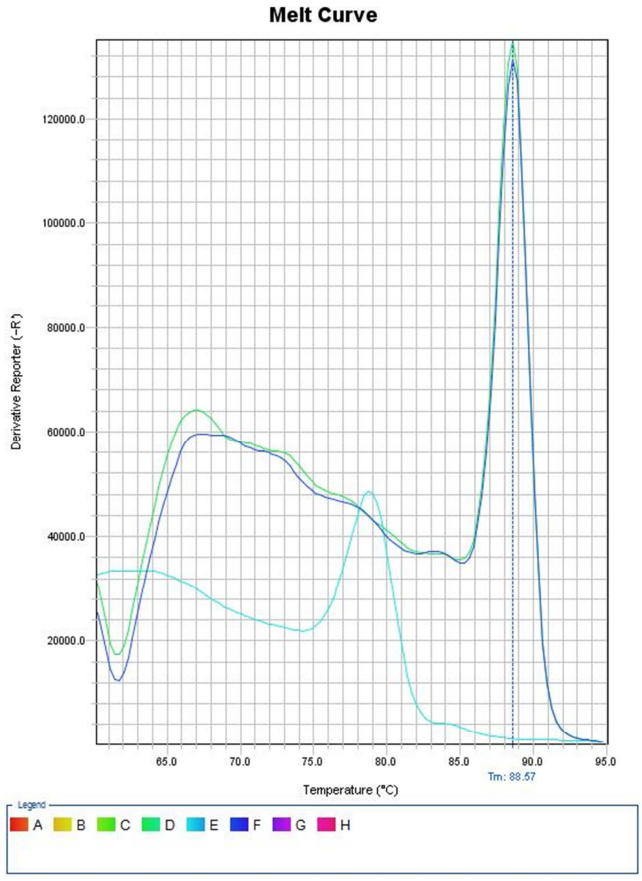

Figure 2 *Represents expression of endotoxin curve encoding gene melt curve E case study sample control (yellow),(blue) marches with F positive control (deep blue), ample (green) and no template for negative control.*

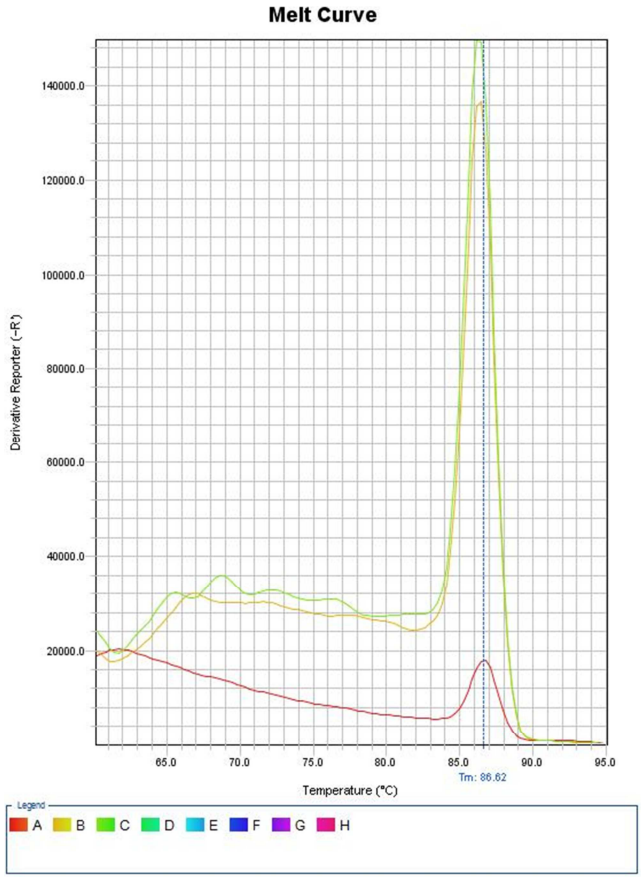

Figure 3. *An expression of gyrase gene melt A negative control (red), B positive and C case study.*

In this case study, we compare the conventional tests with molecular diagnostic test for the detection of *P. aeruginosa*, in which our lab findings show that the conventional culture

test used for the bacterial identification based on morphology, Gram reaction, and biochemical characteristics are all sensitive as molecular test which is considered as an alternative to using for the detection and identification of *P. aeruginosa* in previously healthy burn victim. Observing this from various points, Gram stain can be considered as a rapid test but solely depend on the microbiological culture test, likewise majority of the biochemical test such as oxidase and API 20E tests. Previous decades, several molecular diagnostic tests for the detection of *P. aeruginosa* have been designed. A number of researchers discover highest sensitivity of PCR over culture and or biochemical tests in the detection of *P. aeruginosa* from CF patients while some found no difference or even lower sensitivity in PCR assay [8, 17]. Also, reference [4] suggests that culture based test was considered to be gold standard techniques due to the fact that it is sensitive, allow the identification of viable microorganism and their specific specie under investigation. The culture test requires more time at least 24 hours to produce result, and may not allow the growth of some microorganisms like mycobacterium as reported by [2].

The appropriate technique to be considered in the diagnostic laboratory with large volume of samples from patients may be genetic technique which involves the combination of 16S rRNA gene sequencing, endotoxin, and gyrase genes expression due to its predictive significance for imminent in *P. aeruginosa* infection and antibiotic resistance, it also detects organisms that cannot be isolated on culture media as well as recognising pathogens that are novel as reported by [18] from his findings. Consequently, this technique cannot tell whether the detected fragment of the organism is viable or dead or it is the causative agent of the infections, inaccuracy of some sequences from databases, lack of quantitative meaning of actual identification (genus/specie) on 16S rRNA gene sequence data; there is also a communication problem due to multiplicity in the genotypic and phenotypic variations, the technique cannot be use apart from large volume laboratories or in reference databases due to its cost effectiveness and technical challenges [19].

To conclude, it is better to adopt the combination of the use of conventional microbiological culture test and molecular in the routine diagnostic laboratories for identification detection of *P. aeruginosa*. Moreover, more assessment and validation are required for other identification techniques that were suggested to be more sensitive than culture test [19]. The 16S rRNA which is an orthologs gene, present and highly conserved in all bacteria, endotoxin encoding gene virulent factor in burn infection, and gyrase genes responsible for developing antibiotic resistance in *P. aeruginosa* are the target genes [2]. *P. aeruginosa* is the suspected causative agent of this kind of infection, the culture based, biochemical, and Gram stain methods have shown that they are equally sensitive to each other but also the combination of these with nucleic amplification assay (PCR), both approaches may provide a definitive conclusion in the choice of antibiotics for proper therapeutic [1].

The use of antigenic detection assay such as Enzyme-linked immune sorbent assay (ELISA) may be considered as an alternative to PCR for the detection of bacterial pathogens due to its specificity, less time consuming, easy to use, and economical compared with PCR. ELISA can detect O-antigen, the glycan polymer that is present within LPS of Gram negative bacteria cell wall. LPS roughness in bacteria makes cell membrane more porous to antibiotics that are hydrophobic and that makes O-antigen appear on the bacteria cell wall surface and due to this nature of the O-antigen, it can be detected by antibodies and ELISA can also be used in the determining the suitable antibiotic therapy [20].

In considering the above findings for this case study, the use of Imipenem in combination with gentamicin and ciprofloxacin as the suitable antibiotics to the treatment of infection caused by *P. aeruginosa* in burn patients is suggested. This is due to the fact that gentamicin is a member of aminoglycosides group that inhibits the process of bacterial protein synthesis which binds to the 30s ribosomal molecule, ciprofloxacin is fluoroquinolones group member having anti *Pseudomonal* activity, and Imipenem is a member of beta-lactams group responsible for inactivating the penicillin binding proteins (PBPs) contained in the bacterial cell wall synthesis [21, 22]. Endotoxin encoding gene in Gram negative bacteria responsible [7].

Table 6. *Antibacterial Activity of Pod ethanol extracts of Vachellianilotica against P. aeruginosa.*

S/No	1	2	3	4
Concentration (µg/disc)	10^{-1}	10^{-2}	10^{-3}	10^{-4}
Zone of inhibition	10.0	6.0	3.0	0.3

Table 7. *Antibacterial Activity of Stem bark ethanolic extracts of Prosopisafricana.*

S/NO	1	2	3	4
Concentration (µg/disc)	10^{-1}	10^{-2}	10^{-3}	10^{-4}
Zone of inhibition	7.0	3.0	1.0	0.0

Tables 6 and 7 presents the results for antibacterial properties of the ethanol extracts of pod and back extracts of *V. nilotica* and *P. africana* respectively. The result showsrelatively anti bacterial properties of both plant extracts against *P. aeruginosa.* It is seen that the higher the concentration and the better the antibacterial effect. Therefore, in this study, the result obtained proved that the pod ethanol extract of *V. nilotica* and stem bark, ethanol extract of *P. africana* at different concentrations were reasonably effective against *P. aeruginosa*. This confirmed that the pod ethanol extract of *V. nilotica* constitute chemical compounds that can inhibit the emergence and growth of *P. aeruginosa*. [9] Also reported that pod extract of *A. nilotica* inhibited the growth of various microorganisms including different bacterial strains at different concentrations. Literature reports several recorded use for *V. nilotica* in traditional and orthodox medicine to treat various illnesses [21]. In a research similar to this, the fruits of *V. nilotica* were screened for antimicrobial activity against Five Gram negative bacteria (*Escherichia coli, Shigellaflexneri,*

Salmonella typhi, Klebsiella pneumonia and *Pseudomonas aeruginosa*) and Two Gram positive bacteria (*Listeria monocytogenes* and *Bacillus cereus*) using the cup plate agar diffusion method. The extract obtained from the fruits of *V. nilotica* exerted pronounced activity against several bacteria strains tested as indicated by diameter of growth inhibition zones [6]. These antibacterial property of *V. nilotica* is believed to be based on the phytochemical compounds present in the pod of the plant. It is therefore, important to screen and isolate the active chemical compounds from the pod of this important medicinal plant.

However, with respect to the identified antibacterial properties of the extract of *P. africana* the ascending grades of aqueous extract of *P. africana* acted against the selected Gram positive and Gram negative microorganisms, i.e., *Staphylococcus aureus, Streptococcus pyogenes*, *Escherichia coli* and *Klebsiella pneumonia* [17].

4. Conclusion and Recommendations

This study has shown a high index of wound contamination with prevalence of *Pseudomonas aeruginosa* in all the categories of burn wounds, coliform in second and third degree burn wounds and *Staphylococcus aureus* only in the third degree burn wound. Concentional method of diagnosing burn wound is effective affordable and reliable while molecular diagnosis is expensive, less available and requires the use of high-tech equipments which are not accessible everywhere. The two medicinal plants were both confirmed to be of effective antibiotics against *P. aerigunosa*. It is recommended that wound specimens should be collected for culture and susceptibility testing before the administration of antibiotics. This will help the clinicians in appropriate antibiotic selection and chemotherapeutic management of burn wound infections. It has also been concluded that the therapeutic uses of these analyzed medicinal plants was due to the presence of phytochemicals constituents. Hence preliminary studies to extract, isolate and identify the chemical compound responsible for the antibacterial property is recommended. Similar research can also be carried out on other plants species used traditionally for the treatment of burn wound and other ailments.

Acknowledgements

The authors would like to express their sincere gratitude to Umaru Musa Yaradua University and TETFund, for the encouragement and financial support.

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