MOLECULAR PROFILING OF MICROORGANISMS IN CUTANEOUS WOUNDS OF PATIENTS AT TERTIARY HOSPITALS IN SOUTH-SOUTH NIGERIA

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Abstract: The presence of pathogenic microorganisms in cut wounds often leads to wound infection. This investigation is aimed at identifying microorganisms associated with cut wound infection. A total of 40 cut wound specimens were collected from patients within the age interval 11-70 years with cut wound infection in the surgical unit of Federal Medical Centre, Yenagoa, and Federal Medical Centre, Asaba. The specimens were analyzed utilizing culture, Gram staining, biochemical analysis, Kirby Bauer disc diffusion technique, and molecular technique using DNA sequencing for confirmation of the isolates.The bacterial isolates identified were *Bacillus* sp. 1(6.67%), *Escherichia coli* 1(6.67%), *Klebsiella pneumoniae* 2(13.3%), *Pseudomonas aeruginosa* 3(20%) and *Staphylococcus aureus* 3(20%), while the fungal isolates were *Aspergillus japinocus* 1(6.67%), *Candida albicans* 2(13.3%), *Histoplasma capsulatum* 1(6.67%), and *Madurella* sp. 1(6.67%). The predominant organisms associated with the cut wound infection were *Pseudomonas aeruginosa* (20%) and *Staphylococcus aureus* (20%). Most isolates showed high resistance to antibiotics used in the susceptibility test. Phylogenetic analysis using 16s rRNA sequences identified bacterial isolates; *B. cereus*, *B. thuringiensis*, *K. pneumoniae, P. aeruginosa*, and fungal isolates; *C. albicans* and *A. japinocus.* Regular surveillance and molecular profiling of multidrug-resistant organisms are imperative for controlling the dissemination of these pathogens that do not tolerate antibiotics.

Keywords: Molecular profiling, susceptibility, bacteria, fungi, cut wounds

Introduction

Microbiologically, the main role of healthy, unbroken skin is to regulate the presence of microorganisms residing on its surface and to shield the tissues beneath from colonization and invasion by harmful pathogens. When skin integrity is compromised, such as in the case of a wound, it exposes subcutaneous tissues, creating an ideal habitat for microbial colonization and proliferation (Kemebradikumo *et al.,* 2013). Wound colonization typically involves a variety of microorganisms, many of which can be harmful. Consequently, there is always a risk of infection in any wound (Dowd *et al.*, 2008, Kemebradikumo *et al.,* 2013). Wound infection is among the most prevalent health issues, as noted by Giacometti *et al*. (2000). It often occurs when bacteria enter through compromised skin. Comparable to renal and cardiovascular conditions in significance, wounds can significantly affect the quality of life. According to the Global Wound Care Market report, the industry was valued at approximately 20 billion dollars and is projected to surpass 25 billion dollars globally by 2023 (Weller *et al*., 2020).

Wounds are typically classified based on their underlying causes, which can be either acute or chronic. Acute wounds result from external injuries to intact skin, encompassing various types such as surgical incisions, bites, burns, minor cuts, and abrasions. They also include more severe traumatic injuries like lacerations crush injuries, or those caused by gunshots. On the other hand, chronic wounds usually stem from internal factors associated with an underlying condition that weakens the integrity of both dermal and epidermal layers, as described by Bowler *et al*. (2001). Examples of chronic wounds include leg ulcers and foot ulcers.

Various microorganisms, including bacteria, fungi, and parasites, are implicated in wound infections, as identified by Bowler *et al*. (2001). Additionally, the composition of the wound microbiome may be influenced by ecological factors, leading to the emergence of polymicrobial infections with synergistic effects and increased resistance to antimicrobials (Baishya and Wakeman, 2019; Kalan and Brennan, 2019). Common pathogens associated with wound infections include *S. aureus*, Coagulase-negative Staphylococci, Enterococci, *E. coli*, *P. aeruginosa*, *Enterobacter* species, *Proteus mirabilis*, *K. pneumoniae*, *Propionibacterium*, *C. albicans*, Group D Streptococci, *Bacteroides fragilis*, and *Clostridium tetani*, as reported by NNIS (1996), El-Saed *et al*. (2020), Gupta *et al*. (2019), and Scharschmidt and Fischbach (2013).

Molecular technologies have revolutionized the study of human microbiota by providing researchers with rapid and precise tools, offering a significant advantage over traditional culture-based testing methods (Bowler and Davies, 1999). Most research utilizes universal primers for 16S rRNA genes, complemented by specific identification techniques such as polymerase chain reaction (PCR) (Hill *et al*., 2003), denaturing gradient gel electrophoresis, and sequencing (Dowd *et al*., 2008a, 2008b; Price *et al*., 2009). Additional molecular methods employed in the analysis of microorganisms associated with wounds include Pulsed Field Gel Electrophoresis (PFGE), Random Amplified Polymorphism DNA, and Plasmid Profile Analysis (Frederick *et al*., 2012). However, this study is aimed at identifying the diverse microorganisms associated with cutaneous wounds using molecular techniques.

Materials and Methods

Study area

The areas of study are Federal Medical Centre (FMC), Yenagoa, Bayelsa State, and Federal Medical Centre (FMC), Asaba, Delta State. Both health institutions are

located in the southern part of the country, and they are federally-owned medical center that provides quality tertiary health services for patients.

Ethical Approval

Study participants provided explicit compliance following a thorough explanation of the investigation orally and a guarantee of privacy. Before commencing the study, written permission from the Department of Accidents and Emergencies/Injuries, FMC, Yenagoa, Bayelsa State, and Federal Medical Centre Asaba, Delta State was given.

Specimen collection and processing

A total of 40 wound swabs were obtained from patients with cut wounds from Federal Medical Center, Yenagoa, and Federal Medical Centre, Asaba for two months. Employing the Levine approach, the cut wound specimens were collected before dressing (Huddleston, 2014). The swab stick was then rotated on the wound for 5 seconds while applying enough pressure to produce exudates. The specimens were labeled and transported to the laboratory for analysis under aseptic conditions (Cross, 2014). The cross-streak technique was used to aseptically inoculate the obtained clinical specimens onto the bases of MacConkey agar (Oxoid, England), chocolate agar, and Sabouraud dextrose agar (Oxoid, England), and incubated aerobically at 37° C for 24 hours and Sabouraud dextrose culture plates at 25°C for 3-7 days. Bacterial colonies obtained from the agar plates were gram-stained and subjected to biochemical tests (catalase, oxidase, urease, citrate, citrate, motility, indole, and sugar fermentation) for identification (Cheesbrough, 2006). Fungal colonies were subjected to lactophenol staining and further identification of microbial isolates was done using polymerase chain reaction and DNA sequencing.

Antibiotic Susceptibility Test

The antibiotic susceptibility was determined using the Kirby- Bauer disc diffusion method and interpreted using Clinical Laboratory Standards Institutes guidelines (CLSI, 2020) standards on Muller Hinton Agar to ascertain the antibiotic susceptibility pattern of the bacterial isolates. The bacterial inoculum was generated using nutrient broth (HI Media, India) and the turbidity was adjusted to 0.5 McFarland standard. A sterile swab stick dipped in the solution was used to uniformly cover freshly prepared Muller Hinton Agar. Using sterile forceps, an appropriate antibiotic disc containing ten (10) different antibiotics for gram negative; CN- gentamycin (10µg), PEF-reflacine (10µg), NA- nalidixic acid (20µg), OFX-tarvid (10µg), CEP- ceporex (30µg), PN-ampicillin (10µg), S- streptomycin (30µg), SXT-septrin (30µg), CPX- ciprofloxacin (10µg), AU-augmentin (30µg), and antibiotic disc containing ten (10) different antibiotics for gram positive; S-streptomycin (30µg), NB- norfloxacin (10µg), AMX-amoxil (20µg), RD- rifampicin (20µg), APX_-ampliclox (20µg), CN- gentamycin (10µg), LEV-levofloxacin (20µg), E- erythromycin (30µg), CPX-ciprofloxacin (10µg), CH-chloramphenicol (30µg) was put on the surface of the agar plate seeded with each isolate and softly pressed down to ensure contact with the agar plate. After that, the plate was inverted and incubated at 37°C for 24 hours. The test plates were inspected, and the zone of inhibition in millimeters was measured (mm). The antibiogram profile was interpreted based on the diameter of the zones of inhibition demonstrated by the individual drugs in mm, with the test results classed as Sensitive (S), Intermediate, or Resistant (R). To assess the antibiotic's sensitivity, intermediate, and resistance to bacteria, the Clinical Laboratory Standards Institute guidelines were utilized.

Antifungal Susceptibility Test

The disc diffusion method was used for this purpose. Filter paper discs (4mm diameter) impregnated with 10mg/ml and 20mg/ml concentrations of antifungal agents fluconazole. Itraconazole is placed on SDA plates previously inoculated with the isolates. Filter paper discs soaked in sterile distilled water without any antifungal agent were placed on the culture plate to serve as a control. The culture plates were inoculated at 37°C for 24h. Antimicrobial activity was determined by measurement of the zone of inhibition around the test organisms. The absence of a zone of inhibition was considered resistant by the test against the antifungal agent (Doughari and Sunday, 2008).

Bacterial Detection by Polymerase Chain Reaction

DNA was extracted directly from clinical samples that had been stored at -80°C, using the boiling method described by Weisburg *et al*. (1991). Approximately 1.5 ml of the clinical specimen in broth was centrifuged at 10,000 rpm for 5 minutes, and the supernatant was subsequently discarded. The pellet was resuspended in about 500 µl of sterile water, and the mixture was thoroughly homogenized using a vortex mixer. The suspension was then boiled for 10 minutes in an Eppendorf thermomixer comfort (22331 Hamburg, Germany) set on a heating block (Fisher Scientific). After boiling, the mixture was vortexed again and centrifuged at 10,000 rpm for 5 minutes, followed by vortexing and centrifugation at 10,000 rpm for another 5 minutes. Approximately 500 µl of the resultant DNA solution was transferred to 1.5 ml Eppendorf tubes. The concentration and purity of the DNA were measured using a Nanodrop spectrophotometer (ND-1000, UV/VIS spectrophotometer, USA), with sterile water serving as the blank control in the measurement.

DNA Amplification

Species-specific primers were utilized to perform the amplification. The primer sets employed included 16S rRNA for bacteria: 243F 5'-GGATGAGCCCGCGGCCCTA-3' and A3 3'-CCAGCCCACCTTCGAC-5', and ITS for fungi: ITS-1 5'-TCCGTAGGTAGAACCTGCGG-3' and ITS-4 3'-TCCTCCGTTATTGATATGC-5'. The PCR amplification was conducted in a 25 µl reaction volume that contained 12.5 µl of GoTaq Green master mix, 0.25 µl of each primer (upstream and downstream), 3 µl of DNA template, and 9 µl of nuclease-free water to achieve the desired volume. The amplification was performed in an Eppendorf Master Cycler gradient. The thermal cycling conditions involved an initial denaturation step at 94°C for 3 minutes; followed by 35 cycles of denaturation at 95°C for 1 minute, annealing at 54°C for 1 minute, and extension at 72°C for 2 minutes. A final extension was conducted at 72°C for 5 minutes (Vickerman *et al*., 2007). Following amplification, the PCR products were separated using 1.5% agarose gel electrophoresis at 100 volts for 2.5 hours, employing an

electrophoresis tank (Sigma Chemical Company). The gels were stained with 0.5 µg/ml ethidium bromide and visualized under a UV transilluminator with a digital camera (Optima UVT 260D, S/N-268002). A 100 bp DNA ladder was used as the molecular weight marker.

DNA Sequencing

DNA extraction from cut wound specimens was performed using a Bacterial DNA extraction Kit according to the manufacturer (Zymo Research, South Korea). According to the method, genomic DNA was extracted by transferring 1 ml of the cut wound specimen into a sterile 1.5µl microtube using a micropipette. The sample was washed three times by centrifuging at 8000 x g in a microcentrifuge. The pellets were suspended in 200ul of sterile water and then added into a ZR Bashing Bead ™ Lysis Tube followed by the addition of 750 µl lysis solution to the tube. The content of the 2ml tube was disrupted by mixing in a vortex mixer at maximum speed for 5 minutes. The ZR Bashing Bead ™ Lysis Tube was centrifuged in a micro centrifuge at 10,000 x *g* for 1 minute. About 400 µl of the filtrate was added to a Zymo-Spin ™ IIC Spin column in a collection tube and centrifuged at 7,000 rpm (˜7,000 x *g*) for 1 minute. This was followed by the addition of 1,200 µl of gDNA binding buffer to the filtrate in the collection tube. About 800 µl of the mixture from above was added to a Zymo-Spin ™ IIC Column in a collection tube and centrifuged at 10,000 x *g* for 1 minute. The flow through from the collection tube was discarded and this particular step was repeated with the remaining filtrate. About 200 µl of DNA Pre-Wash Buffer was thereafter added to the Zymo-Spin ™ IIC Column in a new collection tube and centrifuge at 10,000 x *g* for 1 minute after which 500 µl of gDNA wash buffer was added to the Zymo-Spin ™ IIC Column and centrifuged at 10,000 x *g* for 1 minute. The Zymo-Spin ™ IIC Column was transferred into a clean 1.5 ml micro centrifuge tube and 100 µl DNA Elution Buffer was added directly to the column matrix. This was centrifuged at 10,000 x *g* for 30 seconds to elute the DNA which was used for PCR and DNA sequencing.

DNA sequencing was conducted using Next Generation Sequencing techniques with species-specific primers for bacteria 16Rrna; 243F 5'GGATGAGCCCGCGGCCCTA-3' A3 3'CCAGCCCACCTTCGAC-5' and fungi ITS: ITS-1 5' TCCGTAGGTAGAACCTGCGG-3': ITS-4 3' TCCTCCGTTAT TGATA TGC-5' as described by Weisburg *et al*., 1991. The sequences of all microorganisms present in the sample were determined using the Genome Sequencer™ FLX System from 454 Life Sciences™ and Roche Applied. Sequence analysis and alignment were carried out using the Vector NTI Suite 9 (InforMax, Inc.). The obtained nucleotide sequences were compared with those in GenBank using BLAST analysis through CLO Bio software. A resampling support tree derived from 500 resamples (Felsenstein, 1985) was employed to depict the phylogeny of the examined taxonomic group. The tree was produced using BLAST pairwise alignments. Phylogenetic distances were calculated utilizing the Jukes-Cantor method (Jukes and Cantor, 1969). Subsequently, alignment of these sequences was performed utilizing ClustalX. The phylogeny was then deduced using the Neighbor-Joining method within MEGA 6.0 (Saitou and Nei, 1987).

Statistical analysis

The data obtained was subjected to Analysis of Variance (ANOVA) test to determine the significant difference at 95% confidence limit.

Results

Out of the 40 cut wound specimens investigated, 24(60%) were from males and 16(40%) from females of which 15 isolates were obtained. The bacterial isolates identified were *Bacillus* sp. 1(6.67%), *E. coli* 1(6.67%), *K. pneumoniae* 2(13.3%), *P.aeruginosa* 3(20%) and *Staphyloc occus aureus* 3(20%), while the fungal isolates were *A. japinocus* 1(6.67%), *C. albicans* 2(13.3%), *H. capsulatum* 1(6.67%), *Madurella* sp. 1(6.67%) as shown in **Figure1**. The highest number of bacterial isolates were observed with *P. aeruginosa* and *S. aureus,* 3(20%) respectively, while *E. coli* and *B. cereus* had the lowest number with 1(6.67%). The highest number of fungal isolates was seen with *C. alblicans,* 2*(*13.3%).

Table 1 indicates the distribution of bacterial isolates by age and sex. The highest incidence of bacterial isolate was seen within the age bracket of 61-70 years while the least was seen within the bracket 11-20 years, 21-30 years, and 41-50 years. The sex with the highest number of bacterial isolates was male with 7(70%) while the least was female with 3(30%). Statistically, there was no difference between the age and sex of patients with bacteria isolated from cut wounds ($p = 0.22$, $P \geq 0.05$)

The distribution of fungal isolates by age and sex is shown in **Table 2**. The highest incidence of fungal isolate was seen within the age bracket of 11-20 years while the least was seen within the bracket of 21-30 years, and 51-60 years. The sex with the highest number of fungal isolates was male with 4(80%) while the least was female with 1(20%). Statistically, there was a significant difference between the age and sex of patients with fungal isolated from cut wounds ($p = 0.005$, $P \leq 0.05$).

The percentage of antibiotic susceptibility pattern of isolated Gram-negative bacteria from the cut wound is indicated in **Figure 2**. *P. aeruginosa* was susceptible to gentamycin, streptomycin, and ciprofloxacin with 1(33.3%) while resistant to reflacine, nalidixic acid, tarivid, ceporex, ampicillin, septrin, and augmentin. *K. pneumoniae* was susceptible to streptomycin with 1(33.3%) while resistant to gentamycin, reflacine, nalidixic acid, tarivid, ceporex, ampicillin, septrin, augmentin, and ciprofloxacin. *E. coli* displayed resistance to all the antibiotics. There was a significant difference ($p<0.05$) between the antibiotic susceptibility profile of Gram-negative bacteria isolated from patients in Federal Medical Center, Yenagoa, and Federal Medical Center, Asaba.

The isolated gram-positive organisms; *S. aureus* and *B. cereus* were susceptible to streptomycin and levofloxacin with 1(33.3%) respectively as seen in **Figure 3**. There was a significant difference ($p = 0.011$, $p<0.05$) between the antibiotics susceptibility profile of Gram-positive bacteria isolated from a patient in Federal Medical Center, Yenagoa, and Federal Medical Center, Asaba.

Figure 4 displays the antifungal susceptibility test. The fungal isolates exhibited high resistance to the antifungal tested. *C. albicans* was susceptible to fluconazole and resistant to Itraconazole . *Aspergillus japinocus* was resistant to both fluconazole and Itraconazole. Statistically, there was no significant difference (P= 0.29, P>0.05) between the antibiotics susceptibility profile of fungi isolated from patients in Federal Medical Center, Yenagoa, and Federal Medical Center Asaba.

Of the forty specimens, fifteen were identified by culture, and eight comprising *B. thuringiensis* 2(25%), *P. aeruginosa*, 1(12.25%), *K. pneumoniae* 1(12.25%), *B. cereus* 1(12.25%), *C. albicans*, 2(25%) and *A. japinocus* 1(12.25%) were further identified by PCR and DNA sequencing as shown in **(Table 3)**. The phylogenetic tree showing the evolutionary relationship between the *Bacillus* species is shown in Figure 4. The evolutionary distances computed using the Jukes-Cantor method agreed with the phylogenetic placement of the 16S rRNA of the isolates within the *Bacillus* species and revealed a close relatedness to *B. thuringiensis.*

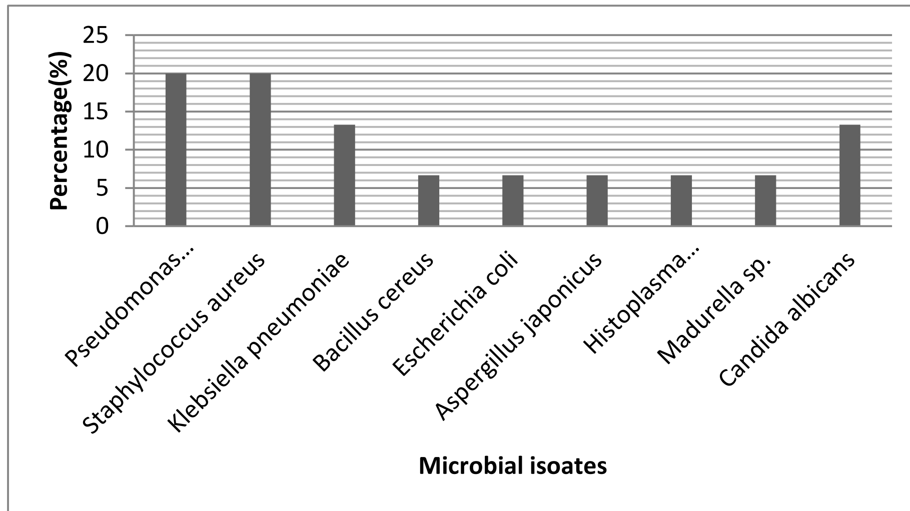

Figure 1: Percentage distribution of the number of microbial isolates

Table 1: Distribution of bacterial isolates by age and sex

Age interval (years)	Male	Female	Total number of specimen	Number of isolates Male	Number of isolates Female	Total number of bacterial isolates
11-20	3	0	3	0	0	0
21-30	5	3	8	0	0	0
31-40	7	4	11	3	1	4
41-50	0	5	5	0	0	0
51-60	3	0	3	1	0	1
61-70	6	3	9	3	2	5
Total	24	16	40	7	3	10

Table 2: Distribution of fungal isolates by age and sex

Age interval (years)	Male	Female	Total number of specimen	Number of isolates Male	Number of isolates Female	Total number of fungal isolates
11-20	3	0	3	1	1	2
21-30	5	3	8	0	0	0
31-40	7	5	11	1	0	1
41-50	0	5	5	1	0	1
51-60	3	0	3	0	0	0
61-70	6	3	9	1	0	1
Total	24	16	40	4	1	5

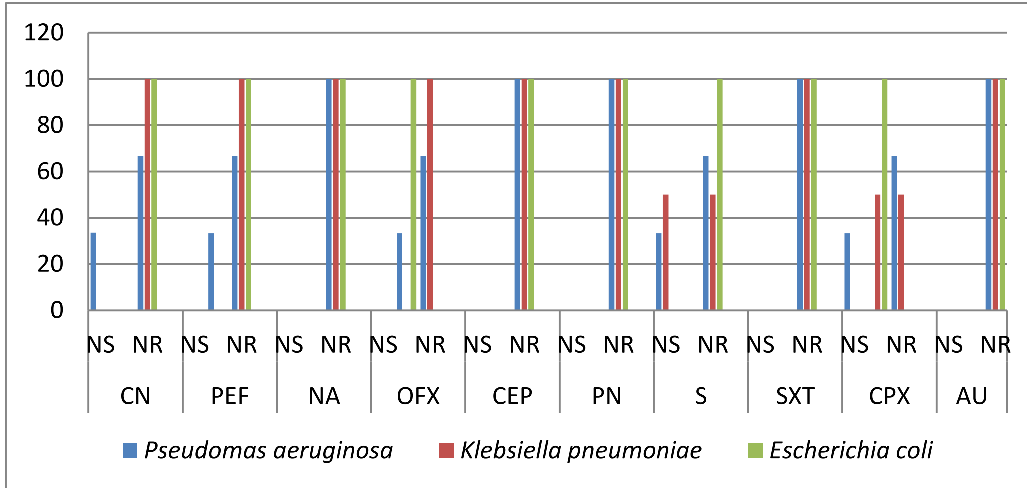

Figure 2: Antibiotic susceptibility pattern of gram negative bacterial isolates from cut wound.
Key: CN- gentamycin, PEF- reflacine. NA- nalidixic acid, OFX-tarvid, CEP- ceporex, PN- ampicillin, S- streptomycin, SXT- septrin, CPX- ciprofloxacin, AU- augmentin
NS-number of sensitive, NI-number of intermediate, NR- number of resistant,

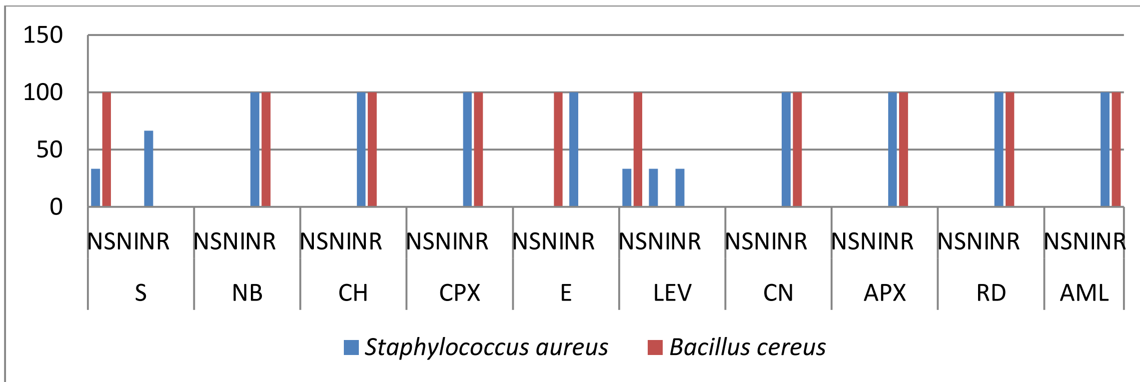

Figure 3: Antibiotic susceptibility pattern of gram positive, isolates from cut wound.
Key: S-streptomycin, NB- norfloxacin, CH-chloramphenicol, CPX- ciprofloxacin, E- erythromycin, LEV- levofloxacin, CN- gentamycin, AML-ampliclox, RD- rifampicin, AMX-amoxil,
NS- number of sensitive, NI- number of intermediate, NR- number of resistant.

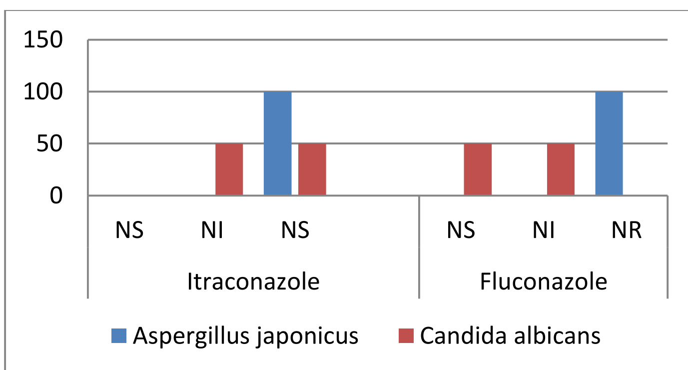

Figure 4: Antifungal susceptibility pattern of fungal isolates from cut wound.
Key: NS- number of sensitive, NI- number of intermediate, NR- number of resistant

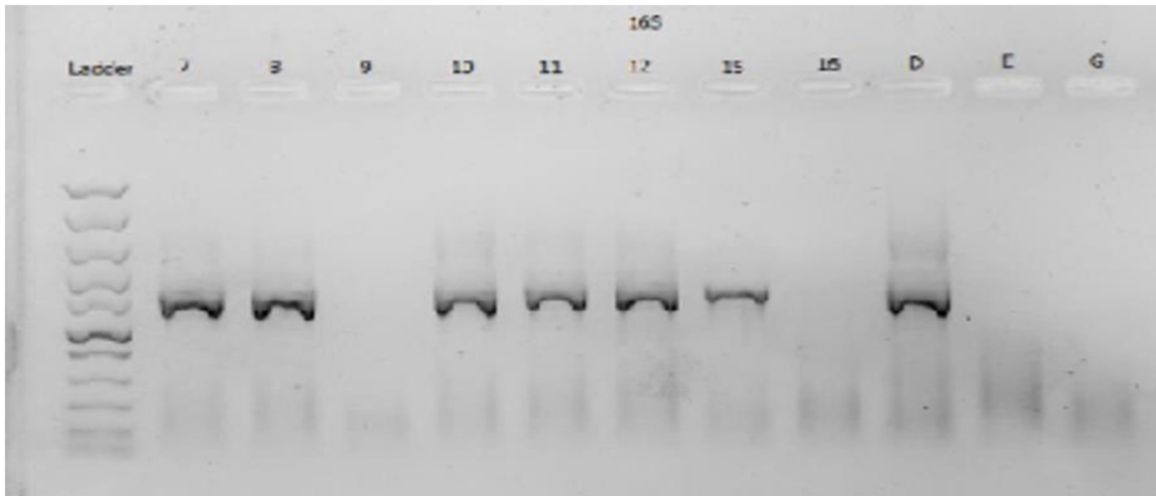

Figure 5: PCR amplicon of 16sRNA conserved region of bacterial isolates before sequencing.

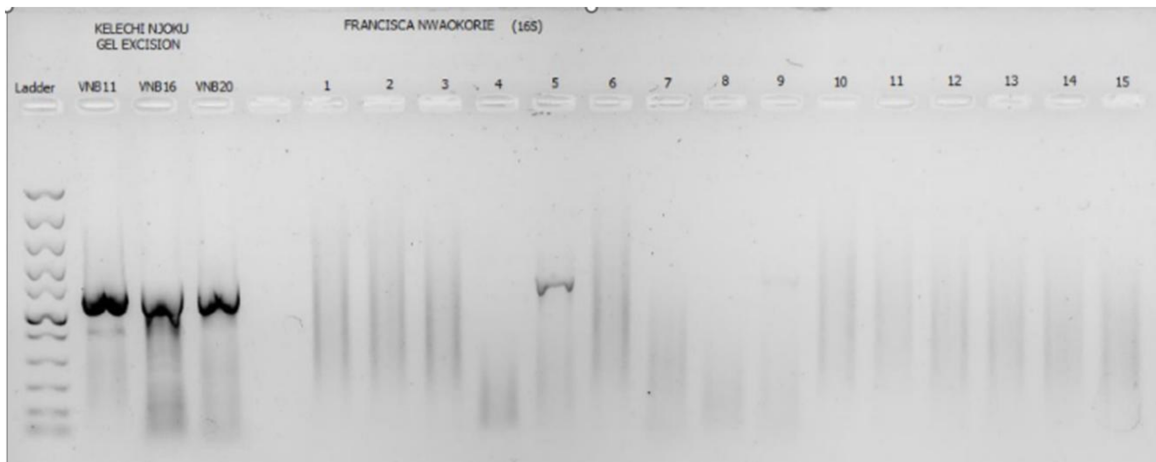

Figure 6: PCR amplicon of 16sRNA conserved region of fungal isolates before sequencing.

Table 3: Characteristics for Blastn analysis and identity of isolates sequenced

S/N	Specimen No./Type	Specimen Number	Sequence Nucleotide Obtained (FASTA format)	Similarity E-score	Gene Bank Accession Number	Identity of Isolate Obtained
1	A	MT	**>A_907R_F05_17** **ACCCAGGGCGGGGTGCTTAATGCGTTACTCAGGCACTAAGGGCGGGAAACCCCTAACACTTAGCACTCATCGTTT** **ACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGTGTCAGTTACAGACCAGA** **AGTCGCCTTCGCCACTGGTGTTCCTSCATCTCTACGCATTTCACCGCTACACTGGAATTCCACTTCCTCTTCTGCAC** **TCA**	94.87/6e-102	MT526248	*Bacillus thuringiensis*
2	D	MT SECRET	>D_907R_E04_13 **CGGGGGGGGCTTAGCGTTACTTCAGCACTAAAGGGGAACATCGTTACGGGGGACTACCAGGGATCTAATCCTGTT** **TGTCCCCACGTTTCCGCCTCAGGCAGTACAGACCAGAAAGCCCTTCGCCACTGGGGTTCCTCCATATTCTACATTT** **CACCGTACCATGAATTCCACTTTCCTCTTCTGACTCAAGTCTCCCAGTCCAATGACCCTCCACGGTGAGCCGGGGT** **TTCCTCAAATTAAGAAACCCCGGGTTTCCTCAAATTAAGAAACCCCTGCGGTTTACGCCCAATAATTCCGGATAAC** **GTTGCCCCTACGATTACCGGGTGTGGACGATTAGCCGTGGTTTTGGTTAGGACCGCAAGGGCCAGTATTAATAACT** **TGTCTTCCCTAAAACAGATTTTACACCCAAAAGCCTTCATCACTCACGCGGGTGGTCCCGGATTTCGCCTTGCGAA** **ATTCCCTACTGTGCCTCCCTAGATTGGCCGTGTCTCATCCAGGGGGCGATCACCCTCTCAGTCGGTACATCGTTGC** **CTTGGTGAGCCTACCTCACCAACTACTAATGGACGCGGGCCATCCATAAGTGACAGCCGAAGCGCCTTTCAATTTC** **AACCATGCGATAAATGTATCCGTATTACCCCGGTTTCCCGATATCCGTTATGGGAGGTAACCCACGTGTACTCACG** **CATCCGCGCTAATTCATAATGAGCAAGCTCTTAATCCATTCGCTCGACTTGCAGATTAGGCAGCCGGCGATCATCC** **TGAGATGATCAAACTTGGG**	85.70/00	OP626157.1	*Bacillus thuringiensis*
3	H	KING STREP	>H_907R_G05_20 ACCAAGGCGGGGTGCTTAATGCGTTAACTTCAGCACTAAAGGGCGGAAACCCTCTAACA**CTTAGCACTCATCGTTTACG** **GCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGTGTCAGTTACAGACCAGAAAG** **TCGCCTTCGCCACTGGTGTTCCTCCATATCTCTACGCATTTCACCGCTACACATGGAATTCCACTTTCCTCTTCTGC** **ACTCAAGTCTCCCAGTTTCCAATGACCCTCCACGGTTGAGTGGGCTTTCACATCAGACTTAAGAAACCACCTGCGC** **GCGCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGTATTACCGCGGGCTGGCACGTAGTTAGCCGTGG** **CTTTCTGGTTAGGTACCGTCAAGGTGCCAGCTTATTCAACTAGCACTTGTTCTTCCCTAACAACAGAGTTTTACGAC** **CCGAAA GCC** **TTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGA** **GTCTGGGCCGTGTCTCAGTCCCAGGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCG** **TTACCTCACCAACTAGCTAATGCGACGCGGGTCCTCATAAGTGACAGCCGAAGCCGCCTTTCAATTTCGAACCATG** **CGGTCAAAATGTTATCCGGTATTAGCCCCGGTTCCCGATTATCCCAGCTTATGGGGGGTACCCCGGGTACTCGCCC** **GCGCCGGTACTTGTAAGAGTCTAGTCCAG**	96.8	GU930360.1	*Bacillus cereus*
4	L	EAO B/D	>L_907R_H05_23 **AGCCGGGCGGGTGACTTAGCGGTTAGCTGCGGCCACTAAGATCTCAAGGATCCCACGGCTAGCGACATCGTTTAC** **GGCGGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTCAGTGTCAGTATCAGCCAGGTGGT** **CGCCTTCGCCACTGGGTTCTTCCTATATCTACGCAGGTATTTCACCGCTACCAGGAAATTCCACCGCCCTCTACCG**	94.64/00	MN538934.1	*Pseudomonas aeruginosa*
5		CAO EC	>M_907R_A06_03 **ACCAGGGGGGGGTCGATTACGCGTTAGCTCGGAAACCCTCAAGGGCACAACCTCCAATCGACATCGTTTACGGCG** **TGGACTACCAGGGTATCTAATCCTGTTGCTCCCCACGCTTTCGCACCTGAGCGTCAGTCTTTGTCCGGGGGCGSCT** **TCGCCACCGGTATTCCTCCAATCTCTACGCATTTCACCGCTACACCTGGAATTCTACCCCCCTCTACGAGACTCTAC** **CTGCCAG**	93.80/00	MT990398.1	*Klebsiella pneumoniae*
6	F2	CANDID	>F2_ITS4_G04_19_copy **CCCGATTTGAGGTCAAAGTTTGAAGATATACGTGGTGGACGTTACCGCCGCAAGCAATGTTTTTGGTTAGACCTAA** **GCCATTGTCAAAGCGATCCCGCCTTACCACTACCGTCTTTCAAGCAAACCCAAGTCGTATTGCTCAACACCAAACC** **CAGCGGTTTGAGGGAGAAACGACGCTCAAACAGGCATGCCCTCCGGAATACCAGAGGGCGCAATGTGCGTTCAAA** **GATTCGATGATTCACGAATATCTGCAATTCATATTACGTATCGCATTTCGCTGCGTTCTTCATCGATGCGAGAACCA** **AGAGATCCGTTGTTGAAAGTTTTGACTATTAGTAATAATCTGGTGTGACAAGTTGATAAAAAATTGGTTGTAAGTT** **TAGACCTCTGGCGGCAGGCTGGGCCCACCGCCAAAGCAAGTTTGTTTCAAAGAAAAACACATGTGGTGCAATTAA** **GCAAATCAGTAA T GATCCTTCCGCAGGTTCACCTACGGAAGAAG**	99.80/00	OK267702.1	*Candida albicans*
7		1B ASPER	>F-4_ITS-1_B06_06 **AGGGGGATCGGTGGGGCCGCCCCCCATCCCCGTTATACCACCCCGTGGTGCTCGGGGGCCCGCTTGTGGGGCCG** **GGGCTGCCCCGGCACCCCGGCCCCTGAACCCATGCAACTCTATCTAAAATGTTTAACGAGTTGATTGATATCTTCA** **TTCAAACTTTTAAAAATGGATCCCTTGGATCCCGTGTCTACGAAAAACAAAGAAAGGAAAATGAATGAATATTGAA** **TTTCGGAATTCTGAAGTTTTGAAGCCTTTGCGCCCCCTGGGCCTCCTGGGGGCTGGGGGGCCGAGCTGCCTTACC** **CCCCTTCTCCCCGCTGGCCGGGGGGGGCCCCCCCCCGCCCCGCCCCCCGAGCCAAGGGG**	67.76/00	JN676110.1	*Aspergillus japonicus*
8		AT AUS	>F5_ITS-1_G04_19 **GTTATTTGCCACATGTGTTTTTCTTTGAACAAACTTGCTTTGGCGGTGGGCCCAGCCTGCCGCCAGAGGTCTAAAC** **TTACAACCAATTTTTTATCAACTTGTCACACCAGATTATTACTAATAGTCAAAACTTTCAACAACGGATCTCTTGGT** **TCTCGCATCGATGAAGAACGCAGCGAAATGCGATACGTAATATGAATTGCAGATATTCGGAATCATCGAATCTTTG** **AACGCACATTGCGCCCTCTGGTATTCCGGAGGGCATGCCTGTTAGCGTCGTTTCTCCCTCAAACCGCTGGGTTTGG** **TGTTGAGCAATACGACTTGGGTTTGCTTGAAAGACGGTAGTGGTAAGGCGGGATCGCTTTGACAATGGCTTAGGT** **CTAACCAAAAACATTGCTTGCGGCGGTAACGTCCACCA.CGTATATCTTCAAACTTTGACCTCAAATCAGGTAGGAC** **TACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAACCG**	99.18	MT640023.1	*Candida albicans*

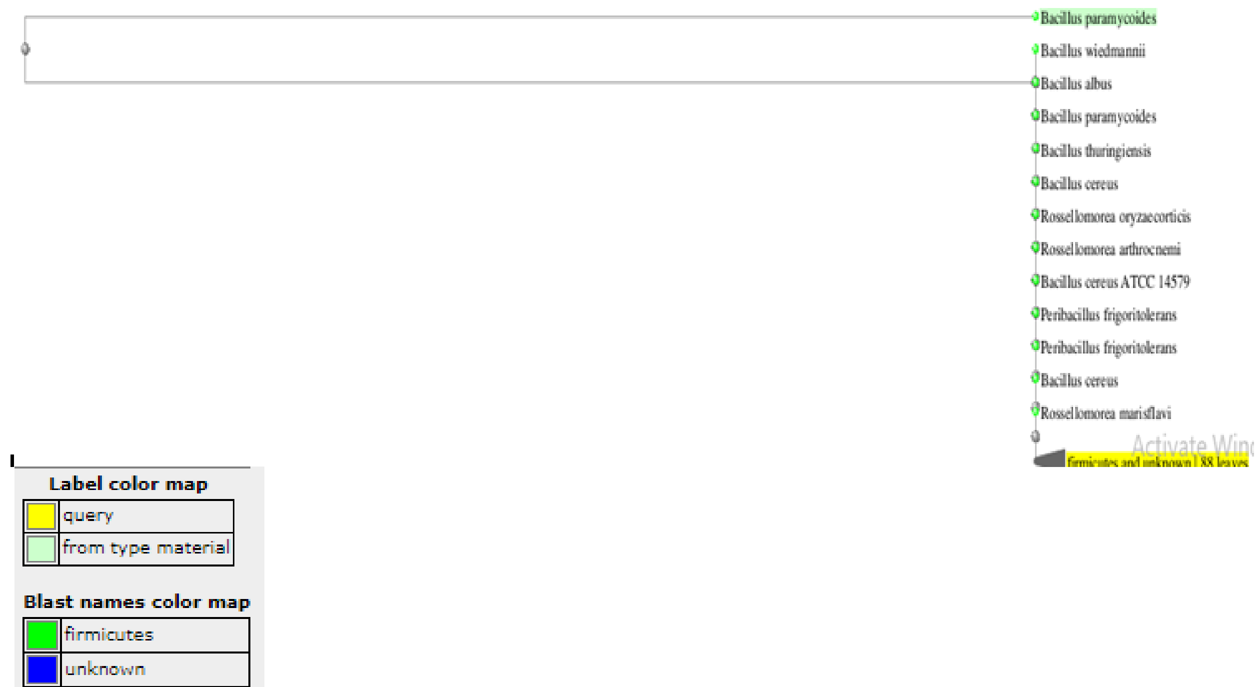

Figure 8: Bacterial phylogenetic tree bases on 16srRNA gene sequences of members of the *Bacillus thuringiensis (*A907R). This tree was produced using BLAST pairwise alignments. Tree method- Neighbor joining.

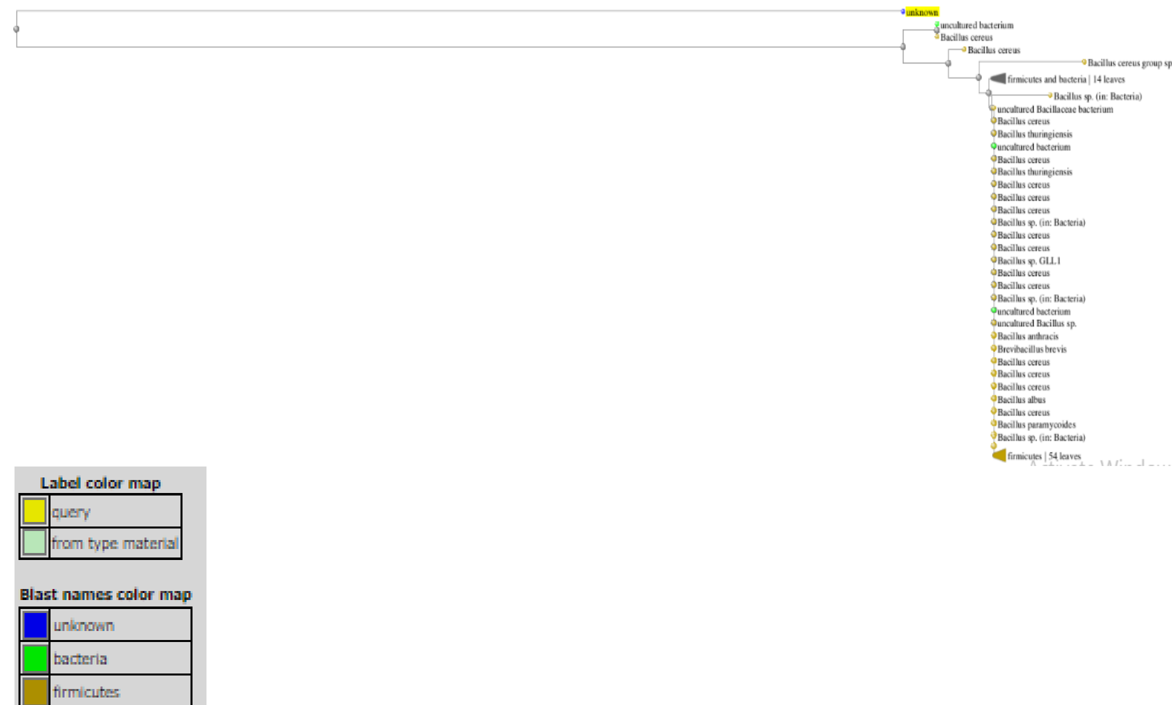

Figure 9: Bacterial phylogenetic tree bases on 16srRNA gene sequences of members of the *Bacillus thuringiensis* (D_907R).

Discussion

Microorganisms, especially harmful microbes in cut wounds lead to wound infection (Kadam and Kaushik, 2020). The contamination of wounds by microorganisms poses a significant challenge in hospital settings, particularly during surgical procedures, where sterile sites can become contaminated, leading to infections (Kemebradikumo *et al*., 2013).

Many overall changes in wound infection and healing capacity are related to age. People at extreme age (age of

60 years and above) are more prone to wound infection. In this study, the finding demonstrated bacterial colonization and wound infection were observed more in the age of 61-70 years, this could be attributed to the weak immune status of these patients resulting in a delayed healing process. Our result is consistent with the findings of the study done in Ethiopia (Mulugeta and Bayeh, 2011). The highest incidence of the fungal isolate was seen within the age bracket of 11-20 years while the least was seen within the bracket of 21-30 years, and 51-60 years. Although more males with 24(60%) as compared to the females 16(40%) sustained cut wounds. The prevalence of bacterial and fungal wound infection accounts for 7(70%) and 4(80%) in males, while 3(30%) and 1(20%) in females respectively. However, there is no correlation between the sex and the prevalence of bacterial wound infection in this present study as compared with a previous study conducted in Nigeria by Kemebradikumo *et al.* (2013).

Gram-negative bacteria were the most commonly isolated pathogen in this study. In addition, our findings demonstrate the predominance of *S. aureus* and *P. aeruginosa* accounting for 3(20%) respectively, followed by *Klebsiella pneumoniae* (13.3%), *Escherichia coli* (6.67%), *B. cereus* (6.67%). These results can be compared with the study conducted by Pandukur *et al*., (2020), and also in agreement with results from previous studies conducted globally and in various parts of Nigeria (Gadzama *et al.,* 2007; Isibor *et al.,* 2008; Egbe *et al.,* 2011, Kemebradikumo *et al.,* 2013). The predominance of *S. aureus* could be due to its presence as an indigenous flora that colonizes the human skin, as such it easily gains access to wounds or by poor hand hygiene and poor handling of patients by health workers during hospital procedures. This predominance aligns with a previous study conducted by Guo *et al*., (2020). The prevalence of *P. aeruginosa* may be due to prolonged hospital stay which can cause colonization, extensively exposed regions of necrotic tissue, lacking protective mechanisms thus presenting optimal conditions for bacterial infection stemming from environmental origin or the indigenous body microbiota, as also reported by Garba *et al*. (2012) or impact of environmental and external factors
affecting *P. aeruginosa* colonization and infections in clinical environments (Hwang *et al.,* 2020). Another study conducted in Nigeria which analyzed the prevalence of *P. aeruginosa* infections in clinical specimens from various hospitals reported that *P. aeruginosa* accounted for 8.2% of all bacterial isolates (Ikwu *et al*., 2020), This percentage is lower than the prevalence reported in the current study.

The gram-negative isolates; *K. pneumoniae, P. aeruginosa, and E. coli* exhibited resistance to a range of antibiotics: reflacine, nalidixic acid, tarivid, ceporex, ampicillin, septrin, and augmentin. This finding contrasts with a study conducted by Goswami *et al*. (2011) on the antibiotic sensitivity profile of bacterial pathogens in postoperative wound infections at a tertiary care hospital in Gujarat, India. In the present study, *P. aeruginosa* was found to be susceptible to ciprofloxacin at a rate of 1(33.3%). According to Goswami *et al*. (2011), ciprofloxacin is considered the most effective drug for treating *P. aeruginosa* infections. *K. pneumoniae* and *E. coli* showed elevated resistance to all the antibiotics except for streptomycin which was effective against *K. pneumoniae*. The high resistance may be due to poor healthcare practices, such as improper management of medical procedures by healthcare givers, creating conditions that allow organisms to thrive and multiply (Anyiam and Uhegwu, 2024). Furthermore, patient non-compliance and overuse of antibiotics, including incorrect prescribing of antibiotic treatments could worsen the situation.

The gram-positive isolates, *S. aureus*, and *B. cereus* were found to be susceptible to levofloxacin and streptomycin, each accounting for susceptibility of 1(33.3%) yet they showed resistance to norfloxacin, chloramphenicol, ciprofloxacin, erythromycin, gentamycin, ampiclox, rifamycin, amoxil. The sensitivity of *S. aureus* to levofloxacin and streptomycin in our study aligns with the findings from previous research by Goswami, *et al.* (2011). Moreover, our results are consistent with the study by Akani *et al*. (2021) which also found *Staphylococcus* species to be sensitive to the observed antibiotics effective against *S. aureus* identified in this research. The significant resistance displayed by *S. aureus* in this study is consistent with previous research by Yusuf *et al*. (2014) as well as earlier investigations conducted in Ghana by Wolters *et al*. (2020). This resistance may suggest antibiotic abuse, and the absence of inadequate infection prevention and control measures, coupled with the potential spread or acquisition of resistant genes from the surrounding clinical or environmental settings.

C. albicans was the predominant fungi isolated in this study with a prevalence rate of 2(13.3%). This result aligns with a study by Dunga *et al*. (2023) which reported *C. albicans* as the predominant fungal isolate with a prevalence of 4(36.4%). *C. albicans* was susceptible to fluconazole with 1(50%) while resistant to itraconazole. Resistance to both fluconazole and itraconazole was observed with *A. japinocus.* The resistance shown by *C. albicans* to itraconazole in this study is in contrast with the study conducted in the Eastern Guangdong region by Bilal *et al.* (2022) which reported *C. albicans* to be susceptible to both fluconazole and itraconazole.

The use of culture technique in this study identified *S. aureus, P. aeruginosa, E. coli, K. pneumoniae, C. albicans Aspergillus* sp., and *Madurella* sp. showing their morphology and physical characteristics but has a limitation because it did not identify some of the organisms up to species level. Of the forty specimens, fifteen were identified by culture and eight were identified by PCR. The reason could be a result of the annealing temperature and high concentration of DNA solution. Cultured and uncultured microorganisms were identified using the 16S rRNA gene sequencing technique. Five(5) cultured organisms identified were *P. aeruginosa, K. pneumoniae, B. cereus, C. albicans, and A. japonicus* while one(1) uncultured organism was; *B. thuringiensis.* PCR was used in the identification of *B. thuringiensis* directly from clinical specimens and was successful up to the species level using a species-specific primer as shown in the result. The isolates' 16S rRNA sequence identified 4 bacterial species; *B. cereus, B. thuringiensis, K.*

pneumoniae, and *P. aeruginosa,* and 2 fungal species; *C. albicans, A. japonicus.* The phylogenetic analysis demonstrated an exact match with *B. cereus, K. pneumoniae* and *P. aeruginosa, C. albicans, A. japonicus* during the megablast search of highly similar sequences within the NCBI non-redundant nucleotide (nr/nt) database. Furthermore, the evolutionary distances were computed using the Jukes-Cantor method, which corroborated the phylogenetic placement of the isolates' 16S rRNA within the microbial species, indicating a significant proximity to these microbial strains (Jukes and Cantor, 1969).

Limitation

It is important to acknowledge several limitations in this study. These include the use of a small sample size, which was necessitated by the limited number of patients available during the research period. Information on the duration of hospital stays and the history of antibiotic usage was not available for analysis. Moreover, the study did not involve the detection of antimicrobial resistance genes.

Conclusion

DNA sequencing or genetic profiling is one of the most sensitive, specific, and rapid molecular techniques in the detection of microbial species in clinical specimens like cut wounds. The results of our investigation illustrate that molecular-based methods are complementary to traditional methods and could provide a more comprehensive understanding of the genetic diversity within microbial strains. Regular surveillance and molecular profiling of multidrug-resistant organisms are imperative for controlling the dissemination of clinical pathogens that do not tolerate antibiotics. Further research could focus on understanding the long-term efficacy of antimicrobial interventions in a healthcare setting and identifying factors that influence the sustainability of reduced antibiotic resistance over time.

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