

Isolation and Identification of *Pseudomonas* spp., *Klebsiella* spp. and *Proteus* spp. Associated with Wound Sepsis and Antimicrobial Susceptibility to Conventional Antibiotics and *Aloe barbadensis*

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Abstract

Background: Wounds and injuries are common experiences in daily lives but an uncommon fact is the presence of microorganisms that could determine the eventual fate of these injuries. This study thus, aimed at isolating few microbial organisms associated with wound sepsis (*Pseudomonas*, *Klebsiella* and *Proteus*) and to determine their antimicrobial profile to conventional antibiotics and *Aloe barbadensis*.

Materials and methods: Patients were randomly sampled in National Orthopedic Hospital, Enugu, Faith Foundation Mission Hospital, Nsukka and Renaissance Hospital, Nsukka. A total of 40 samples were cultured and identified using standard microbiological methods. Microbial strains isolated were *Pseudomonas* species, *Klebsiella* species and *Proteus* species. The pure isolates were then tested for susceptibility to ciprofloxacin which was used as control and aloe vera gel and leaf extract. Solvent extraction of the leaf was done using standard ethanolic extraction method. Dilutions of 100-6.25 mg/ml of the extract and 100% to 6.25% of the gel were made and were used for antimicrobial test.

Result: Both isolates were resistance to aloe vera gel while *Pseudomonas* and *Proteus* were susceptible to the ethanol extract mostly at the concentration of 100 mg/ml with inhibition zone diameter (IZD) ranging from 15-20 mm, while *Klebsiella* isolates were resistant to both the gel and the ethanol extract. Some *Klebsiella* and *Proteus* isolates were also resistant to ciprofloxacin while *Pseudomonas*, *Proteus* 14, 38 and *Klebsiella* 24, 34 isolates were susceptible with inhibition zone diameter (IZD) ranging from 18-30 mm.

Conclusion and recommendation: In conclusion, aloe vera gel and leaf extract has no antimicrobial properties *in vitro* based on the outcome of this study. Further studies should be carried out on the use of herbal plants for wound treatment.

Keywords: *Pseudomonas*; *Klebsiella*; *Proteus*; Antimicrobial; Microbes; Aloe vera gel; Leaf extract; *Aloe barbadensis*

Introduction

Gram-negative infection is a global health concern. Several advances have been registered in the field of intensive care, ventilator support, skin substitution and fluid balance. However, infection has emerged as a major, often unmitigated complication especially in burn injury, which incurs significant morbidity, mortality and healthcare cost [1]. The unselective and extensive use of antibiotics is highly considered as the major cause of invasive procedures. Accordingly, development of resistance mechanisms either intrinsic or acquired has promoted the rapid development of multiple resistance among *Pseudomonas aeruginosa* isolates and other organisms in the clinical settings [2]. Postoperative infections have been found to pose a major problem in the field of surgery for a long time. It is also called Surgical Site Infection (SSI).

Mordi and Momo [3] reported the incidence of *Proteus* species in wound infections and their susceptibility pattern gave results of 26.80% isolation. Seventy isolates (12.5%) were from pathological wounds, 89% were from trauma and 54% from postoperative wounds. Also, 23.6% of *P. aeruginosa* and 6.61% of *Klebsiella* species were isolated. *Proteus* species had the highest frequency of occurrence among the gram-negative bacteria isolated [4].

Uncontrolled and rapidly spreading antimicrobial resistance among bacterial populations has made the management and treatment of postoperative wound infections a serious challenge in clinical and surgical practice [5]. The treatment of bacterial infections is increasingly complicated by the ability of bacteria to develop resistance to antimicrobial agents. World Health Organization (WHO), estimated that in developing countries, about 80% of the population mainly relies on traditional therapies and use of plant extracts as their major medicinal source to treat various infectious diseases [6,7].

In the recent years, extracts or oils of medicinal plants with antimicrobial and anti-inflammatory effects have been used for treatment of many human infectious diseases. Aloe vera is one of these well-known medicinal plants with two distinct products such as yellow latex (exudate) and clear mucilaginous gel (aloe vera gel). Aloe vera is a cactus-like perennial, drought resistant, succulent plant belonging to the *Liliaceae* family of which there are over 360 known species. The elongated and pointed leaves of the plant contain gel which is revealed after removal of the thick outer cuticle [8]. Overall, more than 75 active ingredients have been identified from the inner gel. Aloe vera was first used in the 1930s to heal radiation burns.

Mehdi et al., [9] reported antibacterial activities of aloe vera gel showed that out of 140 *P. aeruginosa* isolates, all of multi drug resistant *P. aeruginosa* strains except five of them were inhibited by aloe vera gel extract at MIC 400 µg/ml. The MIC values of aloe vera gel for the remaining five (10.6%) isolates were 800 µg/ml. None of the multi-drug resistant isolates were sensitive to dilutions of aloe vera gel less than 25 µg/ml. The main factors that determine the antimicrobial activity are the type of the plant extract, composition of the plant extract, amount used, type of microorganism, pH value, temperature of the environment [10].

According to Niranjana et al., [11] solvent used in extracting the various components of medicinal plants also affect antimicrobial activity of the extract. This study therefore is aimed at isolating and identifying *Pseudomonas*, *Klebsiella* and *Proteus* species associated with wound sepsis and antimicrobial susceptibility to conventional antibiotics and *Aloe barbadensis*.

Materials and Methods

A total of 40 swab samples were collected from National orthopedic hospital, Enugu, Faith foundation mission hospital, Nsukka and Renaissance hospital, Nsukka in Enugu State. After collection, swabs were transported to the laboratory; isolates were cultured on blood agar and MacConkey and incubated at 37°C for 24 hours, after which the growth were subsequently sub cultured to obtain distinct colonies.

Preparation of the aloe vera gel

The gel was removed from the plant after washing. Double dilution of the gel was prepared by diluting against distilled water.

Extraction process and preparation of the extract

The active compounds of aloe vera were extracted according to the procedure described by Coats (1979). The aloe vera leaves were washed with distilled water to remove surface contaminants and was allowed to dry. The leaves were cut open and the aloe vera gel was removed and stored in a refrigerator, then the leaves were kept under a shade and allowed to dry. The dry leaves were macerated with mortar and pestle and 100 g of the macerated leaves was weighed and soaked in 500 ml of ethanol, the mixture was poured into a sterilized beaker and was covered with aluminum foil, this was allowed to stand for 48 hours with occasional stirring.

The content was filtered by passing the suspension through a clean muslin cloth, the weight of the extract was determined after evaporation of the ethanol. Serial dilution was carried out within the ranges of 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml.

Standardization of inoculum

The sensitivity test was determined by the use of agar well diffusion method according to Clinical Laboratory Standards Institution (CLSI). Three to five identical colonies from each agar plate were transferred onto a test tube containing 5 ml of nutrient broth. The turbidity of each of the microbial suspension was adjusted to reach an optical comparison of 0.5 McFarland turbidity standards, resulting in a suspension containing approximately 5×10^9 cfu/ml.

Antimicrobial sensitivity assay

A sterile swab stick was used to inoculate standardized microbial test suspension onto the entire surface of Mueller-Hinton Agar using the spread plate method to obtain a lawn culture. The disc was placed carefully on the agar to allow for the diffusion of the antibiotics using sterile forceps. After incubation for 18 hours at 37°C the diameter of the inhibition zone was measured for only ciprofloxacin which was used as a control. The plates were examined for inhibition which is indicated by a cleared zone around the disc. The inhibition zone diameter (IZD) was measured to the nearest millimeter.

Each extract was checked for antimicrobial activity by filling up the wells with different concentrations of the extracts (100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml) into 5 wells. The plate was allowed to stand at room temperature for 15 minutes to allow for the diffusion of the extract into the agar. The agar plates were incubated at 37°C for 18 hours. The same procedure was done for the gel using different concentrations of 100-6.25 mg/ml.

Results and Discussion

Result of antimicrobial susceptibility of ciprofloxacin and aloe vera gel is shown in Table 1.

Samples yielded the isolates	Ciprofloxacin (mm)	Gel (mm) 100% to 6.25%
Pr3	0.00 mm	0.00 mm
K6	0.00 mm	0.00 mm
Ps10	22.00 mm	0.00 mm
Ps11	25.00 mm	0.00 mm
Pr14	20.00 mm	0.00 mm
K24	30.00 mm	0.00 mm
K34	18.00 mm	0.00 mm
Pr38	20.00 mm	0.00 mm
Ps40	25.00 mm	0.00 mm

mm=millimeter, mg/ml=milligram per millilitre, 0=No effect,
Ps=*Pseudomonas*, K=*Klebsiella*, Pr=*Proteus*

Table 1: Comparison of antimicrobial susceptibility profile of ciprofloxacin and gel.

Result of antimicrobial susceptibility of ciprofloxacin and aloe vera leaf extract is shown in Table 2.

Microbial strains	CPX	Extract(mm) 100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml
Pr3	0.00 mm	0.00 mm	0.00 mm	0.00 mm	0.00 mm	0.00 mm
K6	0.00 mm	0.00 mm	0.00 mm	0.00 mm	0.00 mm	0.00 mm
Ps10	22.00 mm	15.00 mm	0.00 mm	0.00 mm	0.00 mm	0.00 mm
Ps11	25.00 mm	18.00 mm	15.00 mm	0.00 mm	0.00 mm	0.00 mm
Pr14	20.00 mm	20.00 mm	0.00 mm	0.00 mm	0.00 mm	0.00 mm
K24	30.00 mm	0.00 mm	0.00 mm	0.00 mm	0.00 mm	0.00 mm
K34	18.00 mm	0.00 mm	0.00 mm	0.00 mm	0.00 mm	0.00 mm
Pr38	20.00 mm	15.00 mm	0.00 mm	0.00 mm	0.00 mm	0.00 mm
Ps40	25.00 mm	16.00 mm	0.00 mm	0.00 mm	0.00 mm	0.00 mm

CPX=Ciprofloxacin, mm=millimeter, mg/ml=milligram per millilitre, 0=No effect, Ps=*Pseudomonas*, K=*Klebsiella*, Pr=*Proteus*

Table 2: Comparison of antimicrobial susceptibility profile of ciprofloxacin and leaf extract.

The results obtained from the antimicrobial activity assay showed that *Aloe barbadensis* has variable but significant antibacterial activity against the three clinical isolates used in this study. It is shown by the results recorded that the gel had no antibacterial activity against the isolates (Table 1). This agreed with the work of Agarry et al., [12] which revealed that aloe vera leaf possesses inhibitory effect with zone of inhibition of 4.0 mm while the gel had no effect. The results of the antibacterial activity of the gel on *Pseudomonas* is in contrary to the study done by Mehdi et al., [9] which showed that the multidrug resistant *P. aeruginosa* was susceptible to the gel but at higher concentrations of MIC 400 µg/ml and 800 µg/ml. The result obtained in this study may likely be due to the lower concentration used; it may also be due to the type of microorganisms tested as this is one of the factors that determine the antimicrobial activity [10].

The results of the ethanol extract showed that *Pseudomonas* 10, 11 and 40 were susceptible to the ethanol extract at concentration of 100 mg/ml with inhibition zones diameter (IZD) ranging from 15-18 mm and only *Pseudomonas* 11 was susceptible to the ethanol extract at concentration of 50 mg/ml. This agreed with the works Agarry et al., and Etusim et al., [12,13].

It was also found that *Proteus* 38 and 14 were susceptible to the ethanol extract with inhibition zone diameter (IZD) ranging from 15-20 mm at concentration of 100 mg/ml, this concurs with the work of Etusim et al., [13]. This portrays ethanol as a good extraction solvent. All the *Klebsiella* spp. were resistant to both the ethanol extract and the gel, this could be due to high resistance among the *Klebsiella* spp. and could also be due to the extraction solvent. The isolates furthermore showed susceptibility to ciprofloxacin which was used as the control with inhibition zone diameter (IZD) ranging from 18-30 mm, though some isolates like *Proteus* 3 and *Klebsiella* 6 were resistant to ciprofloxacin and subsequently to the gel and leave extracts. Abuse of antibiotics therapy through self-medication plays an important role in infection treatment [14].

Self-medication and drug abuse has given rise to antibiotic resistant strains of the organisms concerned. *Klebsiella* isolates showed the highest level of resistance among other isolates while *Pseudomonas*

isolates showed the least with the use of gel and leave extracts. Because of the resistance of *Klebsiella* and *Pseudomonas* spp., they normally act as secondary colonizers of wounds thus preventing fast healing of wounds [15].

Conclusion and Recommendation

It can be concluded that aloe vera gel is not a good substitute for antibiotics against different organisms because they did not prove effective at all in combating microorganisms associated to wound sepsis while the leave extract was relatively sensitive to those isolates. Further study of other plant extracts is highly recommended to ascertain their efficacy on wound isolates while good hygiene practices is recommended for prevention of contamination of wound by secondary sources. Early treatment of wounds and appropriate use of drugs is highly recommended.

References

1. Robson MC, Sternberg BD, Hergers JP (1990) Wound healing alterations caused by infection. *Clinical Plastic Surgery* 17: 485-492.
2. Agwunglefah FD, Nwabunike CC, Nwaju PC (2014) Antibiotic susceptibility pattern of bacteria isolated from surgical wounds of patients attending federal medical centre and christiana specialist hospital, Owerri. *J Natural Sci Res* 4: 2224-3186.
3. Mordi RM, Momoh MI (2009) Incidence of *Proteus* species in wound infections and their susceptibility pattern in the university of Benin teaching hospital. *Afr J Biotechnol* 8: 725-730.
4. Miller CN, Carville K, Newall N (2011) Assessing bacterial burden in wounds: comparing clinical observation and wound swabs. *Int Wound J* 8: 45-55.
5. Adegoke AA, Tom M, Okoh AI, Jacob S (2010) Studies on multiple antibiotic resistant bacteria isolated from surgical site infection. *Science Research Essays* 5: 3876-3881.
6. Akerele O (1992) WHO guidelines for the assessment of herbal medicine. *Fitoterapia* 63: 99-104.
7. Oluyemisi FK, Henry EO, Peter OA (2012) Standardization of herbal medicine: A review. *Int J Biodivers Conserv* 4: 101-112.
8. Surjushe AR, Vasani R, Saple D (2008) Aloevera: A short review. *Indian J Dermatol* 53: 163-166.

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9. Mehdi G, Maryam F, Mehdi A, Sima SS, Reza M (2015) Aloe vera gel: Effective therapeutic agent against multidrug-resistant *Pseudomonas aeruginosa* isolates recovered from burn wound infections. Chemotherapy Research and Practice 20: 1-5.
 10. Sagdic O (2003) Sensitivity of four pathogenic bacteria to turkish thyme and wild marjoram hydrosols. LebensmWiss Technology-Food Science and Technology 36: 467-473.
 11. Niranjana K, Sathiyaseelan V, Jeyaseelan EC (2013) Screening for antimicrobial and phytochemical properties of different solvent extracts of leaves of *Pongamia pinnata*. Int J Sci Res 3: 1-3.
 12. Agarry OO, Olaleye MT, Bello-Micheal CO (2005) Comparative antimicrobial activities of aloe vera extracts and products. Afr J Biotechnol 4: 1413-1414.
 13. Etusim P, Okafor E, Nwachukwu N, Melariri P, Ogbonnaya C (2013) A study on antimicrobial activities of aloe vera leaves, stems and roots on some selected organisms. J Biol Sci 5: 52-59.
 14. Brady RA, Leid JG, Camper AK, Costerton JW, Shirtliff ME (2001) Identification of *Staphylococcus aureus* proteins recognized by the antibody-mediated immune response to a biofilm infection. Infection and immunity 74: 3415-3426.
 15. Bowler PG (2002) Wound pathophysiology, infection and therapeutic options. Annals of Medicine 34: 419-427.