Global Scientific JOURNALS

GSJ: Volume 11, Issue 11, November 2023, Online: ISSN 2320-9186
www.globalscientificjournal.com

# ANTIBACTERIAL ACTIVITY OF AFRICAN KILLER BEE *Apis mellifera adansonnii* VENOM EXTRACT AGAINST ANTIBIOTIC RESISTANT *Acinetobacter species* ISOLATES

[1] Dahunsi, Aisha Ejura, [2*]J.K.P Kwaga [*3]Mohammed Bello

1. Professor, Department Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University
2. Professor, Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University

**Corresponding author:** Dahunsi Aisha Ejura

Email:aisha.dahunsi2022@uniabuja.edu.ng; aishasalsa2017@gmail.com



KeyWords
Antibacterial activity, Acinetobacter species, African killer bee venom, Multidrug resistance, Minimum Inhibitory Concentration (MIC), Column chromatography, High-performance liquid chromatography (HPLC)

## ABSTRACT

This study aimed to assess the antibacterial activity of African killer bee venom against antibiotic-resistant Acinetobacter species isolates, utilizing both extracted and commercial venom from *Apis mellifera adansonnii*. Acinetobacter species were isolated from four diverse sites, given its prevalence in the environment and its role as a nosocomial pathogen, causing infections in hospital ICUs. The study sites included Abattoir soil and effluent, ABUTH Gynecology department, and ABUTH Veterinary hospital examination table and tray. Among the sixty samples collected, 18 (56.28%) isolates of Acinetobacter species were recovered. Identification was based on colonial morphology, standard biochemical tests, and the AP120NE multi-test system. Venom extraction utilized an electric-shock collector device (VC-6F), and sterility was ensured through membrane filtration. Sensitivity tests on positive Acinetobacter species isolates were conducted for both extracted and commercial venom at concentrations of 7.81mg/ml, 15.26mg/ml, 31.25mg/ml, 62.5mg/ml, and 125mg/ml. The disc diffusion method revealed a multidrug-resistant pattern, with 100% resistance to ciprofloxacin, penicillin, sulfamethoxazole, and ampicillin, 90% to chloramphenicol, and 60% to colistin. No resistance was observed (0%) to imipenem. The Minimum Inhibitory Concentration (MIC) required to inhibit Acinetobacter species growth was 31.23mg/ml, while the Minimum Bactericidal Concentration (MBC) was 62.5mg/ml. Column chromatography and high-performance liquid chromatography (HPLC) identified Am2, Am3, and Am5 as active components in the venom. HPLC identified Apamin, Melittin, and Phospholipase A2 as unknown components. Statistical analysis, using one-way analysis of variance (ANOVA), showed

no significant difference in the activity of extracted and commercial venom against Acinetobacter species isolates. However, a significant difference was observed between the activity of both venom types and imipenem.

## INTRODUCTION

An antimicrobial is any substance that kills or inhibits the growth of microorganisms such asbacteria, fungi, or protozoan, as well as killing viruses. The discovery of antimicrobial likepenicillin and tetracycline paved the way for better health for millions around the world. Before1941, the year in which penicillin was discovered, no true cure existed for some diseases such asgonorrhoea,strepthroat,orpneumonia.(Castro*etal*.,2005).Theneedsforresearchofantibacterial activity are important for the health of people globally, because of the importance offindinganewcureforsomediseasesthatoccurduetomicroorganisms.Someofthemicroorganisms,especiallybacteriaarebecomingresistanttomoreandmoreantibacterialagents.By making new research, microorganisms that can kill or inhibit the growth of other bacteria canbefound(Weil*etal*.,1933).Now,mostoftheseinfectionscanbecuredeasilywithashortcourseofantibacterial. (AbdManap, *et al*., 2014).However, the effectivenessof antimicrobialtherapy issomehow indoubt.Microorganisms,especially bacteria are becoming more resistant to antimicrobial agents. They are becomingresistantmorequicklythannewdrugsthatarebeingmadeavailable.Therefore,futureresearchinantimicrobialtherapy mayfocusonfindingthenewantimicrobialthatcanovercomethisproblem,or treat infectionswith alternativemeans(AbdManap*et al*.,2014).The increase in life-threatening infections that are resistant to commonly used antibiotics hasbecomeaworldwideproblem.Itisbecominganimportantcauseofmorbidityinimmunecompromised patients in developing countries (Nablo *et al*., 2005). The increasing prevalence ofmultidrugresistantstrainsofbacteriaandtherecentappearanceofstrainswithreducedsusceptibility to antibiotics has raised the specter of untraceable bacterial infection and addsurgencyto searchfornewinfection-fightingstrategies(Ito*etal*., 1995; Britland*et al*.,2011).Presently, there are global problems of the emergence of multiple antibiotic resistance as well asthe emergence of new and "resurrection" of previously eradicated diseases. There is a need tosearch for new and more potent antimicrobial compounds of natural origin to complement theexisting synthetic antimicrobial drugs that are gradually becoming less potent against pathogenicmicroorganisms(Castro*et al.,* 2005).The continuous spread of multi-drug resistant pathogens has become a serious threat to publichealth and a major concern for infection control practitioners worldwide (Sanders and Sanders,2014). In addition to increasing the cost of drug regimens, this scenario has paved way for the re-emergenceofpreviouslycontrolleddiseasesandhascontributedsubstantiallytothehighfrequency of opportunistic and chronic infection cases in developing countries (Dobrovolsky and Titaeva 2002; Wysocki *et al*., 1999). Abuse and misuse of antibiotics in humans and livestock havespurredthe evolutionarydevelopment ofbacteria thathaveselectiveresistancetoantibiotics.Followingthislead,itisimperativetodevelopnewalternativesubstancestoreplaceantibioticstominimize the damage or loss that human and livestock farming industry may suffer by theexclusion of antibiotic from animal feed and to ensure continuous improvement of human healthandlivestock productivity(Dibner and Richards,2005).Beevenomisamixtureofseveralcomponentswithproventherapeuticbenefits,amongwhichareanti-inflammatory,analgesicandvariouscardiovasculareffects.Thetherapeuticapplicationofbeevenom has been used in traditional medicine to treat diseases since ancient times (Hegazi, 2015).It also has biological activity against arthritis (Jensen *et al.,* 1987), rheumatism, pain, tendonitis,fibromyositis, rheumatoid arthritis and osteoarthritis (Son *et al*., 2007; Han *et al*., 2009). It alsoinhibits mammary carcinoma cell proliferation (Orsolic *et al.,* 2003), and cytotoxic to malignantcellsboth*invitro*and*invivo*(Shaposhnikova*etal.,*2001).Beevenomwasalsousedasacureforbaldness,therapyagainstgoutandwasprescribedtoimprovethef lowofurineandagainstkidneystonesanditisalsousedcosmeticallyto'fool'theskinintothinkingithasbeenlightlystungwiththe toxin melittin. This causes the body to direct blood towards the area and stimulates theproduction of the naturally-occurring chemicals, collagen, and elastin. Collagen strengthens bodytissue while elastin is the protein that helps the skin to remain taut and bounce back into shapeafter being pressed or pinched. The venom also has the effect of relaxing the muscle and againstaging-related human face wrinkles which have been scientifically proven (Doo *et al*., 2015). Kim*et-al*.(2012)reportedthatbeevenomhasactivityagainstdiseasesofcentralandperipheralnervoussystems and other skin diseases and also on HIV virus. However, venom administration wasreported to stimulate the function of the immune system and to affect the release of cortisolproduction,knownasanaturalanti-inflammatoryagent(Castro*etal*.,2005).BeevenomwasalsofoundtohaveantibacterialactivityonsomeGram-positivebacteriaspecieswhichinclude*Staphylococcus aureus, Streptococcus pyogenes,* and gram-negative bacteria such as *Klebsiellapneumonia,Escherichiacoli*and*Pseudomonasaeruginosa*(Holton1983;Hepburn andHepburn.(2005);Pak, 2016; Hegazi*et al.,* 2015b).

### STATEMENTOFTHERESEARCH PROBLEM

Antibiotic resistance is a global problem. It is a trans-boundary problem where the organismrequiresnointernationalpassport.Itisconsideredtobeoneofthegreatestthreatstopublichealth.

According to the Centers for Disease Control and Prevention of the USA, at least 2 millioninfectionsthatoccureachyearintheUSarecausedbyresistantbacteria(Oberoi*etal*.,2018).Theestimated direct economic losses due to resistant bacterial infections in man amounts to US $20billion and additional costs due to loss in productivity as well is over US $20 billion. Apart fromeconomiclosses,antimicrobialresistanceaddsenormouspressureonhealthcaredelivery,requiresa prolonged stay in the hospital, more expensive treatment options, and greater expenditure andpatientturnaroundtimeduetoadditionaltestsincludingsensitivitytests.Suchindividualsoverallhavehigherchances of dying(Olaitan*et al*., 2013).The rapid expansion of *Acinetobacter Species* isolates exhibiting resistance to carbapenems andmostorallavailableantibioticsduringthelastdecadeisaworryingevolution(Doern,2014).Theevolutionaryadvantage of the dominant clonal lineages relies on the capability ofthe *Acinetobacter Species* pangenome to incorporate resistance determinants. In particular, thesimultaneous presence of divergent strains of the international clone II and their increasingprevalence in international hospitals further support the ongoing adaptation of this lineage to thehospital environment (Olaitan *et al*., 2013). Genomic and genetic studies have elucidated the roleof mobile genetic elements in the transfer of antibiotic resistance genes and substantiate the rateof genetic alterations associated with acquisition in *Acinetobacter Species* of various resistancegenes, including OXA- and Metallo-β-lactamase-type carbapenemase genes (Dijkshoorn*et-al*.,2007).Establishment of a network of reference laboratories in different countries would generate a morecompletepictureandafullerunderstandingoftheimportanceofhigh-risk*AcinetobacterSpecies* clones in the international dissemination of antibiotic resistance (Villegas and Hartstein,2003). Several species persist in hospital environments, sewage, effluent, soil and cause severelifethreateninginfectionsinimmune-compromisedpatients(Dijkshoorn*etal*.,2007).These organisms have been implicated in a diverse range of infections and are particularly problematicinintensivecareunits(Gordon*etal*.,2010).Moreover,detectionof*Acinetobacter Species*carriersandtheestablishmentofisolationmeasuresareofcrucialimportanceto preventingthedevelopmentofnosocomialinfections.Thisorganismhasbeenreportedtohavebeenisolatedfrom African countries including Egypt, Algeria, Tunisia, Libya, Senegal, Nigeria, South Africa andMadagascar(Higgins*etal*.,2010).Odewale*etal*.(2017)statedthattheprevalenceof *Acinetobacter Species* isolated from hospital environment was 8.5% and was more prevalentamongpatients(72.7%)intheintensivecareunit(ICU),theorganismshowed100%resistancetoamikacin, ciprofloxacin, ceftriaxone, piperacillin, and was, however, sensitive to colistin andimipenem. Outbreak of *Acinetobacter* infection in Ogun state Tertiary hospital was linked tocontaminatedrespiratoryequipment,intravascularaccessdevices,beddingmaterial,andtransmission via hospital personnel (Neely *et al.,* 1999). *Acinetobacter Species* generally occur inpatientswithdiminishedhostdefence(Example:Alcoholism,tobaccouse,diabetesMellitus,renalfailure,andunderlyingpulmonarydiseases),inpregnantwomen,childrenandtheelderly(Richard*et al*.,2015).



**JUSTIFICATIONOF THESTUDY**

*Acinetobacter Species* has become a problematic nosocomial pathogen, partly due to clonalspread, particularly in intensive care units (ICUs). In the 1970s, *Acinetobacter Species* infectioncould be treated with common antibiotics, now reports of infections with multidrug or even pandrug-resistant isolates are alarmingly increasing. Given the problem associated with antibiotic-resistant,pathogenic*AcinetobacterSpecies*,thedevelopmentofalternativetreatmentstrategiesforthe management of *Acinetobacter* infections is needed. Mortality ranges from an attributable in-hospitalmortalityof7.8%to70%inventilator-associatedpneumoniaand72.7%inpatientssuffering from meningitis. *Acinetobacter Species,* bacteremia is associated with a high mortalityrate, ranging from 29% to 46.9% and a diverse spectrum of comorbidities have been investigatedas potential risk factors for mortality in these patients such as previous severity of organ failure,neutropenia,elevatedAPACHEIIscore,malignancy,inappropriateantimicrobialtreatment,pneumonia as the source of bacteraemia and septic shock. The most serious issue in the treatmentof*AcinetobacterSpecies*infectionismultidrugresistance(MDR)becausethenumberofappropriately active agents is limited. Studies have reported that antibiotic resistance has anadverse impact on mortality rate, duration of hospitalization and healthcare costs in patients with*Acinetobacter Species* bacteremia (Odewale *et al*., 2017). One matched-control study conductedshowed that mortality rate increased from 13% to 34.6% in *Acinetobacter Species* bacteremiawhen pathogen possessed MDR and that duration hospitalization and healthcare costs wereadditionally increased. Given that *Acinetobacter Species* is developing resistance, there is a needtohavebeevenomasanalternativeHoneybeevenomhasbeenusedwithintheorientalregionforthe treatment of several inflammatory diseases and bacterial infections. The venom containspredominantly biologically active compounds. Paul and Shumovsky (2015) reported a study onMethicillin-Resistant*Staphylococcusaureus*(MRSA)agram-positivebacteriawhichwasusedasa model. The study evaluated bee venom and its principal active components, in terms of theirantibacterial activities and *in vivo* protection against MRSA infections. *In vitro,* bee venom orApitoxin exhibited comparable levels of antibacterial activity, which was more marked againstMRSA strains, compared with other Gram-positive bacteria. When MRSA-infected mice weretreated with the venom, only the latter animals were successfully rescued from MRSA- inducedbacteremia or exhibited re-

covery from MRSA-infected skin wounds (Littlewood *et al.,* 2000;Shumovsky,2015).

**AIMOFTHESTUDY**

Theaimofthisstudywastodeterminetheantibacterialactivityofkillerbee(*Apismelliferaadansonii*)venom against antibiotic-resistant*Acinetobacter Species* isolates.

**OBJECTIVESOF THESTUDY**

Theobjectiveswereto:

1. isolate and identify *Acinetobacter Species* from Abattoir soil, abattoir effluent, ABUteachinghospital, andABUVeterinaryTeachinghospital
2. extractthevenomfrom *A.melliferaadansonnii*
3. determinetheantibacterialactivityofextractedvenomofAfricankillerbee*Apismelliferaadansonii*against antibiotic-resistant *Acinetobacter Species*isolates.
4. determine the antibacterial activity of commercial venom from African killer bee *Apismelliferaadansonii*against antibiotic-resistant*Acinetobacter Species*isolates
5. determinetheMinimumInhibitoryConcentration(MIC)andMinimumbactericidalconcentration (MBC) of the extracted venom of the killer bee *Apis mellifera* againstantibiotic-resistant*Acinetobacter Species* isolates.
6. determine the different and active components of the extracted venom using Column andHigh-Performanceliquidchromatography(HPLC)

## MATERIALSANDMETHODS

**STUDYAREA**

The study was carried out in Zaria Metropolis which comprised of Zaria, Sabon Gari and Sobalocal Governments of Kaduna State, Nigeria. The area lies between latitude $11^0$. 00'N to latitude$12^0$ 12'N and longitude $07^0$ 33'E to $08^0$ 03'E with an altitude of 675 meters above the sea level.Theareaischaracterisedbytropicalcontinentalclimatewithtwodistinctseasons,coolhumidwetseason and cold or hot dry season. The area has an average daily temperature range of $17^0$C to$33^0$C and total land area of 5,126km$^2$. (KDSG, 2008). The area has an estimated population of1,279,253 and a density population of 1,400/km$^2$ (NPC, 2006) (Figure 3.1).Majority of theresidents are Hausa-Fulani with an estimated of 40 % to 75 % of the working population derivingtheir livelihood from agriculture. Agriculture in the area can be divided into two types: Rain- fall(fromMayto October) and irrigation farmingin thedryseason(from Novemberto April).

**StudyPopulation**

The bee farms used in this study were maintained by Jorafarm Associates. The farm is located atJahi,Abuja,Nigeria.JahiisapopulatedsettlementinAbujaFederalCapitalTerritory.Itislocatedatanelevationof568metersabovesealevelanditspopulationamountsto250,459.Itscoordinatesare 9º6'15" N and 7º26'33" E in DMS (Degrees Minutes Seconds) or 9.10417 and 7.4425 (indecimaldegrees).Thefarmhasabouttwenty(20)activeWarrenhiveseachcontainingover20,000Killerbees.

**STUDYDESIGN /SAMPLINGMETHOD**

An experimental study design was used to determine the antibacterial activity of venom from *A.melliferaa*gainstantibioticresistant*AcinetobacterSpecies*isolatesusingNon-Probabilitysamplingtechnique based on Purposivewasusedforthe research work.Theexperiment was dividedinto three(3)groupsaccordingto thestudyofHegazi,*et al*., 2015

GroupA-Positivecontrol(*Escherichiacoli* 10788(Standardstrain))

GroupB-negativecontrol(distilledwater)

GroupC-testsample(extractedvenom (Apitoxin))

TheGroupAwasusedasapositivecontrol,groupBservedasthenegativecontrolandtheGroupC was tested against the positive isolates of multi-drug resistant *Acinetobacter Species* at theconcentratio-

nof125mg/ml,62.5mg/ml,31.25mg/ml,15.62mg/mland7.81mg/mlasdescribedbyHegazi*etal*.,2015.Twenty(22)platesofsterile MuellerHiltonagarwereused.TheAgarsurfacewas inoculated by spreading a volume of the microbial inoculums over the entire surface.

Then,wellwithadiameterof6mmwereasepticallyboredandavolumeof20ulofthevenomextractwereintroducedintothetestwell,aswellasforthepositiveandnegativecontrolplates.Thentheagarplateswereincubated at $37^0$C for24 hours.

**SAMPLECOLLECTION**

Fifteensampleseachwerecollectedfromabattoirsoilandeffluent,ABUTHGynaecologydepartment, and ABU Veterinary teaching hospital (examination table). 10grams of the soilsamples were collected at different points in the selected site, while 20ml of effluents were alsocollected from different point at the selected site. Swab sticks were used to collect samples fromABUTH-patientbeds,tables,sinks,floor,andmedicalequipment,whileintheveterinaryenvironment swab sticks were also used to collect sample from the examination table. The swabsticks,soilsamples andeffluentweretransportedto thelaboratoryfor furtherisolation process.

**MEDIAPREPARATION**

The study used a formulation of CHROMagar *Acinetobacter* that was designed for the detectionof *Acinetobacter* species. CHROMagar *Acinetobacter* was prepared from dehydrated powder andliquid supplement according to the manufacturer's instructions (CHROMagar; Paris, France).MacConkeyagarwithChloramphenicolwaspreparedinthelaboratoryfromdehydratedMacConkeypowderaccordingtomanufacturer'sinstructions(BD,Sparks,MD),andsupplemented with 300 µl of 20 mg/ml stock of Chloramphenicol solution per one litre ofMacConkey solution for a final concentration of 6 µg/ml. Chlorampenicol concentration of 6µg/mlwaschosentoselectforintermediateandresistant*Acinetobacterspecies*usingClinicalandLaboratoryStandards Institute(CLSI)breakpoints(CLSI, 2010)

**IsolationandIdentificationof*AcinetobacterSpecies***

In the Laboratory under aseptic conditions, the specimens collected from soil and effluent wereinoculated in Trypton Soya broth and incubated for 24 hours at $37^{\circ}$C, while the swab specimensfromABUTHGynaecologydepartment andABUVeterinaryhospitalweresuspendedin20mlofTrypton Soya broth and incubated for 24 hours at $37^{\circ}$C. After that, the swab specimens werestreaked directly on CHROM-agar and MacConkey agar and incubated for 24 hrs at $37^{\circ}$C. Theconspicuously red, round and smooth colonies on CHROM-agar and non-lactose fermentingcoloniesonMacConkeyagarweresubculturedonMacConkeyagarandincubatedforanother24hrs at $44^{\circ}$C (Fobes *et al*., 2007). *A. species* isolates were identified using the morphological andbiochemical tests. All isolates were examined for gram stainability and conventional biochemicaltests which included the Catalase test, Oxidase test, Indole production test, Motility test, Ureaseproduction test, Lactose fermentation test, and Citrate utilization test. Identification results weredoneusingAPI20NE System (Hegazi,*et al*., 2015).

**GRAMSTAIN**

Asmearoftheindividualcolonywasmadeonacleangrease-freeslide.Slideswerelabelledaccordinglyand stained as follows (Cheesbrough,2000):

I. Asmearoftheculturedorganismwasmadeonagrease-freeslideusingasterilizedwireloop and was air-dried.

Theslidewas fixed bypassingover aflame threetimes and allowedto cool.

2. Slideswerecoveredwithcrystalvioletfor1minute.

3. Slidesweredrainedandwerewashedin slowrunningwater.

4. Slideswere coveredwithLugol'siodinefor30secondsandrinsedinslowrunningwater.

Slidesweredecolourizedwith70%alcohol.

5. Slideswerecoveredwithsafraninreagentfor30secondsandwererinsedunderslowrunningwater.

Slideswereair-dried.Oilimmersionwasappliedoneachslideandwasexaminedunderthemicroscopeusingoil immersion.

**BIOCHEMICALTEST**

**CatalaseTest**

A colony of culture was placed on the slide and a 2-3 drop of 10% hydrogen peroxide was addedto it. The appearance of gas bubbles within 2-3 seconds indicated positive results. It is used todistinguishedcatalaseenzymeproducersfrom non– producer (Cheesbrough, 2000).

**CITRATEUTILIZATIONTEST**

About 2.4g of Simmons citrate was dissolved in 100ml of distilled water and autoclaved. It wasalsoallowedtocoolandpouredintosterilebottlesinaslantingposition.Itwasallowedtosolidifyand the slope surface was inoculated and incubated at 37°C for 96 hours. A change from green toblue indicated an alkaline reaction arising from citrate utilization when the citrate is not utilized,thegreenishcolourremainsunchanged.Thetestisimportantintheidentificationofcitrateutilizer.

**UREASETEST**

This test was performed using urea agar. The test organisms were inoculated and the bottleincubated at 37°C for 24 hours. The appearance of light violet colour indicated urease positiveculture. The test was used to differentiate urease producer from those which do not growth(Cheesbrough,2000).

**INDOLETEST**

Testing indole production is important in the identification of *Enterobacteriaecae*. The organismis cultured in a medium which contains tryptophan. Indole production is detected by Kovac's orEhrlich's reagent which contains 4 (P)-dimethylamino benzaldehyde. They react with the indoletoproducearedcolouredcompound.Kovac'sreagentisrecommendedinpreferencetoEhrlich'sreagentforthe detectionof indole forenterobacteria (Cheesbrough, 2000).

**API20NEMULTI-TESTSYSTEM**

Theidentified*Acinetobacter*spp.isolateswereverifiedbyAPI20Esystems(BioMerieux,France).Thesetestswereusedaccordingtothemanufacturer'sprotocol fornone*Enterobacteriaceae*. Wells of biochemical tests were inoculated with overnight 0.5 McFarlandbacterial suspension and incubated at 37$^{o}$C for 24 hrs. The results were read after the addition ofreagents,and a7 digit numberwasgenerated andidentifybyAPI20NESoftware.

**VENOMCOLLECTIONANDPREPARATION**

Venom were collected using a mild electro-stimulator device VC-6F model, with an adjustedvoltage of DC 12V as described by Towner (2006). The most efficient collection cycle was three15-minutes stimulations at intervals of three days, repeated after 2-3 weeks (Krell, 1996). Aconvenient sampling method was used in the collection for a period of 2 months (April-May,2019).Thedeviceismadeupofavenomcollectorframewithwireelectrodesinstalledinparallelto each other. The device uses electrical impulses connected to an electro-stimulator to stimulatethehoneybeestostingthecollectorsheet.Beesthatcomeintocontactwiththewiresreceivemildelectrical shock through wires above the collecting tray and stung onto the glass sheet. Thecollector device is bee safe. A well-adjusted impulse generator cannot harm more than 5-15 beespercollection.Thealarmodour,whichisevaporatedfromthevenom,mobilizesandirritatesotherbeesandtheyalsostarttosting.Itisunlikelythatabeewillejectallthecontentsofitsvenomsac,even after repeated stinging. The electric shock device frame with the fresh dried bee venom onthe glass was carefully packed into a special container for transportation to the laboratory. Theprocessing of bee venom started right after the frames are brought back to the laboratory and asharp scraper was used to scrape off the dried bee venom from the glass frame and packed up inthedarkglass jarsand stored in a cool dryplace.

**Purificationofbeevenom**

The bee venom was purified by dissolving 125mg, 62.5mg, 31.25mg, 15.62mg and 7.81 mg ofvenom weighed in 1ml of distilled water each and allowed to pass through a membrane filter asdescribedbyHegazi*et-al*.(2015).Thefilterallowswatertoflowthrough,whileittrapssuspendedsolidsand othersubstances throughaselectiveseparation wall.

**EVALUATIONOF ANTI-BACTERIALACTIVITYOFBEEVENOM**

Agarwelldiffusionmethodwasusedtotesttheantibacterialactivityofthevenomontheisolatesof *Acinetobacter Species.* 0.5 McFarland standard, yielding approximately $10^{6}$ CFU/ml was usedand further 0.1ml was spread on sterile agar plates using a sterile cotton swab. The surface of themedium was allowed to dry for 3min and sterile glass borer was used to bore wells to containvenom concentrations of 125mg/ml, 62.5mg/ml, 31.25mg/ml, 15.62mg/ml and 7.81mg/ml. Wellcontaining 0.1ml of distilled water served as negative control while 20µl of (*Escherichia coli*10788(Standardstrain))servedasthepositivecontrol.Thebacterialplateswereincubatedat37°Cfor 24 hours. The diameters of inhibition zones were measured using a transparent ruler and themeasurements recorded. This was repeated at four directions in the inhibition zone and the meanradiuscalculated.

**ANTIBIOTICSSUSCEPTIBILITYTEST**

IsolateswereplatedonMueller–HintonagarandtheirsusceptibilitiestodifferentantibioticsweretestedbyKirby-BauerdiskdiffusionmethodandinterpretedaccordingtotheClinicalandLaboratoryStandardInstituteguide-dLaboratoryStandardInstituteguide-lines(2010).Thefollowingseven(7)differentantibioticswereused:chloramphenicol(C,30µg),imipenem(IMP,10µg),colistin(CT,10µg),Ampicillin(AMP,10µg), Ciprofloxacin (CIP, 10µg), Penicillin G (P,10µg) and Sulphamethoxazole (SXT, 10µg).Inhibition zones developed around the discs were measured to the nearest millimetre (mm) usinga metric ruler according to Clinical and Laboratory Standards Institute CLSI (2010). *Escherichiacoli*10788standardstrainwasusedasqualitycontrolinsusceptibilitydetermination(CLSI,2010).

**DETERMINATIONOFMINIMUMINHIBITORYCONCENTRATIONS(MIC)**

MICwasdeterminedbyusingthedilutionmethodofthevenomextracts.Concentrationsofvenomextracts at (125mg/ml, 62.5mg/ml, 31.25mg/ml, 15.62mg/ml and 7.81mg/ml.) were prepared.One-hundred and forty (140) test tubes were set up; 2ml of nutrient broth was pipetted into eachsteriletest tubes that werelabelledproperly. Control tubes were alsoset up.

Positivecontrol:TubeA(test tubescontaining commercialvenom andbroth),

Organismcontrol:TubeB(testtubecontainingbroth,inoculumsandvenomextract) and

Negative control: Tube C (test tube containing the broth only). Using sterile Pasteur pipettes 0.2ml suspension of the test organisms were introduced into the test tubes according to their labels.Also, 2 drops of the different stock solutions (isolates) were introduced using sterile Pasteurpipettes and 2drops of the venom extract were also introduced into the same test tubes containingbothbrothandinoculum.Thepreparationwasincubatedat37ºCfor24hoursafterwhichthetesttubes were observed for turbidity or clearness. The least concentration where no turbidity wasobservedand notedas theminimal inhibitoryconcentration (MIC)value(Mates *etal.,* 1999)

**DETERMINATIONOFMINIMUMBACTERICIDALCONCENTRATIONS(MBC)**

The least concentrations of the venom extracts that have an antibacterial effect on the organismwasconsideredastheminimumbactericidalconcentration(MBC).Thiswasdeterminedfromthebroth dilution resulting from MIC tubes by sub-culturing on the surface of freshly preparedMuelleragarplatesbyusingasterileinoculatinglooptostreaktheplateas described (Dijkshoorn*et al*., 2007). All the test tubes that showed no microbial growth after 24 hours of incubation i.e.thenonturbidtesttubesweresub-culturedontothesurfaceoffreshlypreparednutrientagarplatesand incubated at 37ºc for 24 hours, after which the plates were observed for growth. The plateswith the lowest concentration of the extract that show no bacterial growth after incubation wasnotedand recordedas theMBC(CLSI, 2010).

**COLUMNCHROMATOGRAPHYOFBEEVENOM**

The bee venom was subjected to qualitative screening for antimicrobial assay. The flash columnwas washed, dried and clamped vertically unto a retort stand. A piece of cotton wool wasintroducedintothecleandrycolumnfollowed-by30gabsorbentsilicagel.Thecolumnwastappedgently to give a uniform packing. Some 15 g of venom sample was weighed and poured into thecolumn followed by the addition of solvent. Solvent elution was started with 100% hexanefollowed by hexane/ethyl acetate (80:20 %, 60:40 %, 40:60 %, 20:80 % v/v, 100% ethyl acetateand 100% methanol respectively) each sample. The fractions were collected in 50 ml beakers andevaporatedand tested on*Acinetobacter Species.*

**DETERMINATIONOF THEDIFFERENTCOMPONENTSOFHONEYBEEVENOMEXTRACTSUSINGHPLC**

HPLCstudieswerecarriedoutattheShedaScienceandTechnologyComplex(SHESTCO)Abujausing the KNAUER (Dr. Ing. Herbert Knauer GmbH, Berlin, Germany) instrument made up ofthe following elements: HPLC PUMP K-1001, detector (UV K-2501), column oven, manualinjector-with20µmloopandEUROCHROM2000V2.05computersoftware.Intheinvestigationsforthedevelopmentofthemethodforseparationandidentificationofbeevenomcomponents,thefollowingparameters werechecked usingliteraturedata (Bouthrin,*et al*., 2018).

(1)-ChromatographiccolumnswithC18packing,25cmx4mm(4.6mm),5µm,ofdifferentporesize: 100 (Knauer Eurosfer-100 C18, Dr. Ing. Herbert Knauer GmbH, Berlin, Germany), 180(DISCOVERY®C18, Supelco Park, Bellefonte PA, USA) and 300 (SUPELCO-SIL™ LC-318,Supelco Park, Bellefonte PA, USA); (2)- Conditions for linear gradient elution: 0%B - 45%B for60min,0%B60%B,for60min,5%B-80%Bfor40min;-flowrateofmobilephase1.5ml/min, 2.0ml/minand2.5 ml/min;

(3)-Separation temperature: 25°C, 30°C and 35°C; Chromatographic separation was performedusing the following eluents: eluent A - 0.1% TFA in water, B - 0.1% TFA in the solution:acetonitrile: water (80:20). Venom compounds were identified using a UV detector at 220 nmwavelength. The external standard method (one-point calibration) was used for quantitation ofApamin, phospholipase A2, and Melittin. The whole procedure was validated by determining thedetection limit, precision, accuracy, repeatability, and linearity of the method. Validation wasperformedfortheassayon the column DISCOVERY®C18.

**DATAANALYSIS**

Descriptive statistics were used to analyse the data into simple percentage and standard deviationof the mean and Microsoft Excel. The data was subjected to one-way analysis of variance(ANOVA) to compare significant difference between extracted venom and commercial venom,andsignificantdifference betweenthecommonlyusedantibioticsandvenomextract.Valuesof$p \leq 0.05$wereconsideredstatisticallysignificant.

**RESULTS**

Outof60samplescollected,32(53.3%)werepositivebasedonthemorphologicalcharacterizationand biochemical behaviour of the isolates. *Acinetobacter species*, which appeared as small, paleand lactose non-fermenter colonies on MacConkey agar, and round smooth red colonies onCHROM agar. Growth at 44°C was positive for all *Acinetobacter species* isolates which showedtheabilitytogrowatthistemperature.Thefrequencyofisolationofpositive*Acinetobacterspecies* isolatesis presented in Table 1

**TABLE1:Frequencyofisolationof*Acinetobacterspecies***

| S/N | Samplingsite | No.ofsamples | No.(%)ofpositive *Acinetobacterspecies* |
|---|---|---|---|
| 1 | Abattoirsoil | 15 | 5(8.3%) |
| 2 | AbattoirEffluent | 15 | 7(11.67%) |
| 3 | ABUTH Gynaecologydepartment | 15 | 11(18.33%) |
| 4 | ABU Veterinary-Teaching hospitalexaminationtable | 15 | 9(15%) |
| **Total** | | **60** | **32(53.3%)** |

Table 2 shows the frequency and sources of *Acinetobacter species* isolates identified by API20E system. It was found that the system identified a total of 18(56.28%) *Acinetobacter Species*isolateswiththefollowingoccurrence:Abattoirsoil3(9.4%),abattoireffluent4(12.5%),ABUTHGynaecology department 6(18.75%), and ABU Veterinary Teaching hospital examination table5(15.63%). The API 20NE system identified species so as to avoid the variability in findings ofconventionalbiochemical tests.

**TABLE 2: Frequency and sources of *Acinetobacter species* isolates identified by multi-testsystemsout of32 presumptive*Acinetobacter*isolates.**

| S/N | Samplesite | No.(%)ofpositive *Acinetobacterspp* | API20NE |
|---|---|---|---|
| 1 | Abattoirsoil | 5 | 3(9.4%) |
| 2 | AbattoirEffluent | 7 | 4(12.5%) |
| 3 | ABUTH Gynaecologydepartment | 11 | 6(18.75%) |
| 4 | ABU VeterinaryTeachinghospital examinationtable | 9 | 5(15.63%) |
| **Total** | | **32** | **18(56.28%)** |

Table3belowshowstheMean±SDinhibitionzoneofdiametersofextractedbeevenomagainst*AcinetobacterSpecies*isolatesatdifferent concentrationsof7.81mg/ml.15.26mg/ml,31.25mg/ml,62.5mg/ml and 125mg/ml respectively. For the purpose of this finding, to interpret the results ofthezonediameterofinhibition,theantibacterialandinhibitoryeffectofthevenomwasconsideredat the concentration of 30mg/ml as compared to that of Chloramphenicol (30µg), a standardantibiotic.WheninterpretedusingCLSI,2004guide,zonediameterofChloramphenicol(30µg)≥18issensitive,≤12isresistant,while13-17isintermediate.

**TABLE 3 MEAN± SD OF INHIBITION ZONE (MM) OF EXTRACTED BEE VENOM AT FIVE DIFFERENTCONCENTRATIONSAGAINSTEIGHTEENPOSITIVE ISOLATESOF*ACINETOBACTERSPECIES***

| | ZI(mm) for venom Concentration(mg/ml) | | | | |
|---|---|---|---|---|---|
| ***AcinetobacterSpp* Isolates** | **7.81** | **15.26** | **31.25** | **62.5** | **125** |
| **A1** | 14.9±0.00 | 15.3±0.00 | 19.4±0.50 | 20.9±0.50 | 22.6±0.03 |
| **A2** | 14.4±0.01 | 15.3±0.01 | 18.7±0.03 | 19.9±0.03 | 22.0±0.05 |
| **A3** | 13.4±0.03 | 14.6±0.01 | 19.6±0.03 | 21.9±0.04 | 22.6±0.02 |
| **B1** | 15.8±0.03 | 16.3±0.05 | 19.4±0.01 | 20.9±0.02 | 22.6±0.05 |
| **B2** | 14.0±0.00 | 15.3±0.03 | 19.7±0.00 | 19.9±0.00 | 22.0±0.50 |
| **B3** | 13.4±0.01 | 14.6±0.03 | 19.6±0.01 | 21.9±0.00 | 22.6±0.50 |
| **B4** | 13.0±0.01 | 15.0±0.02 | 19.4±0.00 | 19.5±0.05 | 21.7±0.50 |
| **C1** | 14.9±0.50 | 15.1±0.05 | 19.3±0.03 | 19.3±0.05 | 22.3±0.3 |
| **C2** | 12.0±0.50 | 13.3±0.01 | 15.0±0.05 | 17.6±0.00 | 19.8±0.01 |
| **C3** | 12.0±0.03 | 13.3±0.00 | 15.3±0.04 | 16.9±0.00 | 18.9±0.00 |
| **C4** | 11.6±0.03 | 12.6±0.00 | 14.9±0.04 | 17.0±0.01 | 18.9±0.00 |
| **C5** | 11.0±0.01 | 12.0±0.01 | 15.4±0.04 | 17.5±0.01 | 19.3±0.03 |
| **C6** | 12.9±0.00 | 13.7±0.00 | 15.2±0.00 | 16.3±0.00 | 18.3±0.03 |
| **D1** | 12.0±0.00 | 14.0±0.02 | 15.0±0.02 | 17.6±0.05 | 20.2±0.03 |
| **D2** | 12.0±0.01 | 14.2±0.00 | 15.3±0.01 | 16.9±0.03 | 18.9±0.05 |
| **D3** | 11.6±0.01 | 13.6±0.00 | 14.9±0.01 | 17.0±0.01 | 18.9±0.05 |
| **D4** | 11.0±0.01 | 14.0±0.50 | 15.4±0.00 | 17.5±0.00 | 19.3±0.00 |
| **D5** | 12.9±0.00 | 13.9±0.02 | 15.2±0.02 | 16.3±0.00 | 18.3±0.01 |

**CLSI,2004Interpretation**

**Key:** A=Abattoir soil, B=Abattoir Effluent, C=ABUTH Gynecology department, D= ABUVeterinaryteachinghospital examination table

Table4showsthemean±SDofinhibitionzoneofcommercialbeevenomagainst*Acinetobacterspecies* isolates at different concentrations of 7.81mg/ml. 15.26mg/ml, 31.25mg/ml, 62.5mg/mland 125mg/ml. For the purpose of this finding, to interpret the results of the zone diameter, theantibacterial effect and inhibitory effect of the venom at the concentration of 30mg/ml was-compared to that of Chloramphenicol (30µg), a standard antibiotic. When interpreted with CLSI,2004guide,zonediameterofChloramphenicol(30µg)≥18issensitive,≤12isresistant,while13-17is intermediate.

**TABLE4:MEAN±SDOFINHIBITIONZONEOFCOMMERCIALBEEVENOMAT5DIFFERENTCONCENTRATIONS AGAINSTEIGHTEEN POSITIVEISOLATES OF*ACINETOBACTERSPECIES***

| | ZI (mm) for venom Concentration(mg/ml) | | | | |
|---|---|---|---|---|---|
| ***Acinetobacterspe-cies*Isolates** | **7.81** | **15.26** | **31.25** | **62.5** | **125** |
| **A1** | 13.8±0.03 | 15.3±0.02 | 19.4±0.03 | 20.9±0.00 | 22.6±0.01 |
| **A2** | 14.4±0.01 | 15.3±0.01 | 18.7±0.03 | 19.9±0.00 | 22.0±0.00 |
| **A3** | 13.4±0.04 | 14.6±0.05 | 19.6±0.01 | 21.9±0.03 | 22.6±0.01 |
| **B1** | 12.8±0.01 | 16.3±0.01 | 19.4±0.02 | 20.9±0.01 | 22.6±0.04 |
| **B2** | 13.0±0.03 | 15.3±0.01 | 19.7±0.02 | 19.9±0.01 | 22.0±0.01 |
| **B3** | 13.4±0.03 | 14.6±0.03 | 19.6±0.02 | 21.9±0.03 | 22.6±0.00 |
| **B4** | 13.0±0.02 | 15.0±0.50 | 19.4±0.00 | 19.5±0.04 | 21.7±0.00 |
| **CI** | 14.9±0.01 | 15.1±0.01 | 19.3±0.00 | 19.3±0.02 | 22.3±0.01 |
| **C2** | 11.0±0.02 | 12.8±0.04 | 15.0±0.00 | 17.6±0.02 | 19.8±0.04 |
| **C3** | 12.0±0.04 | 13.3±0.02 | 15.3±0.01 | 16.9±0.00 | 18.9±0.01 |
| **C4** | 11.6±0.03 | 12.6±0.03 | 14.9±0.03 | 17.0±0.01 | 18.9±0.00 |
| **C5** | 11.0±0.06 | 12.0±0.01 | 15.4±0.50 | 17.5±0.00 | 19.3±0.50 |
| **C6** | 12.9±0.00 | 13.7±0.00 | 15.2±0.03 | 16.3±0.00 | 18.3±0.50 |
| **D1** | 12.0±0.50 | 14.0±0.02 | 15.0±0.50 | 17.6±0.05 | 20.2±0.04 |
| **D2** | 12.0±0.00 | 14.2±0.01 | 15.3±0.04 | 16.9±0.01 | 18.9±0.03 |
| **D3** | 11.6±0.01 | 13.6±0.01 | 14.9±0.50 | 17.0±0.02 | 18.9±0.03 |
| **D4** | 11.0±0.50 | 14.0±0.00 | 15.4±0.00 | 17.5±0.02 | 19.3±0.00 |
| **D5** | 12.9±0.01 | 13.9±0.02 | 15.2±0.01 | 16.3±0.00 | 18.3±0.01 |



**CLSI,2004Interpretation**

Key**:** A=Abattoir soil, B=Abattoir Effluent, C=ABUTH Gynecology departments, D= ABUVeterinaryTeachingHospital examination table

Table 5 shows the Antibiotic Susceptibility profiles of eighteen *Acinetobacter species* isolatesagainst7antibioticsused.Theisolatesshowed100%sensitivetoimipenem,61.1%wereresistanttoColistin,88.8%resistancetoChlorampenicol,100%resistanttoCiprofloxacin,penicillin,SXT,and Ampicillin.

**TABLE5ANTIBIOTICSUSCEPTIBILITYPROFILESOF*ACINETOBACTER*SPECIESISOLATESFROMABATTOIRSOIL,ABATTOIREFFLUENT,ABUTHGYNECOLOGYDEPARTMENT,ANDABU VETERINARY**

**Teachinghospitalexaminationtable against7antibiotics**

| | AntibioticsTested(µg) | | | | | | |
|---|---|---|---|---|---|---|---|
| ***Acinetobacterspe-cies*Isolates** | **IMP** | **C** | **CIP** | **P** | **SXT** | **CT** | **AMP** |
| **A1** | **S** | R | R | R | R | I | R |
| **A2** | **S** | S | R | R | R | S | R |
| **A3** | **S** | S | R | R | R | S | R |
| **B1** | **S** | R | R | R | R | S | R |
| **B2** | **S** | R | R | R | R | S | R |
| **B3** | **S** | R | R | R | R | R | R |
| **B4** | **S** | R | R | R | R | I | R |
| **C1** | **S** | R | R | R | R | R | R |
| **C2** | **S** | R | R | R | R | R | R |
| **C3** | **S** | R | R | R | R | R | R |
| **C4** | **S** | R | R | R | R | R | R |
| **C5** | **S** | R | R | R | R | R | R |
| **C6** | **S** | R | R | R | R | I | R |
| **D1** | **S** | R | R | R | R | R | R |
| **D2** | **S** | R | R | R | R | R | R |
| **D3** | **S** | R | R | R | R | R | R |
| **D4** | **S** | R | R | R | R | R | R |
| **D5** | **S** | R | R | R | R | R | R |

**CLSI,2004INTERPRETATION**

**Key:** IMP Imipenem (30µg), SXT sulfamethoxazole (10µg), CIP ciprofloxacin (10µg), Ppenicillin (10µg), CT colistin (10µg), AMP ampicillin (10µg), and C Chloramphenicol (30µg), Rresistant,S susceptible,Iintermediate.

Table6belowshowstheMinimumInhibitoryConcentration(MIC)forextractedvenomagainst*Acinetobacter species* isolates from Abattoir soil, abattoir effluent was 15.26mg/ml while that ofABUTH Gynecology department and ABU veterinary Teaching hospital examination table was31.25mg/ml. The MIC obtained in the study of bee venom was 31.25mg/ml which indicates thatBVis effectiveagainstMDR*Acinetobacterspecies* strains.

**TABLE6:MINIMUMINHIBITORYCONCENTRATIONS(MIC)OFEXTRACTEDBEEVENOMAGAINST POSITIVE *ACINETOBACTER SPECIES* ISOLATES**

| | Concentration(mg/ml) | | | | |
|---|---|---|---|---|---|
| ***Acinetobacterspecies*Iso-lates** | **7.81** | **15.26** | **31.25** | **62.5** | **125** |
| **A1** | - | + | + | + | + |
| **A2** | - | + | + | + | + |
| **A3** | - | + | + | + | + |
| **B1** | + | + | + | + | + |
| **B2** | - | + | + | + | + |
| **B3** | - | + | + | + | + |
| **B4** | + | + | + | + | + |
| **CI** | - | + | + | + | + |
| **C2** | - | - | + | + | + |
| **C3** | - | - | + | + | + |
| **C4** | - | - | + | + | + |
| **C5** | - | - | + | + | + |
| **C6** | - | - | + | + | + |
| **D1** | - | - | + | + | + |
| **D2** | - | - | + | + | + |
| **D3** | - | - | + | + | + |
| **D4** | - | - | + | + | + |
| **D5** | - | - | + | + | + |



**Key**:+meansnovisible growth,-meansgrowth

The Minimum Bactericidal Concentration (MBC) for bee venom against eighteen *Acinetobacterspecies* isolates from Abattoir Soil and abattoir effluent was 31.25mg/ml, while Isolates fromABUTHGynaecologydepartmentandABUVeterinaryTeaching-hospitalexaminationtablewerefoundto be 62.5mg/ml as shown in table 7 below.

**TABLE7:MINIMUMBACTERICIDALCONCENTRATION(MBC)OFBEEVENOMATDIFFERENTCONCENTRATIONS AGAINST 18POSITIVE*ACINETOBACTER SPECIES*ISOLATES**

| *Acinetobacterspecies*Isolates | 7.81 | 15.26 | 31.25 | 62.5 | 125 |
|---|---|---|---|---|---|
| | - | - | - | + | + |
| **A1** | | | | | |
| **A2** | - | - | - | + | + |
| **A3** | - | - | + | + | + |
| **B1** | - | - | + | + | + |
| **B2** | - | - | - | + | + |
| **B3** | - | - | - | + | + |
| **B4** | - | - | - | + | + |
| **C1** | - | - | + | + | + |
| **C2** | - | - | - | + | + |
| **C3** | - | - | - | + | + |
| **C4** | - | - | - | + | + |
| **C5** | - | - | - | + | + |
| **C6** | - | - | - | + | + |
| **D1** | - | - | - | + | + |
| **D2** | - | - | - | + | + |
| **D3** | - | - | - | + | + |
| **D4** | - | - | - | + | + |
| **D5** | - | - | - | + | + |

Key: +meansnovisiblegrowth,-meansthereisgrowth

Table8showsthegrowthinhibitionofvariouscomponentspresentinthevenomof*A.mellifera*identified by column Chromatography. The column separated the venom into five unknownfractionswhichwereindicatedas*Am*1, *Am*2,*Am*3,*Am*4, and*Am*5.

**TABLE8:GROWTHINHIBITIONOFVARIOUSCOMPONENTSIN*APISMELLIFERA* VENOMAGAINSTFIVE *AcinetobacterSpecies*isolatesbyColumnChromatography**

| Site | *Am*1 | *Am*2 | *Am*3 | *Am*4 | *Am*5 |
|---|---|---|---|---|---|
| A | + | - | - | + | - |
| B | + | - | - | + | - |
| C | + | - | - | + | - |
| D | + | - | - | + | - |

**Key:+=Bacterialgrowth, -=No bacterialgrowth,** *Am*1means *Apismellifera*toxins1

Figure 1 below shows the High-Performance Liquid Chromatograph analysis of *Apis mellifera*venomfraction.TheHPLCidentifiedApamine, PhospholipaseAandMelittinasthethreeactivecomponentspresent in thevenom of*Apis mellifera*.

**Figure1:Separation profileof theproteinfractionof*ApismelliferaAdansonnii***

**DISCUSSION**

*Acinetobacter species* is often associated with epidemic outbreaks of infections. These infectionsare becoming harder to treat due to rising number of nosocomial infections having ability to formbiofilmsandresistallantimicrobialsinuseincludingcolistin(Visca*etal*.,2011;Abou-Raya *et al*., 2013). This study determined the antibacterial activity of bee venom againstmultidrugresistant*Acinetobacterspecies*.Atotalsixtysampleswerecollectedandscreenedfromabattoir soil, abattoir effluent, ABUTH Gynecology department, and ABU Veterinary teachinghospitalexaminationtables,fromwhich32(53.3%)suspected*Acinetobacterspecies*wereisolated. API 20NE system identified 18(56.28%) of the presumptive isolates *Acinetobacterspecies.*Thisfindingcorrespondstothefindingsofsimilarstudiescarriedoutby Siau*etal*.(1996);Atallah*etal*.(2001);andNamita*etal*.(2012)with reportedprevalencesof11%,18.8%,and9.4%respectively.Venomwasextractedforthespeciesof*A.melliferaadansonnii*,usingamildelectrostimulatordeviceVC-6Fmodel,withanadjustedvoltageofDC12VasdescribedTowner,(2006).Twenty(20)warrenbeehiveswereselectedusingnonprobabilityconveniencesamplingtechnique. The extracted venom was further purified and dissolved 125mg, 62.5mg, 31.25mg,15.62mg and 7.81 mg in 1ml of distilled water each and passing it through a membrane filter asdescribed by Hegazi *et al*. (2015). The antibacterial activity of extracted venom from Africanizedkillerbee*Apismelliferaadansonii*againstantibioticresistant*Acinetobacter species*isolatesweredetermined. The analysis showed that the zone diameters of inhibition of extracted bee venom at5 different concentrations (7.81mg/ml, 15.26mg/ml, 31.25mg/ml, 62.5mg/ml and 125mg/ml)respectivelyagainsteighteenisolatesof*Acinetobacterspecies*showedthatzonediameter≥18mmwasconsideredtobesensitive,≤12mmresistant,while13-17mmwasintermediatewhencompared to 30µg of Chloramphenicol (CLSI, 2010). The results of this finding also agrees withthe literature reports (Han, *et al*., 2009) who evaluated the antibacterial effects of whole bee venom and melittin fraction, andutilized inhibitory effect at a concentration of 30mg/ml and compared to a standard antibioticsPenicillin, and the relative sensitivities of the bacteria was qualitatively estimated by measuringthezonesofinhibition.Theantibacterialactivityofcommercialvenomfrom*A.mellifera*wasalsodetermined at 5 different concentrations (7.81mg/ml, 15.26mg/ml, 31.25mg/ml, 62.5mg/ml and125mg/ml) respectively againsttheeighteenisolatesof*Acinetobacterspecies*.Theanalysisshowedthattherewasnosignificantdifferentbetweenthezonediameterofinhibitionofextractedvenompreparationandcommercialpreparation.Theantibioticsusceptibilityprofilesof18*Acinetobacter species* isolates against 7 antibiotics indicated that all the isolates (100%) weresensitive to imipenem, 61.1%resistant to Colistin, 88.8% resistant to Chloramphenicol, 100%resistant to Ciprofloxacin, penicillin, SXT, and Ampicillin. This showed similarity to the findingof Al-Yawer*et al*., (2006), who found that *Acinetobacter Species* isolates were all sensitive toimipenem. Other studies carried out by Peleg *et al*., (2008) showed that all*Acinetobacter Species* isolates were (100%) sensitive to Colistin and resistant imipenem. Thesedifferences may be attributed to the different uses of these two antibiotics inhospitals in the lastfew years, predominate exposure of present isolates to suboptimal levels of antibiotic, prolongeduseofbroadspectrumantibiotics,exposuretoisolatescarryingresistantgenes,lackofhygieneinclinical environments and usage of antibiotics in foods and agriculture (Peleg *et al*., 2008).Regarding colistin, this study showed a very low level of sensitivity. However, many studiesreported that the effective antibiotics used to treat colistin resistant *Acinetobacter Species* isolateswasImipenem (Hello*etal*., 2010;Pongpech *et al*.,2010; Kumaris *et al*., 2011).

*Acinetobacterspecies*isreportedtobeubiquitousgram-negativecoccobacilli,andarewidespreadin nature, water and soil and has the greatest clinical significance and identified as the causativeagent of the majority of nosocomial infections especially in intensive care units (Ortel., *et al*.,2012).Beevenomhasthepotentialtoaddressthisneedduetotheanti-inflammatorypropertiesaswell as anti-microbial activities.A previous study has demonstrated that bee venom inhibited thegrowth of seventeen Gram- positive and two Gram-negative bacterial out of 44 strains isolatedfrom bovine mastitis in Korea (Park *et al*., 2015).Hagazi *et al*. (2015) reported that bee venomhas antibacterial activity against *Escherichia coli,* while Socarras *et al*. (2017) reported effectiveantimicrobial activityagainst*B. burgdorferi* whichis acausative agentof Lymedisease.The MIC of bee venom in the current study was 31.23mglml, which indicate that bee venom iseffectiveagainstMDR*AcinetobacterSpecies*isolates.Theresultsofthesefindingsagreewiththeliterature reports of Safar *et al*. (2018) who determined the antibacterial activity of bee venomagainstmulti-drugresistant*Acinetobacter Species*bacterial strains.

The Minimum Bactericidal Concentration (MBC) for *Apis mellifera adansonnii* venom against*Acinetobacterspecies*isolatedfromabattoirsoilandabattoireffluent-

were31.25mg/ml,whilefor*Acinetobacterspecies*isolatesfromHospitalandveterinaryclinicenvironmentitwas62.5mg/ml.This result also agrees with the findings of Towner (2006) who isolated large number of resistant*Acinetobacter species* isolates from the hospitals and also stated that due to its ability to formbiofilms, they could cause different nosocomial infections and develop resistance to a wide rangeofantibiotics.ColumnChromatographyforbeevenomwereseparatedintofiveunknownfractionswhichwereindicatedas*Am*1,*Am*2,*Am*3,*Am* 4,and*Am*5.Theanalysisshowedthat*Acinetobacterspecies* was sensitive to *Am*2, *Am*3, and *Am5* three of these unknown components. This resultagreeswiththereportofCorzo*et-al*.(2001)whoidentifiedthreeactiveunknownfractionsfromthevenomof*Apismellifera*.Inrecentyears,therehasbeenincreasedfocusonbeevenompeptidesandtheirmechanismsofactionfortargetingandkillingvarioustypesofmicroorganisms(Jenssen*etal*., 1987).TheHigh-

PerformanceLiquidChromatographanalysisof*Apismellifera*venomfraction identifiedApamine,PhospholipaseA2andMelittinasthethreeactivecomponentspresentinthevenom.Theresult of this study is in general agreement with those found by Hegazi *et al*. (2015) and Castro *et-al*.(2006)whoreportedthattheactivepeptidecomponentspresentin*Apismellifera*thatcaninhibitthegrowthofsomeGram-negativeandpositivebacteriaareMeltitin,Apamin,andPhospholipaseA2.The statistical analysis one-way analysis of variance (ANOVA) was employed in this studyindicated that there was no significant difference between extracted and commercial venom, butthereissignificantdifferencebetweenimipenemandbothextractedandcommercial venoms.

**Conclusion**

Basedonthisstudy,the followingconclusionscouldbemade:

Theisolationof*Acinetobacterspecies*fromtheabattoirsoil3(9.4%),effluent4(12.5%),andABUteaching hospital gynaecology department 6(18.75%) and ABU Veterinary Teaching hospital5(15.63%) is an indication that the organism is ubiquitous in the environment and commonnosocomialpathogen in the Hospitals.Antibiotic susceptibility patterns of *Acinetobacter species* isolates showed that Imipenem may beadrugof choicethat canbeused totreat*Acinetobacter species*infection.Venom of *Apis mellifera adansonni* inhibited the growth of *Acinetobacter species* isolates at31.25mg/mland killed them at 62.5mg/ml.TheMICandMBCofthevenomweredeterminedtobe31.25mg/mland62.5mg/mlrespectively.

**RECOMMENDATIONS**

Fromtheresultsofthisstudy,thefollowingrecommendationsareproffered

1. Further researchtodeterminetheextentofthe activityofextract againstotherresistant andpathogenicmicroorganisms should beconducted
2. Thereisneedtodoextensiveresearchonthelethaldoseof*Apismelifera*venomsoastodeterminesafeconcentrationsforhumanuse.
3. It is also recommended that the identification and characterization of the *Apis mellifera*venom5fractionsandtheirmajorantibacterialelements(s)shouldberesearchedandutilizedforotherorganisms
4. Research on bee venom that may lead to the future design of novel antibacterial agentsshouldbeconducted

**CONTRIBUTIONSTOKNOWLEDGE**

1. Thestudyserveasabaselinefortheisolationof*Acinetobacter*speciesinZariaKadunastate
2. Venomof*Apismelliferaadansonnii*haveantibacterialactivitiesagainst*Acinetobacter* species
3. *Acinetobacterspecies*isresistanttomorethanthreeclassesofantibioticsusedandsoitcanbeclassified as multi-drugresistance.

## Acknowledgment

I extend my heartfelt gratitude to the Almighty for the precious gift of life and good health. In Him, I find strength, guidance, and endless inspiration. A special acknowledgment goes to my dedicated supervisors, Prof. J. K. P. Kwaga and Prof. M. Bello, whose fatherly guidance, constructive criticism, and intellectual insights were instrumental in shaping my journey. They not only believed in me but also instilled the confidence needed to overcome challenges and complete this work successfully. To my unwavering husband, you have been my rock, providing constant support and encouragement. Your belief in my abilities, even during the toughest moments, has been my driving force. I appreciate your unwavering belief in me, my best friend. I express profound gratitude to my late father, Elder Ugbenyo John, and my mother, Mrs. Halima John, for their fatherly advice, support, and the confidence they instilled in me to face life's battles. To my dear brothers, Gabriel and David, your prayers and encouragement have been a source of strength. A special mention goes to Pastor Wole Dahunsi for his timely support, even in untimely hours. Dr. M.K. Lawan and Dr. Grace Kia, your words of encouragement, advice, and suggestions have been invaluable. My appreciation extends to the lecturers of the Faculty of Veterinary Medicine at A.B.U., Zaria, especially those in the Department of Veterinary Public Health and Preventive Medicine. Prof. Junaidu Kabir, Prof. E. C. Okolocha, Dr. B. V. Maikai, Dr. Grace. Nok-Kia, and Dr. M.K. Lawan, your impartation of knowledge and support were crucial. I acknowledge the guidance of the laboratory staff of the Department of Veterinary Public Health and Preventive Medicine, particularly Mal. A. Mahmud and Mal. S. Yahuza. Your assistance during my research is deeply appreciated. To my colleagues and friends in the Department of Veterinary Public Health and Preventive Medicine, A.B.U., Zaria, and the entire Postgraduate students (P15) set of the Faculty of Veterinary Medicine A. B. U. Zaria, your camaraderie is cherished.

**References**

Abbo,A.S.,Navon-Venezia,O.,Hammer-Muntz,T.K.,Siegman-Igra,Y.,Carmeli,Y.(2005). Multidrug-resistant*AcinetobacterbaumanniiEmergencyInfectiousDisease.* 11**:**22-29.

Abd Manap, M., Nampak, H., Pradhan, B., Lee, S., Sulaiman, W. N. A. and Ramli, M. F. (2014).Applicationofprobabilistic-basedfrequencyratiomodelingroundwaterpotentialmapping using remote sensing data and GIS. *Arabian Journal of Geosciences*, *7*(2): 711-724.

Abou-Raya, A., Abou-Raya, S. and Helmii, M. (2013). The effect of vitamin D supplementationon inflammatory and hemostatic markers and disease activity in patients with systemiclupuserythematosus:arandomizedplacebo-controlledtrial.*TheJournalof Rheumatology*,*40*(3): 265-272.

Abraham,J.C.,Palmer,D.,Dwyer,B. andSeifert,H.(2002).Community-acquiredbacteremic*Acinetobacter*pneumoniaintropicalAustraliaiscausedbydiversestrainsof*Acinetobacterbaumannii* with carriage in the throat in at-risk groups. *Journal of Clinical Microbiol*ogy, 4: 24-46

A'Court, C. and Garrard, S.C. (1992). Nosocomial pneumonia in the intensive care unit: mechanismsandsignificance. *Thorax.* 47: 465-473.

Adekoya, N., Thurman, D.J., Webb, K.W. and White, D.D. (2002). Surveillance for traumaticbraininjurydeaths United States, 1989-1998.

Al-Bari,Q.,Nablo,B.J.,Prichard,H.L.,Butler,R.D.,Klitzman,B.andSchoenfisch,M.H.(2016).Inhibition of implant-associated infections via nitric oxide release. *Biomedical Journal*.26:6984-6990.Replacewith NabloAllen,D.M. and-Wong,S.Y.(1990).Acinetobacter:Aperspective.*SingaporeMedicalJournal.* 31:511-514

Al-Yawer, M. A., Al-Khateeb, H. M. and Al-Khafaji, F. A. (2006). Garden cress seed could be afactualgalactagogue.*TheIraqiPostgraduate Medical Journal*,*5*(1), 62-67.

Anstey, N.M., Currie, B.J., Hassell, M., Palmer, D., Dwyer, B., Seifert, H. (2002). Community-acquiredbacteremic*Acinetobacter*pneumoniaintropicalAustraliais-causedbydiversestrainsof*Acinetobacter*specieswithcarriageinthethroatinat-riskgroups.*JournalofClinicalMicrobiol*ogy. 40**:**685-686

Arias,G.M.;Moellering,R.C.,Williams,D.V.(2008).*Antimicrobialcombinations*.InAntibioticsin Laboratory Medicine, 5th ed.; Lorian, V., Ed.;Williams & Wilkins Co: Baltimore, MD,USA, 2005; Pp. 432–492.

Arteaga, V., Lamas,A., Regal,P.,Vázquez,B.,Manuel,J.,Cepeda,A., Manuel,C.(2019).Antimicrobial activity of apitoxin from *Apis mellifera* in Salmonella enterica strainsisolated from poultry and its effects on motility, biofilm formation and geneexpres-sion.*Microbial. Pathogenesis*. *137*, 103771–103776.

Atallah,S.F.,El-Dosoky,A.R.,Coker,E.M.,Nabil,K.M.,&El-Islam,M.F.(2001).A22-yearretrospectiveanalysisofthechangingfrequencyandpatternsofre-ligioussymptomsamonginpatientswithpsychoticillnessinEgypt.*SocialPsychiatryandPsychiatricEpidemiology*,*36*(8): 407-415.

Babatunde, R.O., Olorunsanya, E.O., Omotesho, O.A. and Alao, B.I. (2010). Economics ofSmall Scale Honey Production in Nigeria: Case Study from Oyo state. *Journal ofAgriculturalEconomy,* 12:111-132.

Baracchi,D.,Francese,S.,Turillazzi,S.(2011).Beyondtheantipredatorydefence:Honeybeevenomfunction asacomponent ofsocial immuni-ty.*Toxicology. 58*, 550–557.

Baumann, P., Doudoroff, M., Stanier, R.Y. (1968). A study of the *Moraxella* group. II.Oxidative-negative species (genus *Acinetobacter*). *Journal of Bacteriolology.* 95:1520-1541

Becky,S.B.(1999)-ArthropodvenomcitrateinhibitsphospholipaseA2. *Toxicon*,33(6):763-770.

BeekeepingManual.(2017). 10thEdition,Baltimore; andWilkins,USA. Pp. 44-53

Benjamin, K., Ba˘si´c, I. and Mann, R.R. (2008). Anti-tumour activity of bee venom. *Journal ofBiology,* 103:49–54

Ber-nards,A.T.,Dijkshoorn,L.,Vander,Toorn,J.,Bochner,B.R.,VanBoven,C.P.(1995).Phenotypiccharacterisationof*Acinetobacter*strains of13DNA-DNAhybridisationgroupsbymeans of thebiologysystem.*Journal of Medical Microbiol*ogy.42: 113-119.

Bouvet,J.M. andGrimont,A.D.(1986).TaxonomyoftheGenus *Acinetobacter*withtheRecognitionof *Acinetobacter baumannii* sp. nov. *Aci-netobacter haemolyticus* sp. nov. *Acinetobacterjohnsonii* sp. nov. And *Acinetobacter junii* and amended descriptions of *Acine-tobactercalcoaceticus* and *Acinetobacter lwofii. Int. journal of systematic microbiology.* 32:228-240

Boyle, A.W., Morse R.A. and Kosikowski, F.V. (2012). Bioassay and standardization of venom ofthe honeybee. *Nature*, 198: 295 -296.

Brauers, J., Frank, U., Kresken, M., Rodloff, C.A., Seifert, H. (2005). Activities of various beta-lactamsandbeta-lactam/beta-lactamaseinhibitorcombinationsagainst*Acinetobacterbaumannii* and *Acinetobacter* DNAgroup 3 strains.*Clinical Microbiology Infection.*11**:**24-30.

Britland,S.,Smith,A.,Finter,W.,Eagland,D.,Vowden,K.,Vowden,P.andPritchard,D.(2011).Recombinant Lucilia sericata chymotrypsin in a topical hydrogel formulation degradeshumanwound eschar exvivo. *BiotechnologyProgress*, *27*(3): 870-874.

Bryony, D., Francese, S., Turillazzi, S. (2009). Beyond the antipredatory defence: Honey beevenomfunction asacomponent ofsocial immunity.*Toxicology. 58*, 550–557.

Carr,E.L.,Kampfer,P.,Patel,B.K.,Gurtler,V.and Seviour,R.J.(2003).SevennovelspeciesofAcinetobacter isolated from activated sludge. *International Journal of System EvolutionMicrobiology.*53**:**953-963.

Castro, H.J., Mendez-Lnocencio, J.I., Santilli, J., Nielsen, H.S., Pavot, A.P., Richert, J.R. andBellanti,J.A.(2005).Aphase1studyofthesafetyofhoneybeevenomextractasapossibletreatment for patients with progressive forms of multiple sclerosis. Allergy Asthmasprocurement.*Publicmedicine*, 28: 470-476.

Catalano, M., Quelle, L.S., Jeric, P.E., Di Martino, A., and Land, S. M. (1999).Survival of*Acinetobacter baumannii* on bed rails during an outbreak and during sporadic cases.*Journalof Hospital Infections.*42**:**27-35.

CDC. (2004). *Acinetobacter baumannii* infections among patients at military medical facilitiestreating injured U.S. service members, 2002-2004. *MMWR Morbidity Mortality Weekly.*53**:**1063-1066.

Chaisakul,H.J.,Nielsen,H.S.,Pavot,A.P.,Richert,J.R.andBellanti,J.A.(2017).Aphase1studyofthesafetyofhoneybeevenomextractasapossibletreatmentforpatientswithprogressive forms of multiple sclerosis. Allergy Asthmas procurement. *Journal of Publicmedicine*,6: 412-476.

Chaline W.C., Park, T.I., Kim, H.J. and Hyun, S.H. (2004). Outer membrane protein 38 of*Acinetobacterbaumannii*localizestothemitochondriaandinducesapoptosisofepithelialcells.*Cell. Microbiology.*2:111-119

Charles,F.C.,Martino,A.andRock,L.(2017).Survivalof*Acinetobacterbaumannii*onbedrailsduringanoutbreakandduringsporadiccases.*Journalof HospitalInfections.*42**:** 27-35

Cheesbrough, M. (2000). *District laboratory practice in tropical countries, part 2*. CambridgeUniversitypress.

Chen,Y.(2016)*ApicultureinChina.* AgriculturalPublishingHouseBeijing. Pp12-17

Choi, C.H., Lee, E.Y., Lee, Y.C., Park, T.I., Kim, H.J., Hyun, S.H. (2005). Outer membraneprotein38of*Acinetobacterbaumannii*localizestothemitochondriaandinducesapoptosisofepithelial cells. *Cell. Microbiology.***7:**1127-1138.

Choi,J.H.,Jang,A.Y.,Lin,S.,Lim,S.,Kim,D.,Park,K.,Han,S.M.,Yeo,J.H.,Seo,H.S.(2015). Melittin,ahoneybeevenom-derivedantimicrobialpeptide,maytargetmethicillin-resistant *Staphylococcusaureus*.*MolecularMedical Response.12*,6483–6490.

Clinical and Laboratory Standards Institute (CLSI). (2010). *Performance standards for antimicrobialsusceptibilitytesting*: Nineteenth informational supplement.

Corrigan, K.M., Harmis, N.Y., Willcox, M.D. (2001). Association of *Acinetobacter* species withcontactlens-induced adverseresponses.*Cornea.*20**:**463-466.

Corzo, G., Escoubas, P., Villegas, E., Barnham, K. J., HE, W., Norton, R. S., & Nakajima, T.(2001). Characterization of unique amphipathic antimicrobial peptides from venom of thescorpion*Pandinus imperator*. *Biochemical Journal*, *359*(1), 35-45.

Cosgrove, A. and Loucks D.P.(2015). Water management: current and future challenges andresearchdirections.*Water Resources Research*,51, 4823 – 4839

Craven, D.E., Barber, T.W., Steger, K.A., Montecalvo, M.A. (1990). Nosocomial pneumonia inthe 1990s: update of epidemiology and risk factors. *Seminar of Respiratory Infect*ion.5**:**157–172.

Cruz-Landim, H.J., Mendez-Lnocencio, J.I., Omidvar, B.; Omidvar, J., Santilli, J., Nielsen, H.S.,Pavot, A.P., Richert, J.R., Bellanti, J.A. (2000). A phase I study of the safety of honeybeevenom extract as a possible treatment for patients with progressive forms of multiplesclerosis.*AllergyAsthmaProc*. 26, 470–476.

Davis, K.A., Moran, K.A., McAllister, C.K. and Gray, P.J.(2015). Multidrug-resistantAcinetobacter extremity infections in soldiers. *Journal of Emergency of InfectiousDisease*,11(8):1218-24.

DeBarros,E.,Gonçalves,R.M.,Cardoso,M.H.,Santos,N.C.,Franco,O.L. andCândido,E.S.(2019).Snake venom cathelicidins as natural antimicrobial peptides. *Front. Pharmacology*, *10*,1415–1427.

DiCesare,A.,Laiacona,F.,Iorio,R.,Marangi,M.andMenegotto,A.(2016).*Aelurostrongylusabstrusus* inwild felidsofSouth Africa. *Parasitologyresearch*, *115*(10),3731-3735.

Dibner,J.J.andRichards,J.D.(2005).Antibioticgrowthpromotersinagriculture:historyandmodeofaction. *Poultryscience*, *84*(4), 634-643.

Dijkshoorn,L.,Nemec,A.andSeifert,H.(2007).Anincreasingthreatinhospitalmultidrug-resistant*Acinetobacter Species*. *NationalReviewof Microbiology*,5: 939-951

Dobrovolsky,A. B. andTitaeva,E.V.(2002).Thefibrinolysissystem:regulationofactivityandphysiologicfunctionsof itsmaincomponents.*Biochemistry (Moscow)*,*67*(1),99-108.

Doern,C.D.(2014).WhenDoes 2Plus2Equal 5:AReviewofAntimicrobialSynergyTesting.
*Journalof Clinical Microbiology,52:4124–8.*

Dolan,L.C.,Matulka,R.A.andBurdock,G.A.(2010). Naturallyoccurringfoodtoxins.*Toxins*, *2*(9), 2289-2332.

Doo,K.H., Lee,J.H.,ChoS.Y.,JungW.S.,Moon,S.K.,Park,J.M.,Ko,C.N.,Kim,H.,Park,H.J.and Park, S.U. (2015). A Prospective Open-Label Study of Combined Treatment forIdiopathic Parkinson's Disease Using Acupuncture and Bee Venom Acupuncture as anAdjunctiveTreatment.*Journal of AlternativeComplementaryMedicine*, 21: 598-603

Dorsey,C.W.,Beglin,M.S.,Actis,L.A.(2003).Detectionandanalysisofironuptakecomponentsexpressedby*Acinetobacterbaumannii*clinicalisolates.*JournalofClinicalMicrobiology.*41**:**4188-4193.

Douglas, J.H. (2018). Repetitive treatment with diluted bee venom reduces neuropathic pain viapotentiation of locus coeruleus noradrenergic neuronal activity and modulation of spinalNR1phosphorylation inrats.*Journal of Pain,* 13, 155–166.

Engel,LJ.,Peter,M.andMufson.A.(2005).In-Vitro,AntimicrobialAgentsandChemotherapy.
*JournalofMicrobiology,*50(3):111-123.

Erridge, C., Moncayo-Nieto, O.L., Morgan, R., Young, M., Poxton, I.R. (2007). *Acinetobacterbaumannii* lipopolysaccharides are potent stimulators of human monocyte activation viaToll-likereceptor4signalling.*Journal ofMedicalMicrobiol*ogy.56**:**165-171.

Farinde, A. J. and Ajayi, A. O. (2005). Training needs of women farmers in livestock production:ImplicationsforruraldevelopmentinOyoStateofNigeria.*JournalofSocialSciences*,*10*(3), 159-164.

Fitzmaurice, C., Abate, D., Abbasi, N., Abbastabar, H., Abd-Allah, F., Abdel-Rahman, O. andDerakhshani, A. (2019). Global, regional, and national cancer incidence, mortality, yearsof life lost, years lived with disability, and disability-adjusted life-years for 29 cancergroups, 1990 to 2017: a systematic analysis for the global burden of disease study. *JAMAoncology*, *5*(12), 1749-1768.

Flávia, A., Pereira, M., Albano, M., Cristina, F., Alves, B., Fernanda, B., Teles, M., Furlanetto,A., Mores, V.L. (2020). Influence of apitoxin and melittin from *Apis mellifera* bee onStaphylococcusaureus strains.*Microbial Pathology.141*, 104011

Free. J.B. (*1993*). *Insect Pollination of Crops*. 2nd Enlarged Edition, Academic Press, London,684.

Garnacho-Montero,J.,Ortiz-Leyba,C. andFernandez-Hinojosa,E.(2005).*Acinetobacterbaumanniiventilator-associated pneumonia: epidemiological and* clinical findings. *Intensive CareMedicine.*31**:**649-655.

Gaynes.R.,andEdwards,J.R.(2005).OverviewofnosocomialinfectionscausedbyGram-Negative Bacilli. *Clinical Infectious Diseases.* 41:848–854.

GibsonA.A.,Khalid,C,K.andWareham,D.W.(2014).PotentSynergyandSustainedBactericidal Activity of a Vancomycin-Colistin Combination versus Multidrug-ResistantStrains of *Acinetobacter baumannii*. *Journal of Antimicrobial Agents and Chemotherapy*,54, 5316-5322.

Gill, O. B. (2009). Synergy Tests by E test and Checkerboard methods of antimicrobialcombinationsagainst *Brucellamelitensis*.*JournalofClinicalMicrobiology*,43(1):22-25.

Gkrania-Klotsas,E.andHershow,R.C.(2006).Colonizationorinfectionwithmultidrug-resistant*Acinetobacterbaumannii*may beanindependentriskfactor forincreasedmortality.*ClinicalInfectious Dis*ease. 43**:**1224-1225.

Gonzalez,R.,Nusblat,H.A.,Nudel,B.C.(2001).Detectionandcharacterizationofquorumsensingsignal molecules in*Acinetobacter*strains.*MicrobiologyRes*. 155:271-277.

Gordon, N.C., Png, K and Wareham, D.W. (2010) Potent Synergy and Sustained BactericidalActivity of a Vancomycin-Colistin Combination versus Multidrug-Resistant Strains of*Acinetobacter Species*. *Journal of Antimicrobial Agents and Chemotherapy*, 54, 5316-5322.

Gorman, E. K. (2016). A new method for the separation of components of bee venom, especiallythecentrallyactive peptide, apamine.*Journal ofBiochemstry*, 343:192-203.

Hadisoesilo S.Y.; Roh, D.H.; Yoon, S.Y.; Moon, J.Y., Beitz, A.J. (2009). Repetitive treatmentwith diluted bee venom reduces neuropathic pain via potentiation of locus coeruleusnoradrenergic neuronal activity and modulation of spinal NR1 phosphorylation in rats.*Journalof Pain,* 13, 155–166.

Han,S.,Lee,K.,Yeo,J.,Baek,H.andPark,K.(2009).Antibacterialandanti-inflammatoryeffectsof honeybee (*Apis mellifera*) venom against acne-inducing bacteria. *Journal of MedicinalPlantsResearch*, *4*(6), 459-464.

Haris, S. L., Falcot, V., Lacassin, F., Mikulski, M. and Baumann, F. (2015). Risk factors forcarbapenem-resistant*Acinetobacterbaumannii*infectionsatatertiarycarehospitalinNewCaledonia,SouthPacific.*ScandinavianJournal of InfectiousDisease*, 42:821-826.

Harpers, K.K. (2016). Biological effects of treatment of an animal skin wound with honeybee(*Apis mellifera.* L) Venom.*Journal of Plastic Reconstruction Aesthetic Surgery*, 64:67–72.

Head,J.L.(2008).ContributionoftheStaphylococcusaureusAtlAMandGLmureinhydrolaseactivitiesin celldivision,autolysis, andbiofilm formation.*PLoS ONE***,** 7:42244.

Hegazi,A.G.,Fyrouz,M.andAbd-Allah,F.A.(2012).AntimicrobialActivityofDifferentSaudiArabiaHoneys. *Global Veterinarian*, 9 (1): 53-59.

Hegazi, A. G., Moharram, N. Z., Abd-Allah, F. A., Nour, M. S. and Khair, A. M. (2015a).Antibacterial activity of different Egyptian honeys in relation to some bee products.*EgyptianJournal ofVeterinary*Science, 36: 31-42.

Hegazi,A.G.,Abdou,A.M.andAbd-Allah,F.A.(2015b).Evaluationoftheantibacterialactivityofbeevenomfromdifferentsources.*WorldAppliedSciencesJournal*,30 (3):266-270.

Hepburn,H.R.andHepburn,C.(2005).Bibliographyof Apisflorea.*Apidologie*,*36*(3),377-378.

Heritier, .C., Poirel, L., Nordmann, P. (2006). Cephalosporinase over-expression resulting frominsertionofIS*A-ba1*in*Acinetobacterbaumannii*.*ClinicalMicrobiologyInfection.*12:123-130.

Higgins,P.G.,Dannmhayn,C.,Hackel,M.,andSeifert,H.(2010).GlobalSpeadofCarbapenem-resistant*AcinetobacterSpecies. Journalof Antimicrobialchemotherapy*, 15:233-238.

Hoduri J. (2002). A note on the preparation and use of selective and differential medium for theisolation of the *Acinetobacter spp* from clinical sources. *Journal of Applied Bacteriology*,54: 141-142.

Holly, E. (2017). Bee and wasp venoms: The biochemistry and pharmacology of their peptidesandenzymesare reviewed.*Science*, 177: 314 -322.

Holton, J. (1983). Efficient differentiation of functional hepatocytes from human embryonic stemcells.*StemCells*, *26*(5), 1117-1127.

Hude-
wenzJ.G.,Krieg,N.R.,Sneath,H.A.,Stanley,J.T.andWilliams,S.T.(2013).HumanhealthInternationalUnionofBiochemistryandMolecularBiology.*JournalofInfectiousDisease*,6(6):317-318.

Hujer,K.M.,Hamza,N.S.,Hujer,A.M.,Perez,F.,Helfand,M.S.,Bethel,C.R.(2005).Identification of a new allelic variant of the *Acinetobacter baumannii* cephalosporinase,ADC-7beta-lactamase:definingauniquefamilyofclassCenzymes.*AntimicrobialAgentsChemotheraphy.*49:2941-2948

Iregbu, K.C, Ogunsola, F.T, Odugbemi, T.O**. (**2002**).** Infections caused by *Acinetobacter* speciesand their susceptibility to 14 antibiotics in Lagos University Teaching Hospital, Lagos.*WestAfrican Journal of Medicine.*21(3):226-229
Ito, Y., Nakamura, M., Hotani, T.and Imoto, T. (1995). Insect lysozyme from house fly (Muscadomestica) larvae: possible digestive function based on sequence and enzymatic properties. *TheJournalof Biochemistry*,*118*(3), 546-551.

Jamasbi,E.,Mularski,A. andSeparovic,F.(2016).Modelmembraneandcellstudiesofantimicrobialactivityof melittin analogues.*Current Topical MedicalChemotherapy.16*, 40–45.

James, A.G. and Grant.,L. (2016). Do different interpretative methods used for evaluation of thecheckerboardsynergytest affecttheresults?*Journal ofMicrobiology,* 46: 410–420.

Jawad.A.,Seifert,H.,Snelling,A.M.,Heritage,J.,Hawkey,P.M.(1998).Survivalof*Acinetobacter baumannii* on dry surfaces: comparison of outbreak and sporadic isolates.*Journalof Clinical Microbiol*ogy. 36**:**1938-1941.

Jensen, T., Pedersen, S. S., Garne, S., Heilmann, C., Høiby, N. and Koch, C. (1987). Colistininhalation therapy in cystic fibrosis patients with chronic *Pseudomonas aeruginosa* lunginfection.*Journal of Antimicrobial Chemotherapy*, *19*(6), 831-838.

Johnson, E.N., Burns, T.C., Hayda, R.A., Hospenthal, D.R., Murray, C.K. (2007). InfectiouscomplicationsofopentypeIIItibialfracturesamongcombatcasualties.*ClinicalInfectiousDis*eases.45**:**409-415.

Jonas, T., Heilmann, C., Hoiby, N. and Koch, C. (2018). Colistin inhalation therapy in cysticfibrosispatientswithchronic*Pseudomonasaeruginosa*lunginfection.*JournalofAntimicrobialChemotherapy,*7(2): 381–448.

Jyothi.J.V.(1994).IncidenceofthemiteNeocypholaelapsIndia(Evans)inthecoloniesofIndiahivebee(*Apiscerena indica F*)at Bangalore(India). *India beeJournal*, 56:32-34.

Karah,D.M. (2012).Hyaluronidasefrom thevenomof thehoneybee (*Apis mellifera*).*Toxicon*

(Supplement),3:227-228.

Kim, I. H., Hancock, J. D., and Hines, R. H. (2012). Influence of processing methods on the ilealdigestibility of nutrients from soybeans in growing and finishing pigs. *Asian-AustralianJournalAnimal Science,*13 (2): 192-199.

Knapp,S.,Wieland,C.W.,Florquin,S.,Pantophlet,S.R.,Dijkshoorn,L.,Tshimbalanga,N.,Akira,S.,Vander. And Poll,T.(2006).DifferentialrolesofCD14andToll-likereceptors4and2inmurine*Acinetobacter*pneumonia.*AmericanJournalofRespiratoryCriticalCareMedicine.*173**:**122-129

Kosmidis H., Dahabreh, I. J., Kanaloupiti, D., Siannis, F., Bafaloukos, D., Kosmidis, P. andMurray, S. (2012). Assessment of somatic k-RAS mutations as a mechanism associatedwithresistancetoEGFR-targetedagents:asystematicreviewandmeta-analysisofstudiesinadvancednon-small-celllungcancerandmetastaticcolorectalcancer.*Thelancetoncology*, *9*(10), 962-972.

Krell,R.(1996). Beekeepingproductanditsbiochemicalcomposition.FAOFoodandAgricultureOrganization ofthe United NationsRo-ma;Pp122

Kucharski M.W.; Patron, R.L.; Archer, G.L. (2016).Combinations of Vancomycin and beta-lactams are synergistic against staphylococci with reduced susceptibilities to vancomycin.*AntimicrobialAgents Chemotherapy*, 43:1747–1753.

Kwon, K.T., Song, J.H., Chang, H.H., Jung, S.I., Kim, S.W., Ryu, S.Y. (2001).Impact ofimipenem resistance on mortality in patients with Acinetobacter bacteraemia. *Journal ofAntimicrobialChemotheraphy.*59**:**525-530

Lee,D.S.,Kang,M.D.,Hwang,E.J.,Eom,S.H.,Yang,J.Y.,Lee,M.S.,Lee,W.J.,Jeon,Y.J.,Choi, J.S. and Kim, Y.M. (2014). Synergistic effect between dieckol from Ecklonia stoloniferaand _lactamsagainst methicillin-resistant *Staphylococcusaureus*. *England journal ofBiotechnology,*13, 758–764.

Leung,W.S.,Chu,C.M.,Tsang,K.Y.,Lo,F.H.,Lo,K.F.,Ho,PL.(2006).Fulminantcommunity-acquired *Acinetobacter baumannii* pneumonia as a distinct clinical syndrome.*Chest.*129:102-109.

Limansky, A.S., Mussi, M.A., Viale, A.M.(2002). Loss of a 29-kilodalton outer membraneprotein in *Acinetobacter baumannii* is asso-ciated with imipenem resistance. *Journal ofClinicalMicrobiology.*40:4776-4778.

Ling,M.J.,Hut,Y.J.,French,G.L.(1988).EvaluationoftheMicrobact-24Ebacterialidentificationsystem.*Journal of ClinicalPathology.*41:910-914.

Littlewood,J.M.,Koch,C.andLambert,P.A.(2000).Aten-yearreviewofColimycin.*RespiratoryMedicine,*94(7): 632–640.

Lu,X.,Zhang,H.,Ni,Y.,Zhang,Q.andChen,J.(2008).Porousnanosheet-basedZnOmicrospheres for the construction of direct electrochemi-cal biosensors. *Biosensors andbioelectronics*,*24*(1): 93-98.

Lubke, L.L. and Garon, C.F. (1997). The antimicrobial agent melittin exhibits powerful in vitroinhibitoryeffectsonthelymediseasespiro-chete.*ClinicalInfectionDiseases.25*,S48–S51.

Lucky,H.M.,Enty,T.(2009).Isolationofenvironmentalmicroorganismsfromclinicalspecimens: A report of the occurrence of *Acinetobacter anitratus* in blood of hospitalizedpatientsin Jakartaina 7 yearperiod.*MedicalJournalof Indones.*18: 227-232.

Lüderitz, O., Freudenberg, M.A., Galanos, C., Lehmann, V., Rietschel, E.T. and Shaw, D.H. (1982).Lipopolysaccharidesofgram-negativebacteria.*CurrentTopicalMembraneTransportation. 17*, 79–151.

Lye,W.C.,Lee,E.J. and Ang,K.K.(1991).AcinetobacterperitonitisinpatientsonCAPD:characteristicsand out-come.*AdvancePeritonealDialysis.***7:** 176–179.

Magnet, S., Courvalin, P. and Lambert, T**.** (2001). Resistance-nodulation-cell division-type effluxpump involved in aminoglycoside resis-

tance in *Acinetobacter baumannii* strain BM4454.*AntimicrobialAgents Chemotheraphy*. 45**:** 3375-3380

Manikal,V.M.,Landman,D.,Saurian,G.,Odyna,E.,Lal,H. and-Quale,J.(2000).EndemiccarbapenemsresistantAcinetobacterspeciesinBrookylnNewYork:citywideprevalence,interinstitutional spreadandrelationtoantibioticusage.*ClinicalInfectiousDisease.*31: 101-106.

Marchand, I., Damier-Piolle, L., Courvalin, P. and Lambert, T. (2004). Expression of the RND-typeeffluxpumpAdeABCin*Acinetobacterbaumannii*isregulatedbytheAdeRStwo-componentsystem.*Antimicrobial Agents Chemotheraphy*.48: 3298-3304

McGhee, S., Visovksy, C., Zambroski, C. and Finnegn, A. (2018).Lyme disease: Recognition andmanagementfor emergency-nurses.*EmergencyNurse. 28*.

Menezes, M. D. L. L. R., Ribeiro, H. L., Flávia de Oliveira, M. and de Andrade Feitosa, J. P.(2020).Optimizationof thecollagenextraction-fromNiletilapiaskin(Oreochromisniloticus)anditshydrogelwithhyaluronicacid.*ColloidsandSurfacesB:Biointerfaces*,*189*: 110852.

Menon, T., Shanmugasundaram, S., Nandhakumar, B. and Nalina, K. (2006). Infective endocarditisdue to *Acinetobacter baumannii* complex—a case report. *Indian Journal of PathologicalMicrobiology.*49: 576-578

Metan,G.,Alp,E.,Aygen,B. andSumerkan,B**.**(2007).Carbapenem-resistant*Acinetobacterbaumannii*:anemergingthreatforpatientswithpost-neurosurgicalmeningitis.*InternationalJournal of Antimicrobial Agents.*29: 112-113.Michael,A.(1999).SeasonalvariationofAcinetobacterinfections.*ClinicalInfectiousDisease.* 24:222-234.

Michael, A. (2009). Antibacterial and anti-inflammatory effects of honeybee (*Apis mellifera*)venomagainst acne1-inducingbacteria. *Journalof Medicine*, 2(5): 23-55.

MichioR.E.andSakamoyto.N.K.(2009).Infectiveendocarditisdueto*Acinetobacterbaumannii* Complex—acasereport.*IndianJournalof PathologicalMicrobiology.* 44**:** 465-511

Migdal, A.R., Fakhimi, A., Zarrini, G., Dehghan, G. and Iranshahi, M. (2005). Galbanic acid fromFerula szowitsiana enhanced the antibacterial activity of penicillin G and cephalexinagainst*Staphylococcus aureus*.Biological Pharmacy.Bulletin, 30:1805–1807.

Minandri., G., Horvath J., Almas M.,Saftics G., Palocz A. (2012)- Liquid ChromatographicAnalysisandSeparationofPolipeptideComponentsfromHoneyBeeVenoms.*JournalofLiquidChromatography,*17 (16):3333-3349.

Morgan, R., Theobald, S., Hawkins, K., Ssali, S., George, A. and Molyneux, S. (2017). Theimportanceofgender analysis inresearchfor-healthsystems strengthening. *Journal of gender community health*. 166: 44-67

Mosafer, H. K. (2007). *Effect of crude fimbriae extract of Acinetobacter baumannii on biotic andabioticsurfaces*(Doctoraldissertation,Msc.thesis.Biologydepartment.CollegeofScience,AL-MustansiriyahUniversity. Baghdad,Iraq). Pp 66-107

Murray, C.K., Roop, S.A, Hospenthal, D.R, Dooley, D.P, Wenner, K., Hammock, J., Taufen, N. andGourdine,E.(2006).Bacteriologyofwarwounds atthetimeofinjury.*MilitaryMed*icine.171**:** 826-829.

Nablo,B.J.,Prichard,H.L., Butler,R.D.,Klitzman,B. andSchoenfisch,M.H.(2005). Inhibitionofimplant-associatedinfectionsvianitricoxiderelease. *Biomaterials*,*26*(34):6984-6990.

Namita, P., Mukesh, R. and Vijay, K. J. (2012). Camellia sinensis (green tea): a review. *Globaljournalof pharmacology*, *6*(2), 52-59.

Nathan,S.,Sugai,M.,Komatsuzawa,H.,Nakashima,S.,Oshida,T.,Matsumoto,A. and Suginaka,

H.(2009).AnautolysinringassociatedwithcellseparationofStaphylococcusaureus.Journalof Bacteriology, 178:1568–1571.

Navarre,W.W. andSchneewind,O.(1999).Surfaceproteinsofgram-positivebacteriaandmechanisms of their targeting to the cell wall envelope. *Microbiology Molecular BiologyRev.63*, 174–229

Neely,A.N.,Male,M.P.andWarden,G.D.(1999).Computerkeyboardsasareservoirfor *AcinetobacterSpecies*inaburnhospital.*Clinicalinfectiousdisease,* 29:1358-13560.

Oberoi, A., Aggarwal, A. and Lai, S. (2018). A decade of underestimated nosocomial pathogen-AcinetobacterinatertiarycarehospitalinPunjab.*JournalofMedicalScience*,24(11):34-36.

Odewale,G.,Adefioye,O.J.,Ojo,J.,Adewumi,F.A.andOlowe,A.O.(2017).MultidrugResistance of *Acinetobacter Species* in Ladoke Akintola University Teaching Hospital,Osogbo,Nigeria. *EuropeanJournal of Microbiologyand immunology*,6(3): 238-243.

Ojeleye,B.(2003).HoneyproductioninNigeria.In*Athree-daybeekeepingandhoneyproductiontraining workshop conducted by Centre for Bee Research and Development (CEBRAD),Ibadan,Held at theFacultyof Agriculture, Universityof Ilorin, Nigeria*.

Olaitan, A.O., Berraze, M., Fagade, O.E., Adelowo, O.O., Alli, J.A. and Rolain, J.M. (2013).Emergenceofmultidrug-resistant*AcinetobacterSpecies*producingOXA-23Carbapenemase,Nigeria.*International JournalofInfectious Disease*,17:495-470.

Orsolic, N., Sver, L., Terzic, S., Tadic, Z. and Basic, I. (2003). Inhibitory effect of water-solublederivativeofpropolisanditspolyphenoliccompoundsontumorgrowthandmetastasizingability:apossiblemode of antitumoraction. *Nutrition andcancer*, *47*(2): 156-163.

Owen, W.R. and Bridge, R. (1999**).** The bee venom test: A new tonic-pain test. *Pain*, 66(2):271–277.

Pak, S. C. (2016). An introduction to the Toxins special issue on “Bee and wasp venoms:Biologicalcharacteristicsand therapeuticapplication”.*Toxins. 8*,315

Park,S.,Kim,H.,Lee,K.M.,Yoo,J.S.,Yoo,J.I.I,Lee,Y.S.andChung,G.T.(2015).Molecularand Epidemiological Characterization of Carbapenem-Resistant *Acinetobacter baumannii*inNon-TertiaryKoreanHospitals.*Yonsei Medical Journal*,54(1): 177-182.

Paul, S. and Shumosky (2015). Bee venom component of promising Antimicrobial agent toenhance healing of MRSA induced wound. Phospholipase A from bee venom. *EuropeanJournalof Biochem*istry,20: 479.

Peleg, A,Y., Bell, J.M., Hofmeyr, A. and Wiese, P. (2008). Inter-country transfer of gram-negativeorganismscarryingtheVIM-4andOXA-58carbapenem-hydrolysingenzymes.*JournalofAntimicrobialChemother*aphy. 57**:** 794-795.

Picoli,T.,Peter,C.M.,Zani,J.L.,Waller,S.B.,Lopes,M.G.,Boesche,K.N.,D’ÁvilaVargas,G.,deOliveiraHübner,S. and Fischer,G.(2017).Melittinanditspotentialinthedestructionandinhibitionofthebiofilmformationby*Staphylococcusaureus*,*Escherichiacoli*and*Pseudomonasaeruginosa* isolatedfrom bovinemilk. *Microbial Pathogenesis.112*,57–62

Putz,T.,Aga.M.,Hino,K. and Koya-Miyata.,S.(2006).Apoptosisofhumanleukemiacellsinducedbyartepillin C,an active ingredient ofBrazilian propolis.*Anticancer Res,*21:221–228.

Radloff,S. E. (1998).Review on Honeybeesof African.*Nature*, 6: 33-46

Rainey, W. E., Saner, K. and Schimmer, B.P. (2004). Adrenocortical cell lines. *Molecular andcellularendocrinology*, *228*(1-2), 23-38.

Ramanadham,M.,NassarM.andLafiS.(2015).Theincidenceofpan-drugresistanceinasampleof*Acinetobacterbaumannii*clinicalisolatesobtainedfromAl-RamadiTeachingHospital.*IraqiJournal of*

*Science*,3: 262-266.

Rello. J. (1999). *Acinetobacter baumannii* infections in the ICU. *Chest.* 115**:**1226-1229

Richard,O.G.,Okolocha,E.C.,Odinna,A.V.,Paul,M.P.,Audu,D.F.,Adamu,D.A.,Uwanibe, E.L. and Costa da Silva, R. (2015). Public health risk of Abattoir operation in ZangoAbattoirZaria, KadunaStateNigeria.*AcademicJournal*, 5: 139.

Rizvi, N., Rao, N. A. and Hussain, M. (2000). Yield of gastric lavage and bronchial wash inpulmonarytuberculosis.*TheInternationalJournalofTuberculosisandLungDisease*,*4*(2), 147-151.

Samy,R.P.,Gopalakrishnakone,P.,Thwin,M.M.,Chow,T.K.,Bow,H.,Yap,E.H. and Thong,T.W.(2007).Antibacterial activity of snake, scorpion and bee venoms: A comparison withpurified venom phospholipase A2 enzymes. *Journal of Applied Microbiology. 102*, 650–659.

Sanders, K. and Sanders, T. (2014). *The Future of Wound Healing*. In: Leaper, D.J., Harding K.J.Wounds:Biologyand Management.Oxford: UniversityPress:Oxford. Page191.

SSchumach.C.C.(1989).Nutritionalandantinutritionalcomponentsof*Pennisetumpurpureum* *PakistanJournalofnutrition*,*8*(1):32-34.

Schumancher,M.J.,Schmidt,J.O.andEgen,W.B.(2002).“Lethalityofkillerbeestings”.*Nature*. 337: 413.

Shaposhnikova,V.V.,Kublik,L.N.,Narimanov,A.A.,MKh, L.,Orlova, O.E.,Kudriavtsev,A. A.andIuN,K.(2001).GrowthinhibitionandinductionoftumorcelldeathbyphospholipaseA2andlipoxygenaseinhibitors.*IzvestiiaAkademiiNauk.SeriiaBiologicheskaia*,(2), 249-252.

Shugart, A.A., Leshchenko, V.V. and Korystov, I.N. (2016). Growth inhibition and induction oftumor cell death by phospholipase A2 and lipoxygenase inhibitors. Izvestiia AkademiiNauk.*Seriia Biologicheskaia*. 7: 214-333.

Siau, H., Yuen, K. Y., Wong, S. S. Y., Ho, P. L. and Luk, W. K. (1996). The epidemiology ofacinetobacterinfectionsinHong-Kong.*Journalofmedical microbiology*,*44*(5): 340-347.

Smith, A.W. and Alpar, K.E. (1991). Immune response to *Acinetobacter calcoaceticus* infectioninman. *Journal of Medical Microbiogy*. 34**:**83-88.

Smith, M.G., Gianoulis,T.A., Pukatzki,S.,Mekalanos, J.J., Ornston,L.N.,Gerstein,M. and Snyder, M. (2009). New insights into *Acinetobacter baumannii* pathogenesis revealed by high-densitypyrosequencingandtransposon mutagenesis.*Genes Development.* 21:601-614

Socarras, K.M., Theophilus, P.A., Torres, J.P., Gupta, K., Sapi, E. (2017). Antimicrobial activityofbeevenomandmelittin against borrelia-burgdorferi.*Antibiotics. 6*: 31.

Sohrabi, A.W., West P.R., Odell G.V., Hudiburg S.M., Ownby C.L., Mills J.N., Scroggins B.T. andShannonS.B.(2012): Arthropodvenomcitrateinhibitsphospholipase A2.*Toxicon*(Oxford),33(6): 763-770.

Son, D. J., Lee, J. W., Lee, Y. H., Song, H. S., Lee, C. K. and Hong, J. T. (2007). Therapeuticapplication of anti-arthritis, pain-releasing, and anti-cancer effects of bee venom and itsconstituentcompounds.*Pharmacology&therapeutics*, *115*(2), 246-270.

Talbot, G.H., Bradley, J., Edwards, J.E., Gilbert, D., Scheld, M. and Bartlett, J.G. (2006). Bad bugsneed drugs: an update on the development pipeline from the Antimicrobial AvailabilityTask Force of the Infectious Diseases Society of American *Clinical Infectious Disease.*42**:** 657-668.

Tarpy,K.J.(2016).BiologyofAcinetobacterspp.in:Acinetobacter:Microbiology,epidemiology,infections,and management.*Journal of bacteriology,* 11: 13-14.

Tomaras, A.P., Dorsey, C.W., Edelmann, R.E., Actis, L.A. (2003). Attachment to and biofilmformation on antibiotic surfaces by *Acinetobacter baumannii* involvement of a novelchaperone-usherpili assemblysystem.*Microbiology.* 149**:**3473-3484

Towner,K.J.(2006).BiologyofAcinetobacterspp.in:Acinetobacter:Microbiology,epidemiology,infections, andmanagement.*Journalof bacteriology,* 11: 13-14.

Trottier, V., Segura, P.G., Namias, N., King, D., Pizano, L.R., Schulman, C.I. (2007). Outcomesof*Acinetobacterbaumannii*infectionincriticallyillburnedpatients.*JournalofBurnCareRes*.28**:**248-254.

Villegas, M.V. and Hartstein, A.I. (2003).*Acinetobacter*outbreaks. *Infectious Control HospitalEpidemiology*,24: 284–295.

Villers, D., Espaze, E., Coste-Burel, M., Giauffret, F., Ninin, E., Nicolas, F., Richet, H**. (**1998**).**Nosocomial*Acinetobacterbaumannii*infections:microbiologicalandclinicalepidemiology.*Annual International Medicine.*129**:**182-189.

Visca, P., Antunes, L. and Towner, K. J. (2011). *Acinetobacter baumannii*: evolution of a globalpathogen.*Pathogens anddisease*, *71*(3), 292-301.

Walter,S.(2005).Anautolysin ringassociatedwithcell separationof*Staphylococcusaureus*.*JournalBacteriology*, 178: 1568–1571.

Wang, L., Zhao, X., Zhu, C., Zhao, Y., Liu, S., Xia, X., Liu, X., Zhang, H., Xu, Y. and Hang,

B.(2020).TheantimicrobialpeptideMPXkills*Actinobacilluspleuropneumoniae*andreducesits pathogenicityin mice.*Veterinary Microbiology.243*, 108634

Wareham, R., Tappin M. J., Pastore A., Harvey T. S., Carver J. A., Campbell I. D. (1988). Thestructureof melittin.*Euroupean. Journal Biochemistry*, 173: 139–146.

Weil, G. C., Simon, R. J. and Sweadner, W. R. (1933). A biological, bacteriological and clinicalstudyoflarvalormaggottherapyinthetreatmentofacuteandchronicpyogenicinfections.*TheAmericanJournal of Surgery*, *19*(1), 36-48.

Wenseleers, E.E, Harris, K. and Kim, D. (2004). Potent Synergy and Sustained BactericidalActivity of a Vancomycin-Colistin Combination versus Multidrug-Resistant Strains of*Acinetobacter baumannii*. *Journal of Antimicrobial Agents and Chemotherapy*, 54, 5316-5322.

Wilson, M. L. (2014). Clinical impact and pathogenicity of *Acinetobacter. Journal of ClinicalmicrobiologyInfections,*14:432-509.

Wisplinghoff, H., Bischoff, T., Tallent, S.M., Seifert, H., Wenzel, R.P. and Edmond, M.B. (2004).Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from aprospectivenationwidesurveillancestudy.*ClinicalInfectious Disease.* 39**:** 309-317.

Wysocki, A. B., Kusakabe, A. O., Chang, S. and Tuan, T. L. (1999). Temporal expression ofurokinaseplasminogenactivator,plasminogenactivatorinhibitorandgelatinase-Binchronic wound fluid switches from a chronic to acute wound profile with progression tohealing.*Wound Repair and Regeneration*,*7*(3): 154-165.

Yacoub,T.,Rima,M.,Karam,M. and Fajloun,J.M.(2020).Antimicrobialsfromvenomousanimals:Anoverview.*Molecules.25*: 2402

Zavascki, P.A., Goldani, Z.L., Jian, Li. and Nation, L.R. (2007). Polymyxin B for the treatment ofmultidrug-resistant pathogens: a critical review. *Journal of Antimicrobial Chemotherapy.*60: 1206–1215.

Beeculture.com (2016).Https://www.beeculture.com. Accessed 23th, February, 2021

Littleladiesofthehive.com (2016).Https://www.littleladiesofthehive.com. Accessed 20th, March, 2021

Yorkshirebeeandhoney.co.uk (2015). Https//www.yorkshirebeeandhoney.com. Accessed 10th, February, 2021