# ISOLATION AND CHARACTERIZATION OF MICROBES FROM DIABETES MELLITUS CELLULITIS WOUND SAMPLE AND ESTIITS ANTIMICROBIAL PROPERTIES

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**ABSTRACT:**

Bacterial Cellulitis is a spreading skin infection and causes skin lesions, necrotizing fasciitis, septic Arthritis and Osteomyelitis. It Damage the skin causes an abscess, Folliculitis. In the present study carriedout isolation and characterization of microbes from Cellulitis wounds samples from Diabetes Mellitus patients and testing its antimicrobial activity against the herbal plants, medicinal

plants *Biden spilosa, Aloe barbadensis ,Rauvolfia serpentina.* *Streptococcus sp.,* shows maximum zone of Inhibition (13mm) and minimum in *Aloe barbadensis* (1mm). In the present study it concludes *Rauvolfia serpentina*and *Biden spilosa,* maximum zone of inhibition against Cellulitis wound infection in *Klebsiella* shows maximum zone of inhibition. And *Rauvolfia serpentine* (22mm) and *Bidens pilosa* (19mm) were observed.

**INTRODUCTION**

Diabetes Mellitus is Prolonged disease. If the Pancreas Cannot Produce Sufficient Insulin. This Condition is known as Diabetes Mellitus. The Person who affected by Diabetes Mellitus faced many critical problems by microvascular dysfunction.

Bacterial Cellulitis and erysipelas which means burst, Spreading skin infection and other infection belongs with occurring suppurative foci like Skin lesions, necrotizing fasciitis Septic arthritis and Osteomyelitis. Normally innermost occurred skin infection is called Cellulitis and outermost layer infection is known as Erysipelas. Therefore the difference between these two diseases is not vivid properly and other two conditions distributed the medical properties. Group B and rarely ,*Staphylococcus sp.*, can also cause these diseas**e(*BonnetblancJM,BedaneC.2003,Chartier C,Groshans E 1990;Eriksson B et al 1996)* .**Result of patient blood culture normally positive for Beta-hemolytic Streptococcus sp., in <5% of cases (***Bonnet blanc JM et al.,2003).( Chartier et al.,1990.Eriksson B et al.***

Streptococcus sp., are classified under their Hemolytic properties and origin blood typing The explanation of wound healing is group of completive process . ***(Ballers S., et al.,2012).***The antibiotics, antiseptics and chemical properties are the several agent that cure the infection. *Streptococcus sp.,* is a Beta-hemolytic *Streptococci* and it is originated group A highly medical vital Species. Siddha Medicine have the capacity to recur from infections are ulcers, wound healing, skin lesions, Scabies, leprosy and venereal disease ***(kirthikar KR and Basu BD 2001.***

The anaerobic bacterium causes wound on foot of Diabetes Mellitus persons. (**Aherrao N et al*., 2012)****.* Isolation and characterization of microbes on wound infection and testing its antimicrobial activity against medicinal plants*.(* ***Krishnaveni et. al.(2020)***into different sp., by their ability to Heamolyse blood by serology and or by biochemical tests.

All *Staphylococcus aureus.,* produce the enzyme catalase which is used in the laboratory for rapid identification ***Kumar et. al.,(2006).***Inflence of *Aloe vera* on wound healing properties was explained by (**Chithra,P., et. al.,(1998)**.

**Habitat and Herbal powders**



## 

Bidens pilosa Bidenspilosa



## 



## Aloe barbadensis Aloebarbadensis extract



***Rauvolfia serpentine Rauvolfia serpentina leaf powder***

**Cellulitis Wound on Isolated Microbes from Cellulitis Diabetic Patients Diabetic Wound Sample**



# RESULTS

**Table 1:Isolation of Microbes from Diabetic Wound**

| **s. no** | **Isolated microbes** | **Colony forming units**  **CFU/ ml** | |
| --- | --- | --- | --- |
| **Colonies** | **Units** |
| 1.  2.  3.  4. | *E-coli*  *Staphylococcus aureus*  *Streptococcus sp.,*  *Klebsiella* | 180  200  280  160 | 1.8X103  2.0X103  2.8X103  1.6X103 |

**Table – 1 a): Biological Characteristics of *E.Coli*on Cellulitic Diabetic Wound**

| **S.No** | **Biological Test/ Staining** | **Positive/Negative** |
| --- | --- | --- |
| 1 | Gram staining | (Rod shape) negative |
| 2 | Culture characteristics on agar | White, moist glistening |
|  | slant | appearance |
| 3 | Gelatin liquification | Negative |
| 4 | Starch hydrolysis | Negative |
| 5 | Liquid hydrolysis | Negative |
| 6 | Lactose | AG |
| 7 | Dextrose | AG |
| 8 | Sucrose | A+ |
| 9 | H2s production | Negative |
| 10 | No3 reduction | Positive |
| 11 | Indole production | Positive |
| 12 | MR reaction | Positive |
| 13 | VP reaction | Negative |
| 14 | Citrate utilization | Negative |
| 15 | Urease activity | Negative |
| 16 | Catalase activity | Positive |

Acid +, gas +, reduction +

**Fig – 1a): Biochemical Characters of *Escherichia coli***

***E.coli* on EMB Agar*E .coli* on Blood Agar**



| **S.No** | **Biochemical Characters** | | **Positive/ Negative** |
| --- | --- | --- | --- |
| 1 | Gram staining |  | Rod (Negative) |
| 2 | Culture characteristics | on | Slimy, white somewhat |
|  | agar slant |  | translucent raised growth. |
| 3 | Gelatin liquification |  | Negative |
| 4 | Starch liquification |  | Negative |
| 5 | Liquid liquification |  | Negative |
| 6 | Lactose |  | AG |
| 7 | Dextrose |  | AG |
| 8 | Sucrose |  | AG |
| 9 | H2s production |  | Negative |
| 10 | No3 reduction |  | Positive |
| 11 | Indole production |  | Negative |
| 12 | MR reaction |  | Negative |
| 13 | VP reaction |  | + acid gas, curd + |
| 14 | Citrate use |  | Positive |
| 15 | Urease activity |  | Positive |
|  |  |  |  |
|  |  |  |  |

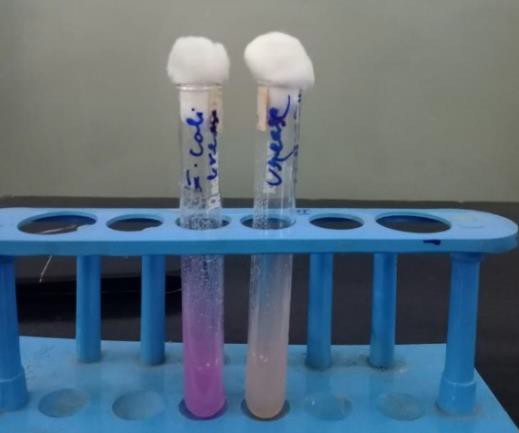
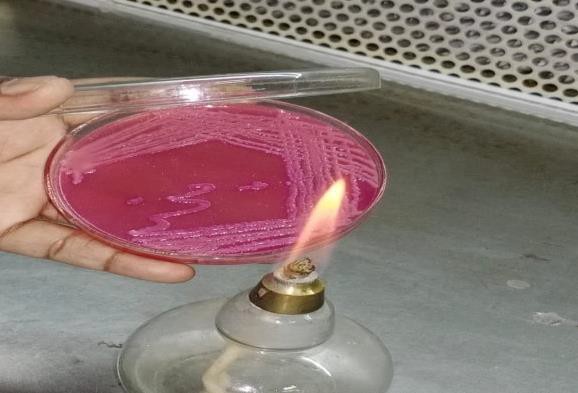
**Table - 2: Biological Characteristics of *Klebsiella sp.,* on Cellulitic Diabetic**

**Fig – 2: Biochemical characters of Klebsiella sp.**

**a) *Klebsiella*sp., on HectoneEntric agar b) Citrate Test – Positive**



1. ***Klebsiella*sp., on Macconkey Agar**



**Urease Test - Positive Nitrate Reduction Tes**



**Table - 3: Biological Characteristics of *Staphylococcus* Sp*.,* on Cellulitic Diabetic Wound**

| **s.no** | **Biochemical test/staining** | **Positive/ Negative** |
| --- | --- | --- |
| 1 | Gram staining | Positive coccus |
| 2 | Culture characteristic on agar | Abundant, opaque golden |
|  | slant | growth |
| 3 | Gelatin liquification | Positive |
| 4 | Starch liquification | Negative |
| 5 | Liquid liquification | Positive |
| 6 | Lactose | Absence |
| 7 | Dextrose | Absence |
| 8 | Sucrose | Absence |
| 9 | H2s production | Negative |
| 10 | No3 reduction | Positive |
| 11 | Indole production | Negative |
| 12 | MR reaction | Positive |
| 13 | VP reaction | + |
| 14 | Citrate use | Negative |
| 15 | Urease activity | Negative |
| 16 | Catalase activity | Positive |
| 17 | Oxidase activity | Negative |

Acid reduction +

**Fig – 3: Biochemical Characters of *Staphylococcus aureus***

***S.aureus*on Blood Agar Medium**



**Nitrate Test - Positive MR Test - Positive**



**Table - 4: Biological Characters of *Streptococcus Sp.,* on Cellulitic Diabetic Wound**

| **S.NO** | **BIOLOGICAL TESTING**  **/ STAINING** | **POSITIVE / NEGATIVE** |
| --- | --- | --- |
| 1 | CAMP (Christie – Alkins | Negative |
|  | munch Peterson) |  |
| 2 | Capsule formation | Capsulated |
| 3 | Catalase | Negative |
| 4 | Gram staining | Positive |
| 5 | Hemolysis | Beta hemolysis |
| 6 | Motility | Non -Motile |
| 7 | OF(Oxidative fermentative) | Facultative anaerobes |
| 8 | Shape | Cocci |
| 9 | Spore | Non- sporing |
| 10 | Urease | Negative |
| 11 | VP (VogesProskauer) | Negative |
| 12 | Fructose | Positive |
| 13 | Galactose | Positive |
| 14 | Glucose | Positive |
| 15 | Lactose | Positive |
| 16 | Gelatin liquification | Negative |
| 17 | Starch hydrolysis | Negative |

**Fig – 4: Bio chemical characters of *Streptococcus* sp.,**

***Streptococcus* sp., on Blood Agar Medium**



1. **Table - 5: Antibiotic Sensitivity:**

| **S.NO** | **ANTIBIOTICS** | ***E .coli*** | ***Klebsiella sp.,*** | ***Streptococcus sp.,*** | ***Staphylococcus aureus.,*** |
| --- | --- | --- | --- | --- | --- |
| 1 | Ciproflaxin | 36mm | 27mm | 12mm | 35mm |
| 2 | Tetracycline | 20mm | 20mm | 19mm | 12mm |
| 3 | Erythromycin | 18mm | 21mm | 11mm | 25mm |
| 4 | Penicillin | No  zone | No zone | 10mm | No zone |
| 5 | Ampicillin | No  zone | No zone | 8mm | 9mm |

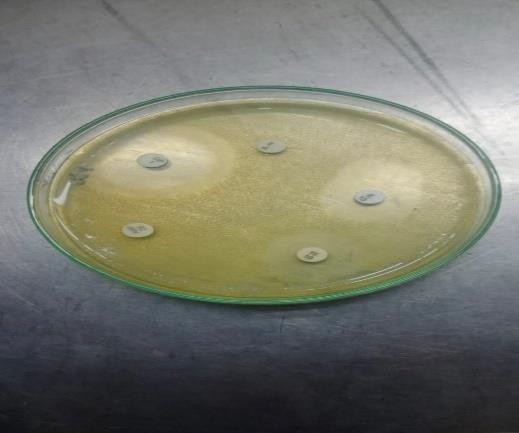
1. **Methyl Red Test - Positive c) Urease Test - Negative**



**Fig – 5: Antimicrobial Activity of Isolated Microbes**

**b) Disk Diffusion Method on) Disk Diffusion Method on**

***Streptoccocus*sp.,**



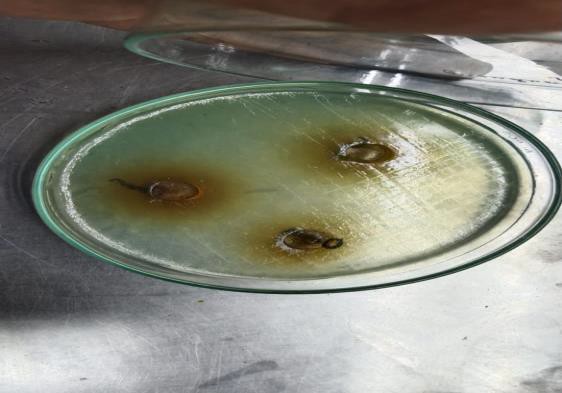
***Klebsiella*sp., Staphylococcus *aureus.,***

**Table - 6: Antimicrobial Activity of Isolated Microbes**

| **Tested Herbal Extract** | ***E.coli.,*** | ***Klepsiella sp.,*** | ***Streptococcus sp.,*** | ***Staphylococcus aureus.,*** |
| --- | --- | --- | --- | --- |
| ***Terminalia arjuna*** | 6mm | 14mm | 12mm | 13mm |
| ***Rauvolfiaserpentina*** | 19mm | 22mm | 10mm | 12mm |
| ***Aleobarbadebnsis*** | 4mm | 2mm | 1mm | 3mm |
| ***Bidenspilosa*** | 14mm | 19mm | 13mm | 15mm |

**Fig -6: Anti microbial Activity of E.coli Against Herbal Plants**

**d) *Rovolfia serpentine*., aginst*E.coli* b) *Bisenspilosa*against *E.coli***

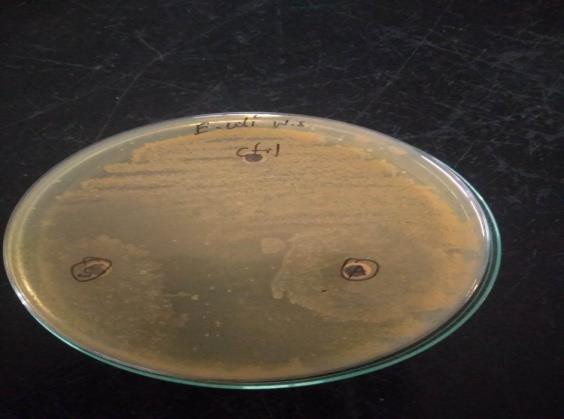


**Fig -8: Anti microbial Activity of *Streptococcus* sp.,**

**Against Herbal Plants**

**c) *Aloe barbadencis*against d) *Rovolfia serpentine***

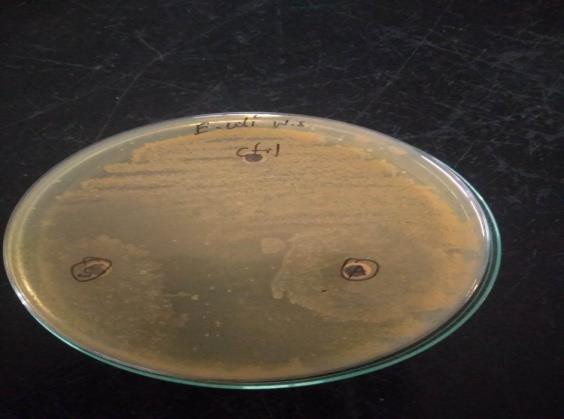
**S*treptococcus* sp., *Streptococcus* sp.**



**Fig -7: Anti microbial Activity of *Staphylococcus aureus.,* Against Herbal Plants**

***a)Terminalia arjuna*against**

***streptococcus* sp., c) *Aloe barbadencis*against**



**Fig -7: Anti microbial Activity of *Staphylococcus aureus.,* Against Herbal Plants**

**d) *Rovolfiaserpentina*against**



SUMMARY AND CONCLUSION

Total Number of Colonies was tabulated CFU/ml of isolated microbes. In the findings *Staphylococcus aureus* (2.8X103) maximum no of colonies (280) and minimum Number of colonies (1.6x103) observed in *klebsiella sp.,* 160 colonies. Followed by *E.coli* (1.8x103) colonies and *streptococcus* (2.0x103) colonies noted.the biochemical characteristics of *E.coli*. It is gram negative rods in staining in cultural character. It is white moist glistening appearance. It shows positive on MR reaction, Catalyse active, Indole production and No3 reduction test. It is Negative on Gelatin Liquification, Starch Hydrolysis and Liquid Hydrolysis. It produces Acid and Gas in Lactose and Dextrose Test. It shows the Negative result in H2s production.(Fig-1a)the Biochemical Characters of *Klebsiella sp.,.,* its Gram-Negative Rod in Staining in culture character. It is Slimy, white somewhat translucent raised growth. It shows Positive reaction on Urease Activity, Citrate Use, Catalase Activity test. It is Negative on Gelatin Liquification, Starch Hydrolysis, Liquid Hydrolysis, H2s Production, No3 Reduction, Indole Production, MR Reaction and Oxidase activity. (Fig-2).the biochemical characters of *Staphylococcus aureous,* its Gram-Positive coccus in Staining in cultural character. It is abundant, opaque golden growth. It shows Positive reaction on Gelatin Liquification, Liquid Hydrolysis, No3 reduction and Catalase Test. It is Negative on Starch Hydrolysis, H2s production, Indole Production, Citrate Utilization Test, Urease Activity and Oxidase Activity. It makes absents in Lactose, Dextrose and Sucrose .Streptococcus it is a Gram Negative, Non motile, Non spore forming cocci. It is catalase Positive and shows β hemolysis in Blood Agar Medium. Its Positive to Fructose, Galactose, Glucose, Lactose, test. It is Negative on Starch hydrolysis, Gelatin liquification, Urease and Catalase test. (Fig4-)

CONCLUSION

From the cellulitis wound infection microbes S*taphylococcus* sp *Streptococcu*s sp,*Klesiella s*p and *E.coli* was isolated. *Streptococcus* sp.*,* shows Higher effective zone of inhibition (13mm) and lower in *Aloe barbadensis*(1mm).In the present study it concludes *Rauvolfia serpentine* and *Bidens pilosa,* higher effective to treat cellulitis wound infection.

*Streptococcus* sp.*,* shows maximum zone of inhibition (13mm) and minimum in *Aloe barbadensis*(1mm).In the present study it concludes *Rauvolfia serpentine* and *Bidens pilosa,* maximum zone of inhibition against cellulitis wound infection in *Klebsiella* shows maximum zone of inhibition. And *Rauvolfia serpentina*(22mm) and *Bidens pilosa* shows (19mm) were observed. So, *Rauvolfia serpentine* and *Bidens pilosa*

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