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

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.

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I. 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 disease**(*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.***

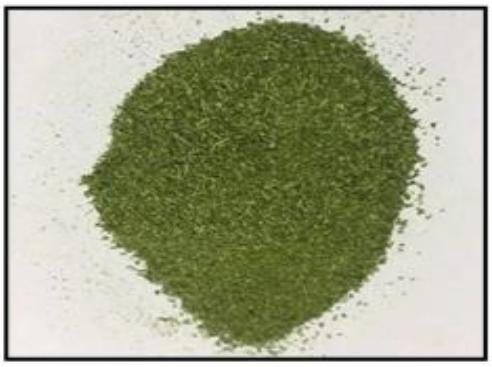
Streptococcus sp., are classified under their Hemolytic properties and origin blood typing The explanation of wound healing is group of completive process. ***(Ballers S., et al.,2012).***The antibiotics, antiseptics and chemical properties are the several agent that cure the infection. *Streptococcus 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

Herbal Powders

Bidens pilosa



Bidenspilosa

Aloe barbadensis

Aloebarbadensis extract

Rauvolfia serpentine

Rauvolfia serpentina leaf powder

Cellulitis Wound on Diabetic Patients

Isolated Microbes from Cellulitis

Diabetic Wound Sample

II. RESULTS

Table 1: Isolation of Microbes from Diabetic Wound

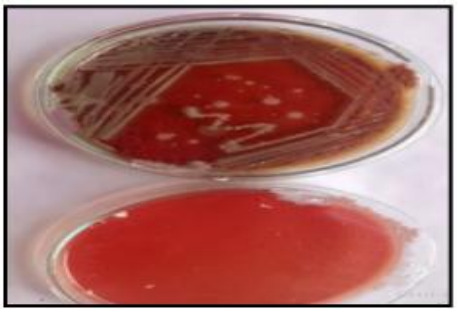
SL. No	Isolated microbes	Colony forming units CFU/ ml	
		Colonies	Units
1.	*E-coli*	180	$1.8X10^3$
2.	*Staphylococcus aureus*	200	$2.0X10^3$
3.	*Streptococcus sp.,*	280	$2.8X10^3$
4.	*Klebsiella*	160	$1.6X10^3$

Table 2: Biological Characteristics of *E.Coli*on Cellulitic Diabetic Wound

SL. No	Biological Test/ Staining	Positive/Negative
1	Gram staining	(Rod shape) negative
2	Culture characteristics on agar slant	White, moist glistening 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

1. Acid ±
2. gas ±
3. reduction ±

1. Biochemical characters of *Escherichia Coli*



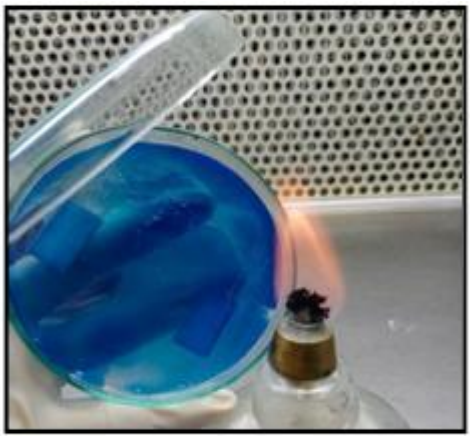
E.Coli **On Emb Agar*e*** ***Coli*** **On Blood Agar**

Table 3: Biological Characteristics of *Klebsiella sp.*, on Cellulitic Diabeti

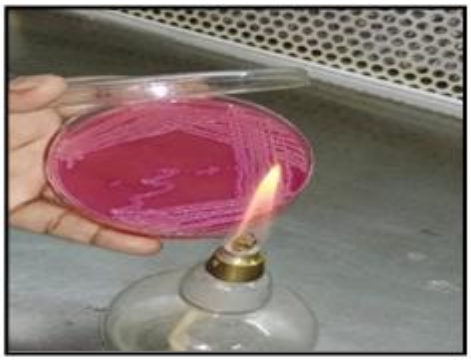
S.No	Biochemical Characters		Positive/ Negative
1	Gram staining		Rod (Negative)
2	Culture characteristics	on	Slimy, white somewhat
	agar slant		translucent raised growth.
3	Gelatin liquification		Negative
4	Starch liquification		Negative
5	Liquid liquification		Negative
6	Lactose		AG
7	Dextrose		AG
8	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

2. BIOCHEMICAL CHARACTERS OF KLEBSIELLA SP

***Klebsiella*sp., on HectoneEntric agar**

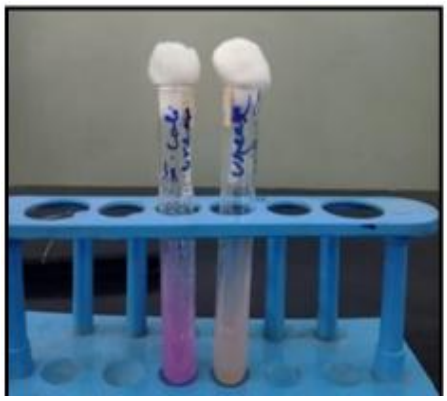


Citrate Test – Positive

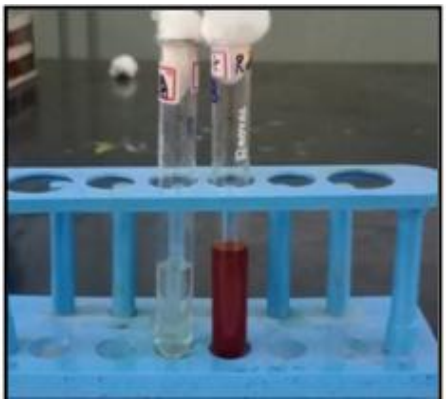


***Klebsiella*sp**

on Macconkey Agar



Urease Test - Positive



Nitrate Reduction Tes

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

SL.no	Biochemical test/staining	Positive/ Negative
1	Gram staining	Positive coccus
2	Culture characteristic on agar slant	Abundant, opaque golden 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 +

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

Sl.No	Biological Testing / Staining	Positive / Negative
1	CAMP (Christie – Alkins munch Peterson)	Negative
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

4. Bio chemical characters oF *Streptococcus* Sp.,

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

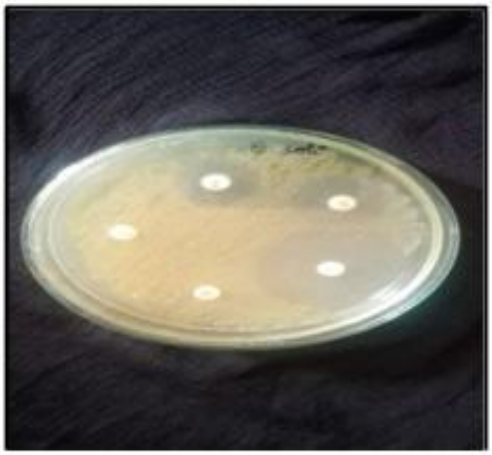
Table 5: Antibiotic Sensitivity

SL.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

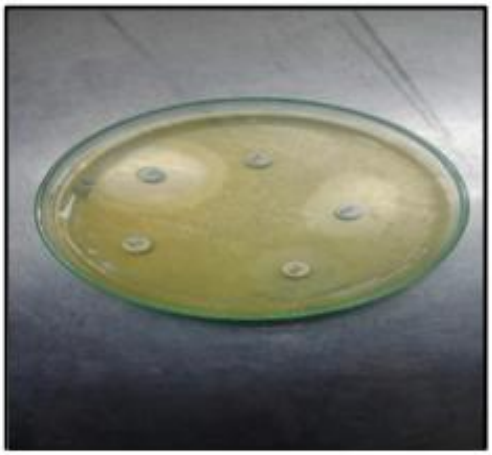
Methyl Red Test - Positive

Urease Test – Negative

5. ANTIMICROBIAL ACTIVITY OF ISOLATED MICROBES



Disk Diffusion Method on) *Klebsiella*sp.,



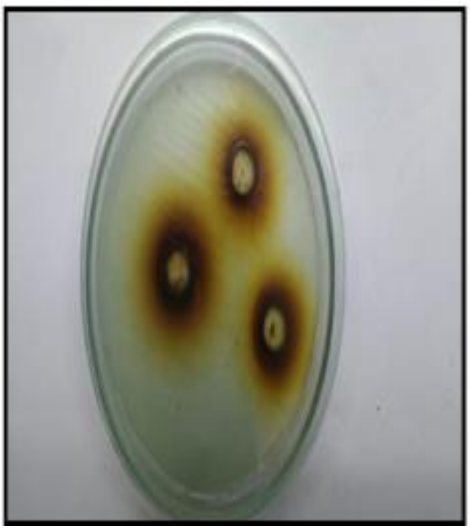
Disk Diffusion Method on *Streptoccocus*sp., Staphylococcus *aureus.,*

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

6. Anti microbial activity of e.coli against herbal plants

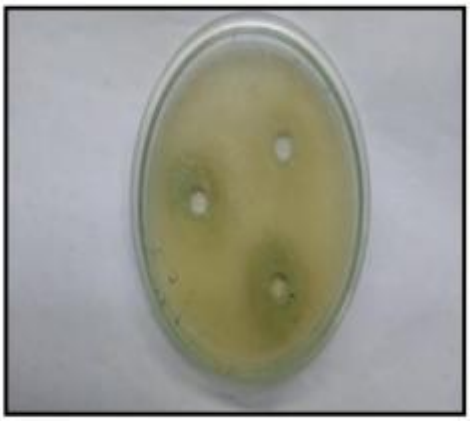
Rovolfia serpentine*., aginst*E.coli



Bisenspilosa*against *E.coli

7. A nti microbial activity of *StreptococcuS* sp., against herbal plants

***Aloe barbadencis*against S*treptococcus* sp.,**



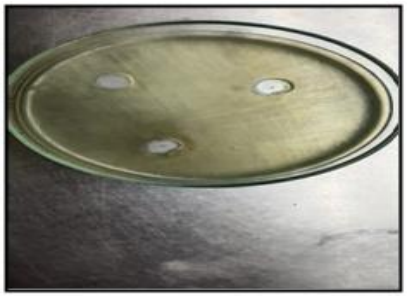
***Rovolfia serpentine Streptococcus* sp.**

8. Anti microbial activity of *Staphylococcus Aureus.*, against herbal plants

***Terminalia arjuna* against *streptococcus* sp.,**

***Aloe barbadencis* against**

9. Anti microbial activity Of *STAPHYLOCOCCUS AUREUS.*, against herbal plants



***Rovolfiaserpentina*against**

III. SUMMARY AND CONCLUSION

Total Number of Colonies was tabulated CFU/ml of isolated microbes. In the findings *Staphylococcus aureus* ($2.8X10^3$) maximum no of colonies (280) and minimum Number of colonies ($1.6x10^3$) observed in *klebsiella sp.,* 160 colonies. Followed by *E.coli* ($1.8x10^3$) colonies and *streptococcus* ($2.0x10^3$) 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-)

IV. 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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