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AEROBIC BACTERIOLOGICAL PROFILE OF SKIN AND SOFT TISSUE INFECTIONS (SSTI`S) AND IT`S ANTIMICROBIAL SUSCEPTIBILITY PATTERN AT M. B. GOVT. HOSPITAL IN UDAIPUR, RAJASTHAN

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Abstract:- Introduction: Skin and soft tissue infections (SSTI`s) are a common type of infection. The overall incidence of wound sepsis in India is from 10-33%. A predictable bacterial profile in the wound infections is very important for clinicians, who intend to start empirical treatment for patients, while laboratory culture reports are awaited. **Objectives:** To identify the common aerobic bacterial isolates and their antimicrobial susceptibility pattern along with methicillin resistance in Staphylococcus aureus and CoNS, production of extended spectrum beta lactamases in Escherichia coli and Klebsiella spp and Biofilm production in Staphylococcus aureus and CoNS. **Material and Methods:** A total of randomly 207 pus samples received for aerobic bacteriological culture and sensitivity in the microbiology department of R.N.T. Medical College Udaipur, from various departments (OPD/IPD) of M.B.G. Hospital, Udaipur. This prospective study was done the period of six month (Dec.2014 to June 2015). The samples were processed in the laboratory by standard techniques. **Results:** Out of 207 samples 178(86%) were positive for aerobic bacterial culture while 29 (14%) samples had no growth. Among the 178 culture positive pus samples, 151(84.83%) yielded pure bacterial isolates and 27(15.17%) yielded mixed infection. In the present study, Staphylococcus aureus 77 (37.5%) was the commonest organism isolated. Out of 77 Staphylococcus aureus isolated, 31 (40.25%) were MRSA and ESBL producers among Escherichia coli and Klebsiella species were 30 (85.71%) and 4(50%) respectively. Out of 103 Staphylococcus aureus (77) and CoNS (26) isolates, 33 (32.03%) were biofilm producer. **Conclusion:** The commonest isolates of skin and soft tissue infections are Staphylococcus Aureus (37.5%) followed by Escherichia Coli (17%), CoNS (13%) and Pseudomonas spp (12%) and there is a high level of resistance against commonly used antimicrobials due to methicillin resistance, ESBL and Biofilm production. Staphylococcus aureus were 100% sensitive to vancomycin and Escherichia coli were 80% sensitive to amikacin so these antimicrobials can be included in empirical treatment of skin and soft tissue infections.

Keywords: Antimicrobial Susceptibility, Biofilm, ESBL, MRSA, Staphylococcus aureus, skin and soft tissue infections (SSTI`S).

INTRODUCTION:

Skin and soft tissue infections (SSTI`s) are a common type of infection. Common exampleSSTIs includes cellulitis, abscesses, impetigo, folliculitis, furuncle, carbuncle, necrotizing fasciitis, diabetic foot ulcer and surgical site infections. The development of

wound infection depends on the integrity and prospective function of skin.**(1)** The chances of infection depends on patient conditions such as the state of nutrition and existing diabetes mellitus like medical conditions and pre, intra and postoperative care if the patient has undergone surgery. Thus it is difficult to predict which wound will become infected.**(2)** In India incidence of wound sepsis is 10-33%.(**3)** Wound infections can be caused by different groups of microorganisms, most commonly isolated aerobic microorganisms includes Staphylococcus aureus, Coagulase negative staphylococcus, Enterococci, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacter, Proteus mirabilis, other streptococci, Candida and Acinetobacter.**(4)** Coagulase positive Staphylococcus aureus has been found to be more dominant organism in pus.**(5)** Staphylococci are ubiquitous and most common cause of localized suppurative lesion in human beings.

Wound infection is one of the most common hospital acquired infections and important cause of morbidity and accounts for 70-80 %.**(6)** Developments of such infections represent delayed healing, cause anxiety and discomfort for patient. The importance of wound infections in both economic and human terms, should not be underestimated.**(7)** In a study on an average, patients with wound infections stays about 6-10days more in hospital than if the wound had heal without infections.**(8)**This additional stay doubles the hospital cost.

Relative resistance to antibiotics relatively more virulent strains and capacity to adapt quickly to changing environment make the pathogens acquired in hospitals a matter of concern.**(9)**Effective treatment of wound infections depends upon proper understanding of causative pathogen, pathophysiology of the infectious process and pharmacology of the therapeutic agents. The inadvertent use of antibiotics leads to emergence of drug resistant pathogens, which in turns acts as a great challenge to the health services.

A predictable bacterial profile in the wound infections is very important for clinicians who intend to start empirical treatment for patients, while laboratory culture reports are awaited. The purpose of this study is to show the spectrum of aerobic bacterial profile and its sensitivity pattern from skin and soft tissues infections.

MATERIAL AND METHOD:-

A total of randomly 207 pus samples received for aerobic bacteriological culture and sensitivity in the bacteriology section of microbiology department of R.N.T. Medical College Udaipur, from various departments (OPD/IPD) of M.B.G. Hospital, Udaipur. This study was done the period of six month (Dec.2014 to June 2015).The samples were transported immediately and processed in the laboratory as per standard protocol.(**10)**Gram staining was done and the samples were inoculated into blood agar, MacConkey agar and Glucose Broth by standard techniques. The plates were incubated at 37^{o}C for 24-48 hours and growth was observed. On correlating the gram stain and culture report, further identification were done with biochemical tests(**10)** such as catalase, coagulase, oxidase, indole, methyl red, voges-proskauer, citrate, urease, triple sugar iron, mannitol salt agar, motility by hanging drop method, sugar fermentation test and Amino acid decarboxylase and Arginine dihydrolase test. Kirby-Bauer disc diffusion assay on Mueller

Hinton agar were carried out to determine the antimicrobial susceptibility profiles as per CLSI guidelines.(**11,12)** Separate set of antibiotic were used for gram positive organisms and gram negative organisms. Detection of Methicillin Resistant Staphylococcus Aureus (MRSA) was done with Oxacillin (1µg) disc by disc diffusion test.(**13)** Extended spectrum β- lactamases (ESBL) production in E. coli and Klebsiella spp was detected by phenotypic confirmatory test(**14)** with the help of Ceftazidime (30 µg) discs alone and in combination with Clavulanic acid (Ceftazidime + Clavulanic Acid, 30/10 µg) disc. The control strains used were *Escherichia coli* ATCC 25922 as a non-ESBL producing organism and *Klebsiella pneumoniae* ATCC 700603 as an ESBL producing organism. Biofilm production was determined by using two methods, Congo red agar method(**15)** and Christensen`s tube method.(**16)** Biofilm producing Staphylococcus epidermidis ATCC 35984 used as positive control and negative control was un-inoculated plate and tube respectively.

RESULTS : -

The period of study is from December 2014 to June 2015 with total of 207 pus samples. Out of 207 samples 178(86%) were positive for aerobic bacterial culture while 29 (14%) samples had no growth. Of the positive culture, 129 (72.48%) patients were males and 49 (27.52%) were females yielding a ratio of 2.63 (**Graph 1**). Among the 178 culture positive pus samples, 151(84.83%) yielded pure bacterial (mono-microbial) isolates and 27(15.17%) yielded mixed infection (two organisms- polymicrobial); so a total number of 205 organisms were isolated out of 207 pus samples (**Graph 2**). The department wise distribution of pus samples revealed that surgery dept. was the highest contributor (40%), followed by Skin & V.D.(32%), ENT (16%), Obs & Gynae (5%) (**Table 1**).

In the present study among the 205 isolates, Staphylococcus aureus was the commonest organism isolated. Table shows that Staphylococcus aureus was isolated in 77 samples accounting for 37.5% in the total isolated organism. The second commonest organism was E. coli 35 (17%) followed by Coagulase negative staphylococcus 26 (13%), Pseudomonas spp. 24 (12%), Citrobacter spp 13 (6%), Klebsiella spp 8 (4%), Enterococcus faecalis 6(3%), Streptococcus spp 3 (2%), Proteus mirabilis 2 (1%), Acinetobacter spp 3 (1%), Enterobacter spp 2 (1%), GPB 4 (2%) and Candida albicans was observed in 1 cases (**Table 2**).

The Antibiogram of Gram Positive Cocci (**Table 3**) revealed that the Vancomycin (99%) was the most susceptible drug followed by Gentamicin (85%), Amikacin (82%), Doxycycline (78%), Tetracycline (78%) and Oxacillin (58%). Gram Negative Bacilli are most susceptible to Amikacin(76%) followed by Ceftazidime + Clavulanic acid (74%), Gentamicin (63%), Ciprofloxacin (47%) and Carbenicilline (39%) (**Table 4)**.

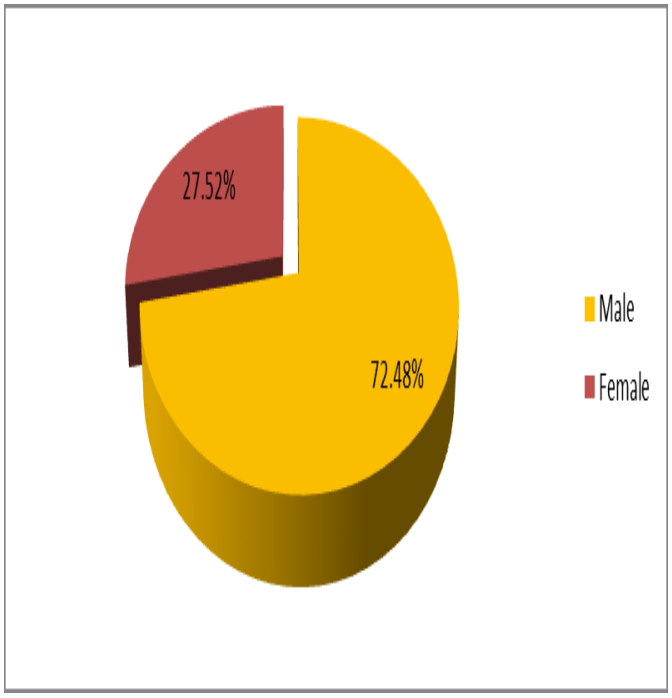
MRSA was detected with the help of Oxacillin discs (1µg) and Out of 77 Staphylococcus aureus isolated, 31 (40.25%) were **MRSA** and 46 (59.75%) were **MSSA** and among CoNS (26), Methicillin resistance CoNS were 13(50%) (**Graph 3, 4**).

ESBL producers among Escherichia coli and Klebsiella species was detected with Ceftazidime (30 µg) and Ceftazidime + Clavulanic acid (30/10 µg) disc and found to be

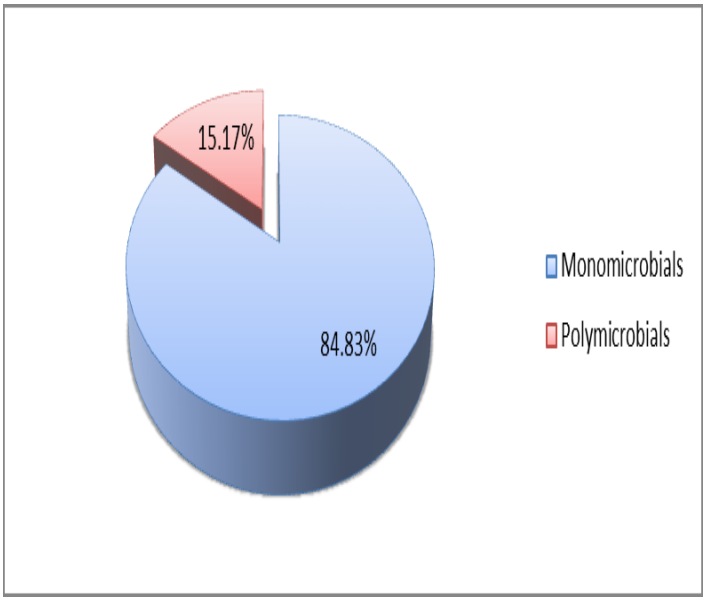
30 (85.71%) and 4(50%) respectively (**Graph 5, 6**).

Out of 103 S. aureus (77) and CoNS (26) isolates, 33 (32.03%) were biofilm producer and 70 (67.97%) were non-biofilm producer (**Table 5**). Biofilm production was more in MRSA (54.83%) than MR CoNS (38.46%) in present study. Biofilm production was more in Methicillin resistant strains than Methicillin sensitive strains in both Staphylococcus aureus and CoNS (**Table 6**).

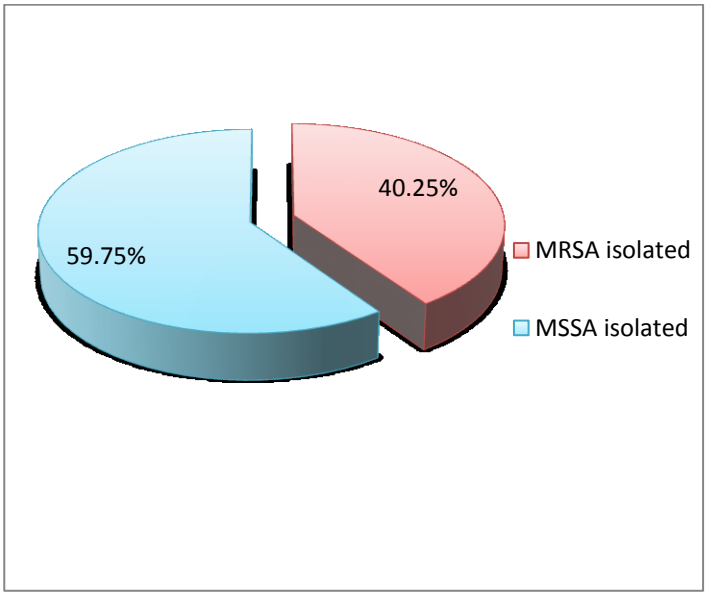
Graph 1: Showing Sex wise distribution



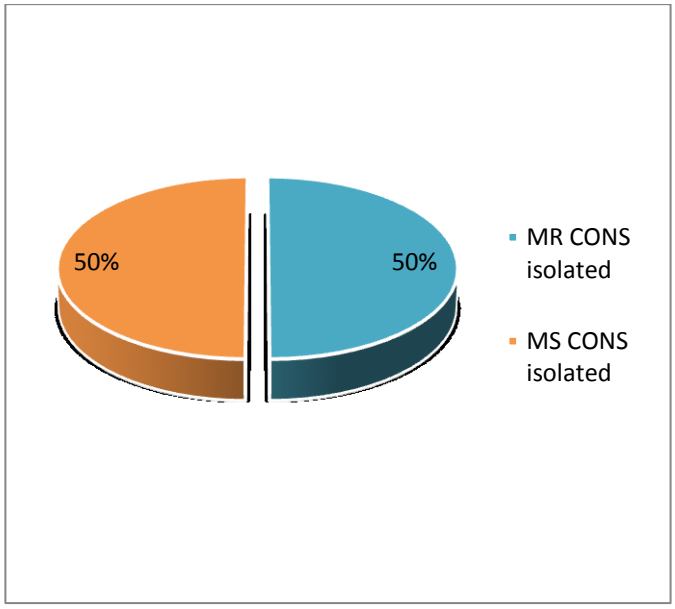
Graph 2: Percentage of Pure and Mixed Cultures



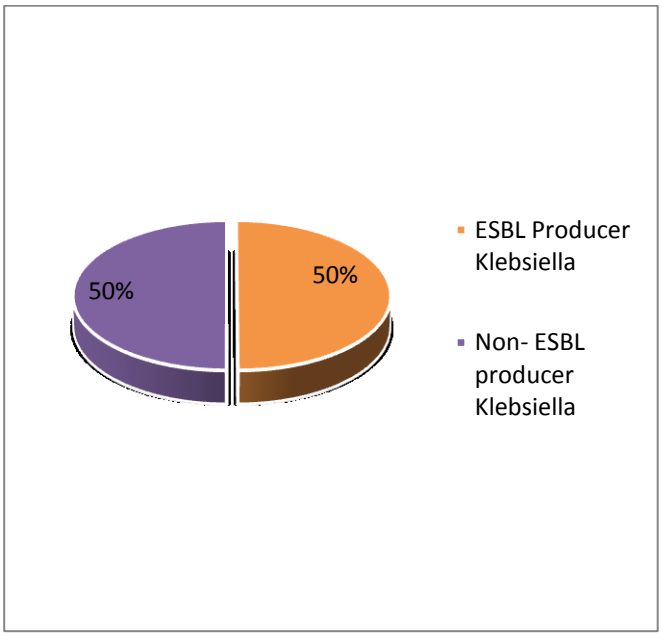
Graph 3: Staphylococcus aureus: MRSA Producer



Graph 4: Coagulase Negative Stayphylococcus (CoNS) isolates: Methicillin resistant



Graph 6: ESBL producing and Non-ESBL producing Klebsiella spp



Graph 5: ESBL Producer and non-ESBL Producer in E. coli

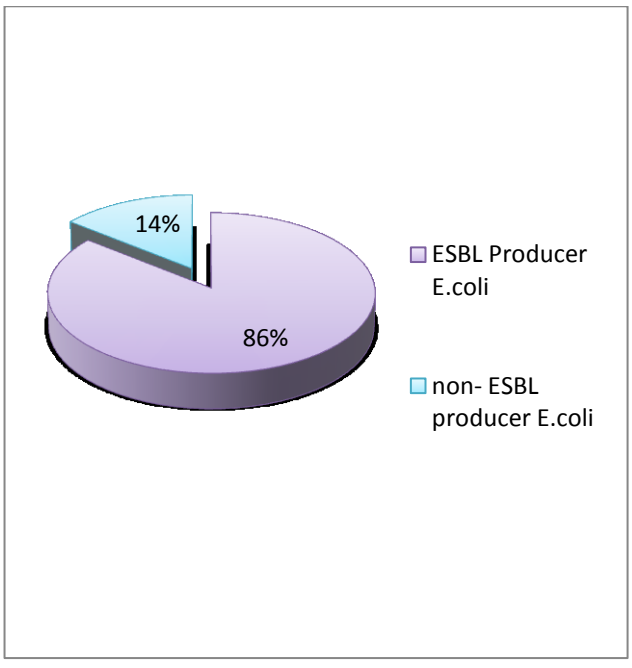


Table 1: Department wise distribution

Name of Department	No. of patients	Percentage
General Surgery	82	40%
Skin & V.D.	66	32%
ENT	33	16%
Obs. & Gynae.	11	5%
Radiotherapy	5	2%
Neurosurgery	4	2%
Orthopedics	3	1%
Paediatric surgery	2	1%
Medicine	1	1%
Total	**207**	**100%**

Table 2: Percentage of the total organisms (Mono-microbial + Poly-microbial) isolates in the present study

S. No.	Organisms	No. of organisms (n=205)	Percentage (%) (n=205))
1	Staphylococcus aureus	77	37.5%
2	Escherichia coli	35	17%
3	CoNS	26	13%
4	Pseudomonas spp	24	12%
5	Citrobacter spp	13	6%
6	Klebsiella spp	8	4%
7	Enterococcus spp	6	3%
8	Streptococcus spp	4	2%
19	Acinetobacter spp	3	1%
10	Proteus spp	2	1%
11	Enterobacter spp	2	1%
12	Candida albicans	1	0.5%
13	GPB	4	2%
	Total	205	100%

Table 3: Antibiotic susceptibility pattern of different gram positive cocci (n=113)

S.NO	Antibiotic	Staphylococcus aureus n=77	CoNS n=26	Enterococcus spp n=6	Streptococcus spp n=4
1	AK	88%	73%	50%	75%
2	GEN	86%	88%	50%	100%
3	CN	49%	50%	33%	100%
4	CTX	56%	50%	50%	100%
5	CAZ	39%	38%	17%	75%
6	CTR	51%	50%	50%	100%
7	ERY	31%	38%	17%	100%
8	AMX	13%	23%	0%	100%
9	AMC	27%	35%	50%	100%
10	CIP	51%	50%	50%	100%
11	COT	25%	38%	0%	25%
12	DO	81%	77%	50%	75%
13	TE	81%	77%	50%	75%
14	OX	60%	50%	50%	75%
15	VAN	100%	100%	83%	100%

Table 4: Antibiotic susceptibility pattern of different gram negative bacilli (n=87)

S.NO	Antibiotic	E.coli n=35	Pseudomonas spp n=24	Citrobacter spp n=13	Klebsiella spp n=8	Acinetobacter spp n=3	Enterobacter spp n=2	Proteus spp n=2
1	AK	66%	75%	69%	75%	67%	50%	100%
2	GEN	57%	63%	46%	75%	33%	50%	100%
3	CN	3%	0%	23%	25%	0%	0%	0%
4	CTX	3%	63%	23%	50%	33%	50%	0%
5	CAZ	3%	67%	23%	50%	33%	50%	0%
6	CTR	3%	54%	23%	50%	33%	50%	0%
7	AMX	3%	4%	15%	0%	0%	0%	0%
8	AMC	3%	4%	15%	25%	0%	0%	100%
9	CIP	9%	79%	54%	63%	33%	50%	100%
10	COT	17%	13%	46%	63%	0%	50%	100%
11	DO	23%	4%	46%	88%	67%	50%	50%
12	NA	0%	0%	15%	38%	0%	0%	0%
13	TE	23%	0%	46%	88%	67%	50%	50%
14	CB	6%	88%	23%	50%	33%	50%	50%
15	CAC	43%	92%	85%	88%	33%	100%	100%

Table 5: Shows distribution of biofilm producing strains (Staphylococcus aureus & CoNS)

Total isolates=103			
Biofilm producers=33		Non biofilm producers=70	
Staphylococcus aureus=27	CoNS=6	Staphylococcus aureus=50	CoNS=20

Table 6: Shows relation of methicillin resistance in relation to biofilm production

Biofilm production	MRSA	MSSA	MR CoNS	MS CoNS
Biofilm producers	17	10	5	1
Non-biofilm producers	14	36	8	12
% Biofilm producers	54.83	21.73	38.46	7.7
% Non-biofilm producers	45.17	78.27	61.54	92.3

DISCUSSION:

Infection of wound is one of the common cause to increase hospital stay and treatment cost. Emerging drug resistant organisms further increases the threat. The identification of common causative bacteria of wound infections with their antimicrobial sensitivity pattern will be helpful to the clinician in choosing an empirical antibiotic therapy. In the present study an attempt was made to know the identification of various pus isolates with their antibiotic susceptibility testing. In our study, the wound infection was more common in male than female (2.63:1). N.

Sowmya and S. Savitha et al.(**17)** (2014) also observed males to be commonly infected than females (2:1). In the present study incidence of wound isolates cases was more common in General surgery department (40%) followed by Skin & V.D. department (32%), ENT (16%), Obs. & Gynae. (5%), Radiotherapy (2%) and Neurosurgery (2%). My study is correlated with other workers like V.M.V.S.V Raghav Rao et al.(**18**) (2014) have found the department wise distribution of pus samples that revealed that surgery dept. was the highest contributors (35.29%), followed by Orthopedics (29.42%), Gynae & Obs. (11.76%), Medicine (9.80%), Skin (7.85%) and ENT (5.88%) departments.

Majority of our results are mono-microbial (85%) and Staphylococcus aureus was found to be the most common pathogen in our study (37.5%), similar reports was also observed by N. Sowmya and S. Savitha et al.(**17)** (2014), A.Ananth and S.Rajan(**19)** (2014), A.R.Kumar(**20)** (2013). The second common pathogen in our study was E.coli (17%). D.V.M.V.S.V et al.(**18)** (2014) and S.Mohanty and A.Kapil et al.(**21)** (2004), also reported Escherichia coli to be the second most commonly occurring pathogens in wound infections. Gram positive organisms obtained in our study were 99% sensitive to vancomycin. In our study, MRSA (Oxacillin resistant) accounts for 40.25% which is in comparison with other workers like N. Lakshmi et al.**(22)** (2015) 39.1%, INSAR (2008) 36% in pus, INSAR (2009) 40% and Rajeshwar et al.(**23)** (2014) 32.70%. So overall we see that the prevalence of MRSA is similar or on a higher level in our region as compared to other studies ranging from 27-40%.

In present study, Escherichia coli is 80% sensitive to amikacin, 69% sensitive to gentamicin, 54% sensitive to ceftazidime + clavulanic acid and 34% sensitive to tetracycline and doxycycline followed by cotrimoxazole 29%.

In our study ESBL production was higher in E. coli in comparison to Klebsiella spp. Out of the total 35 E. coli isolates ESBL production was seen in 30 (85.71%) Escherichia coli isolates. While out of 8 Klebsiella spp. isolates ESBL production was seen in 4 (50%) isolates. ESBL producers Escherichia coli accounts for 34.48% among GNB which is in comparison with other workers like Rajeshwar Rao et al.(**23)** (2014) which showed Extended Spectrum Beta Lactamase were found in variety of Enterobacteriaceae members (30.9%). B Fouzia et al.(**24)** (2013) demonstrate Enterobacteriaceae family showed 32% ESBL (Extended spectrum beta lactamase) producer.

In our study, we isolated 33(32%) biofilm producers out of 103 GPC (Staphylococcus aureus and CoNS) isolates. In which 27(81.81%) were Staphylococcus aureus and 6 (18.19%) were CoNS. Biofilm production was observed in 54.83%(17/31) MRSA and 21.73%(10/46) MSSA while 38.46%(5/13) MRCoNS and 7.69%(1/13) MSCoNS were found to be biofilm producers. A study by Charankaur et al.(**25)** concludes 78.8% MRSA as biofilm producer in various clinical isolates in Pune. Maximum biofilm production was seen in Pus samples followed by Urine. Aggrawal et al.(**26)** reported 79% biofilm producing Staphylococcus aureus and 43% biofilm producing CoNS from blood samples in Lukhnow. 82% of biofilm producing S. aureus and 71.4% biofilm positive CoNS were methicillin resistant which is higher in no. as compared to our study. Biofilm producer S. aureus showed maximum susceptibility to Vancomycin (100%), doxycycline, tetracycline, amikacin (81.48%)

each and gentamicin (77.77%) followed by ciprofloxacin (44.44%), cefotaxime and oxacillin (37.03%). On the contrary, non-biofilm producing S. aureus were comparatively much more sensitive to these antimicrobials.

CONCLUSION: -

This study revealed the presence of wound infection causing bacteria, those are capable of causing various human illness. The bacterial isolate screened in various skin and soft tissue infections collected from various wards. A total of randomly 207 samples received in the bacteriology section of microbiology department of R.N.T. Medical College, Udaipur, from various departments (OPD/IPD) of M.B.G. Hospital, Udaipur.

The commonest isolates of Wound infection are Staphylococcus Aureus (37.5%) followed by Escherichia Coli (17%), CoNS (13%) and Pseudomonas spp (12%) and there is a high level of resistance against commonly used antimicrobials.

Among the Staphylococcus aureus, MRSA accounts for 40.25% and among CoNS Methicillin resistance accounts for 50%.Methicillin resistant Staphylococcus aureus (MRSA) is now endemic in India. The incidence of MRSA varies from 25 per cent in western part of India and 2 to 50 per cent in South India. Community acquired MRSA (CA-MRSA) has been increasingly reported from India.**(27)** The prolonged hospital stay, indiscriminate use of antibiotics, lack of awareness, receipt of antibiotics before coming to the hospital etc. are the possible predisposing factors of MRSA emergence.

Among Gram negative bacilli, ESBL producer Escherichia coli accounts for 34.48% and ESBL producer Klebsiella spp accounts for 4.59%. Among Escherichia coli, ESBL producer E. coli accounts for 85.71% and among Klebsiella spp, ESBL producer Klebsiella spp accounts for 50%.

Out of 103 Staphylococcus aureus (77) and CoNS (26) isolates, 33(32.03%) were biofilm producer and 70 (67.97%) were non-biofilm producer. Biofilm production was more in MRSA (54.83%) than MRCoNS (38.46%) in present study. Biofilm production was more in Methicillin resistant strains than Methicillin sensitive strains in both Staphylococcus aureus and CoNS.

In our study biofilm production was lower than other studies, so it is right time to take action like strict implementation of good infection control programs against spreading of these more resistant biofilm producing isolates.

CONCLUSION

Hence, Knowledge of the most common causative agents of infection and their antimicrobial susceptibility pattern is very essential for the judicious administration of empirical treatment before the culture results are available. Antimicrobial susceptibility of microorganisms varies from time to time and from place to place. Hence regular monitoring of bacterial susceptibility to antibiotics in skin and soft tissues infections is essential for appropriate therapy. Good infection control programs are to be maintained and avoid indiscriminate use of broad spectrum antibiotics to curtail emergence of MRSA, ESBL and Biofilm producers. Antibiogram should be prepared regularly and made readily available to the clinicians to guide them in therapy. There is a need for a central database in India where various laboratories can upload their antibiogram regularly and this data

can be very useful in formulating guidelines for treatment of various infectious diseases.

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