A STUDY OF ALCOHOL TOLERANCE IN COMMON HOSPITAL ACQUIRED MICRO-ORGANISMS.

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Abstract : Use of hand sanitizers has been a usual practice for sanitation in urban population. Mostly, these sanitizers are alcohol based which help in proper disinfection by killing micro-organisms and stop transmission of infectious diseases. However, in recent times, alcohol tolerance has been observed in commonly found micro-organisms which is a matter of concern. In the present study, alcohol tolerance of most commonly found micro-organisms responsible for hospital acquired infections (HAI) has been studied using commercially available hand sanitizers.

Keywords: Alcohol tolerance, Hospital acquired infections.

1. INTRODUCTION

Hospital acquired infections (HAIs) has been a growing cause of concern in last few decades. The Center for Disease Control and Prevention (CDC) estimates that approximately 2 million people acquire Hospital Acquired Infection each year and that approximately 90,000 of these patients die as a result of their infections.

Opportunistic micro-organisms are the primary cause of nosocomial infections. These organisms include *Enterococcus spp., Escherichia coli, Pseudomonas spp.,* and *Staphylococcus aureus*. These infections occur usually at sites like (in order from most to least common) urinary tract, surgical wounds, respiratory tract, skin (especially burns), blood (bacteremia), gastrointestinal tract, and central nervous system. However, HAIs caused by multidrug-resistant pathogens (Vancomycin resistant *Enterococci*, Vancomycin intermediately resistant *Staphylococcus aureus*, or Vancomycin resistant *Staphylococcus aureus*) have been on the rise as they are difficult to treat. *Pseudomonas aeruginosa* has been the causative organism of HAIs in hospitalized and immunosuppressed individuals (Shimizu et. 2002).

Hand hygiene plays an important role in the transmission of these HAIs (Deepak et al,2015). Hand washing removes body's own fatty acids from the skin, which may result in cracked skin that provides an entry portal for pathogens. To overcome the limitations of plain hand washing, hand sanitizers were introduced claiming to be effective against those pathogenic micro-organisms as well as to improve skin condition due to the addition of emollients in it. Hand sanitizers were also effective in reducing gastrointestinal illnesses in households, respiratory tract infections, and skin infections, in curbing absentee rates in elementary schools, and in reducing illnesses in university dormitories. Furthermore, to reduce infections in healthcare settings, alcohol-based hand sanitizers are recommended as a component of hand hygiene. Thus, in this study, the effectiveness of four commercially available hand sanitizers in disinfection of *Methicillin Resistant Saccharomyces cerevisiae (MRSA), Vancomycin resistant Enterococci (VRE), , B. cereus* and *Pseudomonas aeruginosa ATCC 15442* were explored.

2. MATERIAL AND METHODS

2.1.Materials

Bacterial cultures of *Methicillin Resistant Saccharomyces cerevisiae (MRSA), Vancomycin resistant Enterococci (VRE)* were obtained from Suburban Diagnostic Lab, Mumbai, *B. cereus* and *Pseudomonas aeruginosa ATCC 15442* were obtained from Bhavan's Research Centre, Mumbai. Mueller Hinton agar, tryptic soy agar and nutrient agar were obtained from Hi Media. Four commercially available hand sanitizers were bought from local pharmacy. Two were alcohol- based sanitizers while two was non-alcoholic sanitizer.

2.2.Antimicrobial activity by Agar diffusion method.

Antimicrobial activity of the four commercial sanitizers labelled Lifebuoy® handwash (LHS) , Himalaya® handwash (HHS) , Dettol® handwash DHS and Dr. Batra's hand sanitizer (Dr-BHS) where, LHS,DHS are alcohol based hand sanitizers. HHS and Dr-BHS is non-alcohol based sanitizers were studied. The antimicrobial activity were studied against *Methicillin Resistant Saccharomyces cerevisiae (MRSA), Vancomycin resistant Enterococci (VRE), B. cereus* and *Pseudomonas aeruginosa ATCC 15442.* 24 hr culture with OD of 0.1 at 600nm of these culture were spread on sterile Mueller Hinton agar plate and 50 µl of different hand sanitizers were loaded into the well made in the plate. Plates were observed after 24 hr incubation at 37 °C.

2.3.Minimum Inhibitory Concentration (MIC) and Minimum Lethal Concentration (MLC).

MIC and MLC of the hand sanitizers were studied. MIC was studied for different concentrations- 1:2, 1:4, 1:8, 1:16, 1: 32, 1: 64, 1: 128, 1: 256. As per given in Table 1. MLC was studied by inoculating a loopful of cultures from the MIC tubes into fresh sterile nutrient broth tubes and the tubes were incubated at 37 °C for 24 hrs.

Table 1: Preparation for Minimum Inhibitory Concentration (MIC) study.

Sr. no.	Dilutions	Media (ml)	Sample (ml)	Culture (ml)	
1.	1:2	5	5	0.1	
2.	1:4	5	5	0.1	
3.	1:8	5	5	0.1	
4.	1:16	5	5	0.1	Incubate at 37°C for 24 hr.
5.	1:32	5	5	0.1	
6.	1:64	5	5	0.1	
7.	1:128	5	5	0.1	
8.	1:256	5	5	0.1	

9.	Positive Control	5	-	0.1	
10.	Negative Control	5	-	0.1	
11.	Medium Control	5	-	-	

2.4.Time Course study: Quantitative suspension Test

Dilution of sample which showed inhibition in MIC assay were prepared and were inoculated with 0.1 ml of the four test micro-organisms each. The tubes were incubated for different time duration- 30 seconds, 5 minutes and 15 minutes respectively. 20 µl of the sample from these tubes were spread on sterile tryptic soy agar plate. The plates were incubated at 37°C for 24 hrs.

2.5.Bacterial protein detection using SDS – PAGE electrophoresis

SDS – PAGE electrophoresis was carried out for detection of proteins from all the four bacterial samples. SDS - PAGE electrophoresis was carried out as per protocol mentioned in _____.

3. Result and Discussion

Antimicrobial activity of all the four hand sanitizers were studied. Dr-BHS showed antimicrobial activity against *Methicillin Resistant Saccharomyces cerevisiae (MRSA), Vancomycin resistant Enterococci (VRE), B. cereus.* This can be attributed to the presence of antibacterial nature of *Ocimum Sanctum* (basil) in the formulation of the sanitizer. However, it showed no activity against *Pseudomonas aeruginosa ATCC 15442.* Other alcohol-based hand sanitizers – LHS, DHS and non-alcohol based HHS showed no activity against the test organisms suggesting that these organisms were tolerant to alcohol. Antibacterial activity of Results of antimicrobial study is shown in Table 2.

Table 2. Antimicrobial study of hand sanitizers

CULTURES	**SANITIZERS**												
	Dr- BHS (in mm)				HHS			DHS			LHS		
	1	2	3	Mean	1	2	3	1	2	3	1	2	3
Bacillus Cereus	17	20	19	18.67	-	-	-	-	-	-	-	-	-
Vancomycin Resistant *Enterococci*	15	15	17	15.67	-	-	-	-	-	-	-	-	-
***Pseudomonas aeruginosa* ATCC 15442**	-	-	-	-	-	-	-	-	-	-	-	-	-
Methicillin Resistant *S.aureus*	2	2	2	2	-	-	-	-	-	-	-	-	-

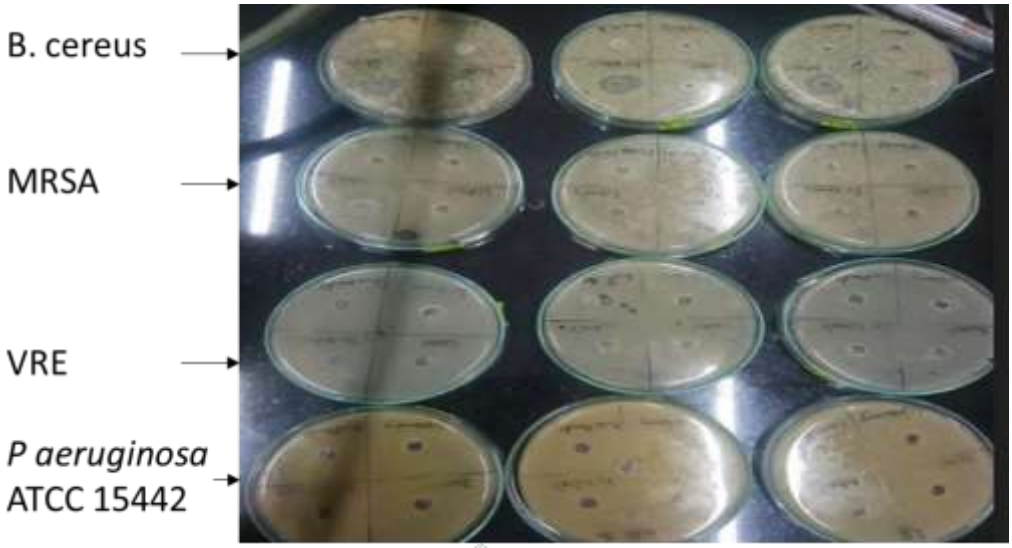

Fig. 1. Antimicrobial activity of hand sanitizers

MIC study showed inhibitory activity of hand sanitizers at 1:16 dilution for all the sanitizers against the test organisms (Table 3). MLC study showed bactericidal activity of hand sanitizers at dilution of 1:4(Table 5 & 6). This indicates that a fairly low concentration of hand sanitizer is required to inhibit the growth of nosocomial pathogens where as a higher concentration of hand sanitizer is required for killing these pathogens.

Table 3: Minimum Inhibitory Concentration study of hand sanitizers

Sr.no.	Dilutions	RESULT							
		B.cereus				*VRE*			
		DHS	LHS.	HHS.	Dr.BHS	DHS	LGS.	HHS.	Dr.BHS.
1	1:2	-	-	-	-	-	-	-	-
2	1:4	-	-	-	-	-	-	-	-
3	1:8	-	-	-	-	-	-	-	-
4	1:16	-	-	-	-	-	-	-	-
5	1:32	+	+	+	+	+	+	+	+
6	1:64	+	+	+	+	+	+	+	+
7	1:128	+	+	+	+	+	+	+	+
8	1:256	+	+	+	+	+	+	+	+
9	**P.C.**	+	+	+	+	+	+	+	+
10	**N.C.**	-	-	-	-	-	-	-	-
11	**Medium Control**	-	-	-	-	-	-	-	-

Table 4: Minimum Inhibitory Concentration study of hand sanitizers

Sr.No.	Dilutions	RESULT							
		MRSA				*P.aeruginosa*			
		DHS	LHS.	H.HS.	Dr.BHS	DHS	LHS.	HHS.	Dr.BHS
1	1:2	-	-	-	-	-	-	-	-
2	1:4	-	-	-	-	-	-	-	-
3	1:8	-	-	-	-	-	-	-	-
4	1:16	-	-	-	-	-	-	-	-
5	1:32	+	+	+	+	+	+	+	+
6	1:64	+	+	+	+	+	+	+	+
7	1:128	+	+	+	+	+	+	+	+
8	1:256	+	-	-	-	-	+	+	+
9	P.C.	+	+	+	+	+	+	+	+
10	N.C.	-	-	-	-	-	-	-	-
11	Medium Control	-	-	-	-	-	-	-	-

KEY: + Growth observed;

- No growth observed**;**

PC – Positive Control

NC- Negative Control.

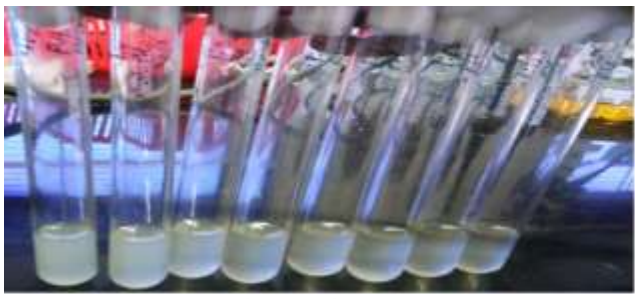

Fig. 2 : HHS against MRSA

Fig 3. :LHS against MRSA

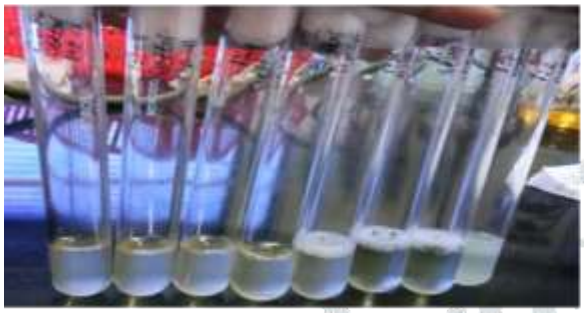

Fig 4 :DHS against MRSA

Fig 5: DR.BHS against MRSA

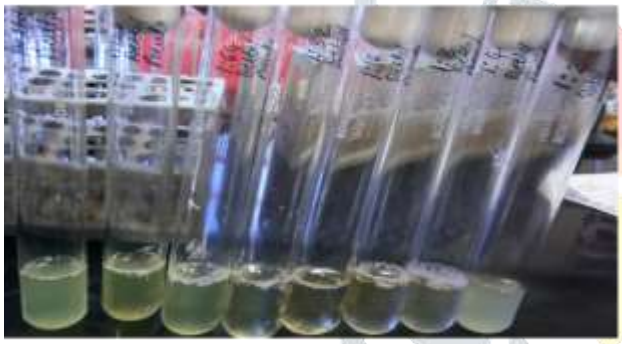

Fig 5: DHS against *P.aeruginosa*

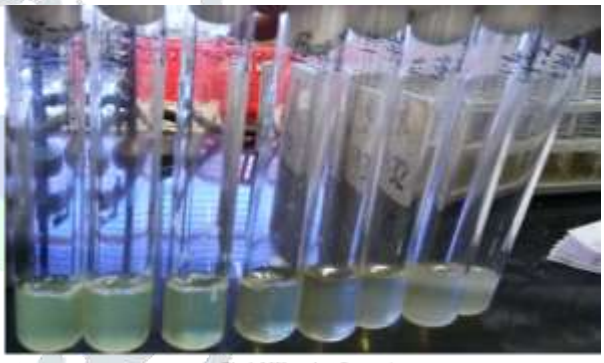

Fig 6: LHS against *P.aeruginosa*

Minimum Lethal Concentration

Table 5: Minimum Lethal Concentration study of hand sanitizers

Sr. no.	Dilutions	Results							
		B. Cereus				VRE			
		DHS	LHS	HHS	DrBHS	DHS	LHS	HHS	DrBHS
1	1:2	+	+	+	+	-	-	-	+
2	1:4	+	+	+	+	-	-	-	+
3	1:8	+	+	+	+	+	+	+	+
4	1:16	+	+	+	+	+	+	+	+
5	1:32	+	+	+	+	+	+	+	+
6	1:64	+	+	+	+	+	+	+	+
7	1:128	+	+	+	+	+	+	+	+

Table 6: Minimum Lethal Concentration study of hand sanitizers

Sr. no.	Dilutions	Results							
		MRSA				P. aeruginosa			
		DHS	LHS	HHS	DrBHS	DHS	LHS	HHS	DrBHS
1	1:2	-	-	-	+	-	-	-	+
2	1:4	-	-	-	+	-	-	-	+
3	1:8	+	+	+	+	+	+	+	+
4	1:16	+	+	+	+	+	+	+	+
5	1:32	+	+	+	+	+	+	+	+
6	1:64	+	+	+	+	+	+	+	+
7	1:128	+	+	+	+	+	+	+	+

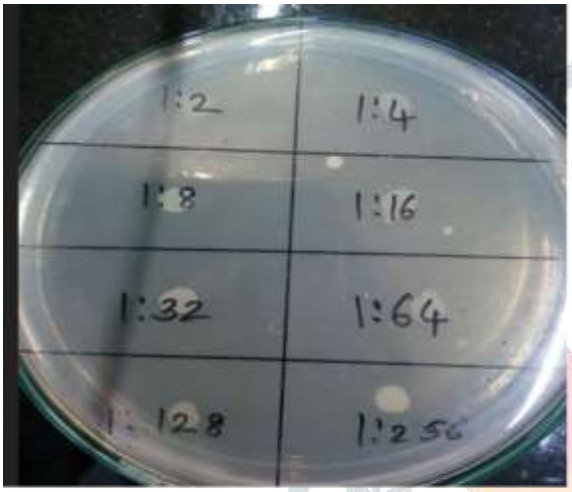

Fig.7 : MBC of Dr.BHS against *VRE*

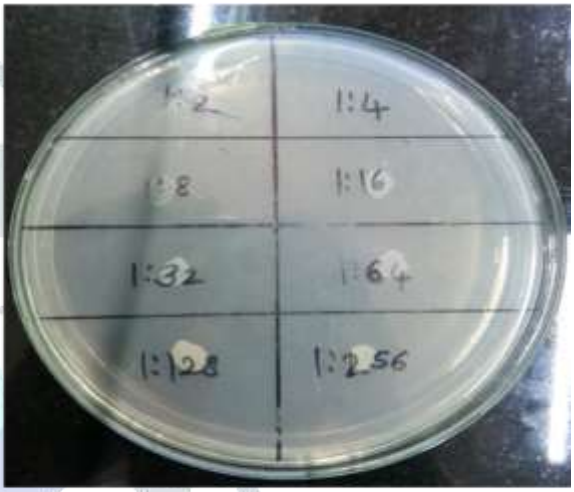

Fig.8 : MBC of Lifebuoy against *VRE*

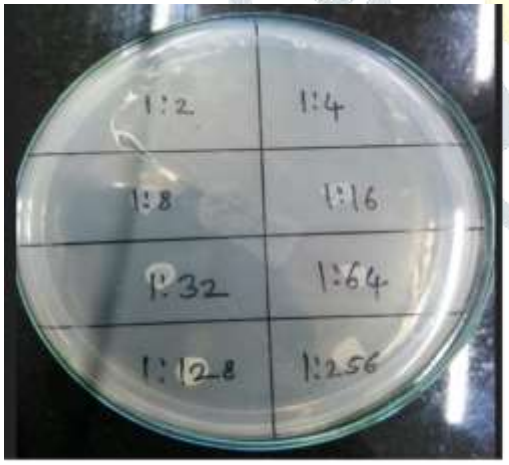

Fig: 9 : MBC of DHS against VRE.

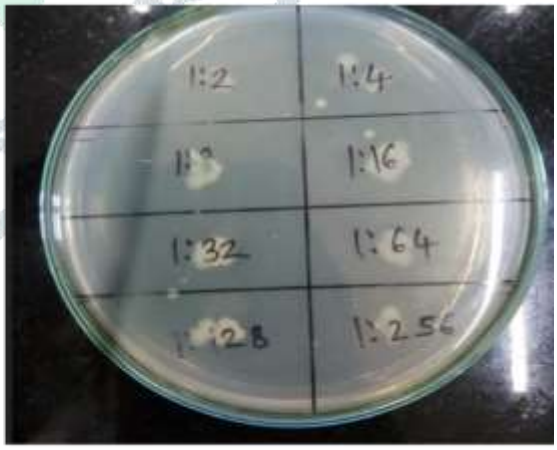

Fig: 10: MBC of Dr. BHS against *B.cereus*

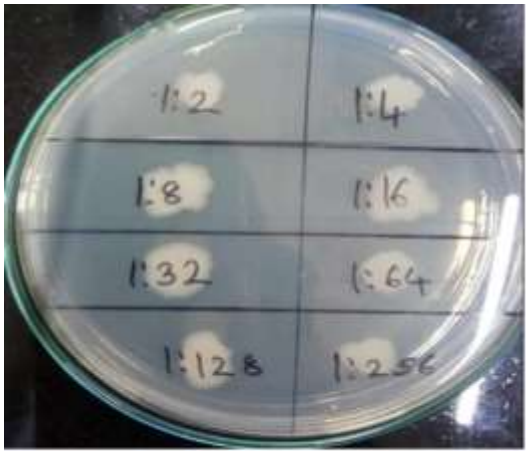

Fig . 11: MBC of HHS against B. cereus

Fig. 12: MBC of LHS against *B.cereus*

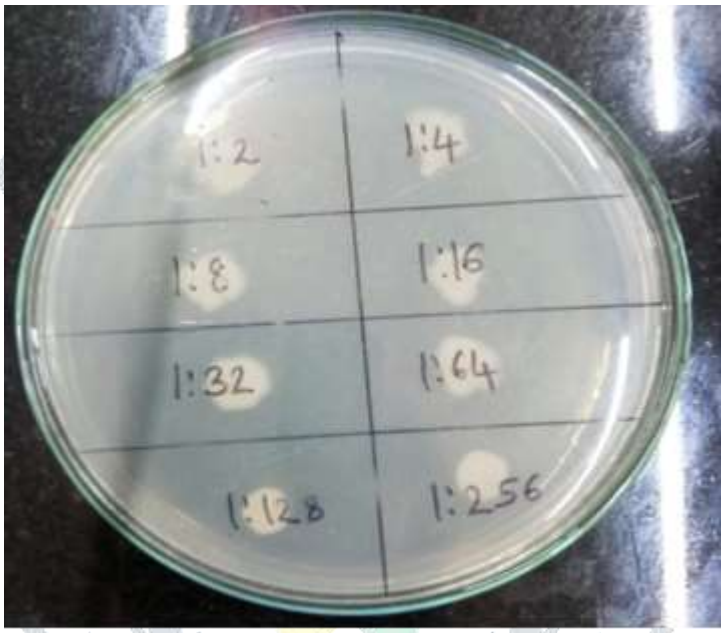

Fig 13: MBC of DHS against *B.cereus*

Time Course study: Quantitative suspension Test

Table 7: Quantitative suspension Test of DHS and LHS

DHS	T.O.C.	*VRE*	*B.cereus*	*MRSA*	*P.aeruginosa*
1:2	30sec	24	86	1	No growth
	5min	5	33	3	No growth
	15min	120	TNTC	22	55
1:4	30sec	220	100	220	No growth
	5min	120	42	180	No growth
	15min	160	TNTC	200	100

1:8	30sec	>300	>300	>300	TNTC
	5min	200	>300	200	TNTC
	15min	TNTC	TNTC	240	TNTC
1:16	30sec	>300	>300	>300	TNTC
	5min	TNTC	>300	>300	TNTC
	15min	TNTC	TNTC	>300	TNTC
LHS	**T.O.C.**	**VRE**	**B.cereus**	**MRSA**	**P.aeruginosa**
1:2	30sec	7	57	86	No growth
	5min	20	87	150	TNTC
	15min	3	TNTC	100	TNTC
1:4	30sec	120	77	224	No growth
	5min	90	100	200	TNTC
	15min	100	TNTC	114	TNTC
1:8	30s	200	>300	236	TNTC
	5min	TNTC	150	220	TNTC
	15min	TNTC	TNTC	140	TNTC
	30s	250	>300	200	TNTC

HHS	T.O.C.	VRE	B.CEREUS	MRSA	P.aeruginosa
Undiluted	30s	0	0	TNTC	1
	5min	1	1	TNTC	3
	15min	1	1	TNTC	2
1:2	30s	140	69	TNTC	TNTC
	5min	69	100	TNTC	26
	15min	67	TNTC	TNTC	32
1:4	30s	160	150	TNTC	35
	5min	TNTC	>300	TNTC	100
	15min	100	TNTC	TNTC	TNTC
1:8	30s	200	>300	TNTC	100
	5min	TNTC	>300	TNTC	128
	15min	TNTC	TNTC	TNTC	TNTC
1:16	30s	250	>300	TNTC	130
	5min	TNTC	>300	TNTC	220
	15min	TNTC	TNTC	TNTC	TNTC
1:16	5min	TNTC	200	180	TNTC
	15min	TNTC	TNTC	150	TNTC

Table 8: Quantitative suspension Test of HHS

Dr. BHS	T.O.C.	*VRE*	*B.cereus*	*MRSA*	*P.aeruginosa*
Undiluted	30s	0	0	>300	TNTC
	5min	1	1	>300	TNTC
	15min	1	1	>300	>300
1:2	30s	100	70	TNTC	TNTC
	5min	80	160	TNTC	TNTC
	15min	150	120	TNTC	TNTC
1:4	30s	120	100	TNTC	TNTC
	5min	100	200	TNTC	TNTC
	15min	190	180	TNTC	TNTC
1:8	30s	140	140	TNTC	TNTC
	5min	220	220	TNTC	TNTC
	15min	240	240	TNTC	TNTC
1:16	30s	160	160	TNTC	TNTC
	5min	>300	>300	TNTC	TNTC
	15min	TNTC	TNTC	TNTC	TNTC

Table 6: Quantitative suspension Test of Dr.BHS

Bacterial protein detection by SDS-PAGE Electrophoresis

Bacterial proteins were analyzed as per protocol given by Hi media. SDS-PAGE was run for the samples of MIC concentrations which showed resistance against the pathogens. It was run to observe the stress conditions if any caused due to the sanitizers on the proteins present in the respective cultures. Protein bands were observed for *VRE* and *P.aeruginosa* for lane 1 which consisted of sample *P.aeruginosa* without sanitizer, lane 2 *P.aeruginosa* with DHS and lane 5 *P.aeruginosa* with Dr.BHS. Protein bands for *MRSA* and *B.cereus* was also observed for lane1 consisting of sample MRSA without sanitizer, lane 2 *MRSA* with DHS, lane 3 *MRSA* with LHS, lane 3 *MRSA* LHS, lane 4 MRSA with HHS, lane 5 with Dr. BHS, lane 6 B.cereus without sanitizer, lane 7 B.cereus with DHS, lane 8 B.cereus with LHS, lane 9 B.cereus with HHS and lane 10 B.cereus with Dr. BHS. Fig 14&15 shows the results of SDS PAGE electrophoresis.

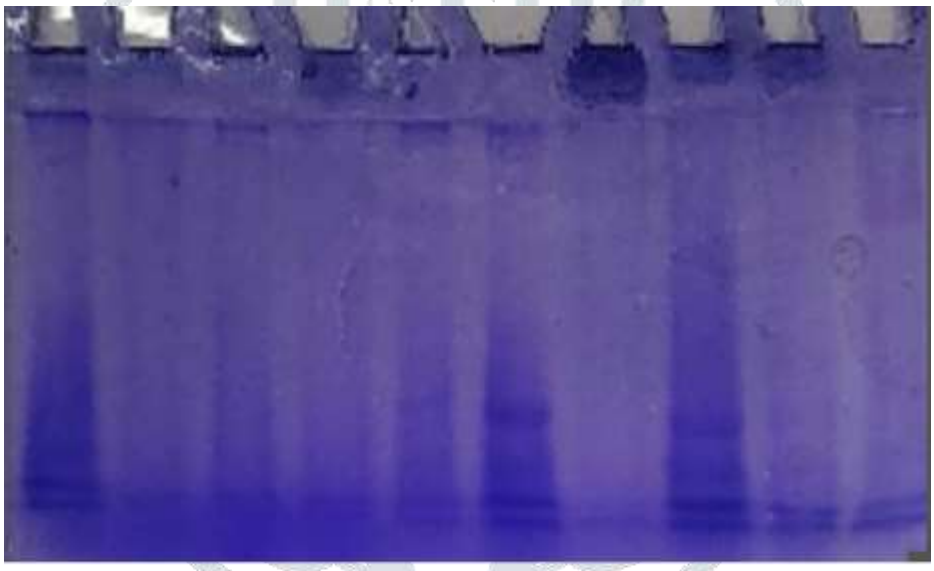

Fig 14: Result for SDS-PAGE for *MRSA & B.cereus*

Key:

Sr.no.	Lanes	Conc.used	PARTICULARS
1	**1**	1:10	MRSA without sanitizer, grown in NB.
2	**2**	1:32	MRSA with DHS.
3	**3**	1:32	MRSA with LHS.
4	**4**	1:32	MRSA with HHS
5	**5**	1:32	MRSA with Dr. BHS

6	**6**	1:10	B.cereus without sanitizer, grown in NB.
7	**7**	1:32	B.cereus with DHS.
8	**8**	1:32	B.cereus with LHS.
9	**9**	1:32	B.cereus with HHS.
10	**10**	1:32	B.cereus with Dr. BHS

Fig 15: SDS-PAGE for *VRE* & *P.aeruginosa* ATCC 15442

Key:

Sr.no.	Lanes	Conc.used	PARTICULARS
1	**1**	1:10	*P.aeruginosa* without sanitizer, grown in NB.
2	**2**	1:32	*P.aeruginosa* with DHS.
3	**3**	1:32	*P.aeruginosa* with LHS.
4	**4**	1:32	*P.aeruginosa* with HHS
5	**5**	1:32	*P.aeruginosa* with Dr. BHS
6	**6**	1:10	*VRE* with Dr.BHS
7	**7**	1:32	*VRE* with HHS
8	**8**	1:32	*VRE* with LHS
9	**9**	1:32	*VRE* with DHS
10	**10**	1:32	.

Conclusion

According to the study and the results observed it was seen that the sanitizers which claimed 99.99% killing of bacteria also was not able of giving satisfactory results or desired efficacy. From these findings, it can be concluded that MRSA, B.cereus, P.aeruginosa and VRE have grown tolerant to alcohol to some extent. Hence more research needs to be carried out to understand the alcohol tolerance and its mechanism.

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