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Comparison of Staphylococcal Virulence Factors, Biofilm Formation and Antibiotic Susceptibility Test Among *Staphylococcus aureus* Strains Isolated from Various Clinical Specimens

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Abstract: *Staphylococcus aureus* isolates from clinical samples such as urine, pus, wound swab, blood and other body fluids were included in this study. Detection of Coagulase enzyme, Haemolysin production, Lecithinase enzyme, Staphylokinase activity, protease enzyme assay, Lipase enzyme, Biofilm production and Antibiotic susceptibility test were detected. In this study a number of 135 *Staphylococcus aureus* isolates were collected from various clinical samples. Isolates were phenotypically investigated for some virulence factors (Coagulase, Clumping factor, Hemolysis, Staphylokinase, Lipase, Protease, Lecithinase and Biofilm production). The antibiotic resistance and virulence factors were correlated statistically by chi-square test. It is evidenced that Gentamicin and Cloxacillin resistance is significant with the Protease activity. Cloxacillin, Ciprofloxacin and Erythromycin resistance is significant with Lipase activity. Cloxacillin resistance is significant with Lecithinase activity. Cloxacillin resistance is significant with Staphylokinase activity. Ciprofloxacin resistance is significant with Hemolysin activity. The antibiotic resistance and Biofilm formation were correlated statistically by chi-square test. It is evidenced that Ciprofloxacin resistance is significant with the Biofilm formation. It will be helpful for clinicians for better understanding about antibiotics resistance exhibited by the *Staphylococcus species*, also it will provide a better understanding about different types of virulence factors produced and it's correlation with antibiotic resistance.

Keywords: *Staphylococcus aureus*, Biofilm, Antibacterial susceptibility, Virulence factors

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Introduction

A relatively unspectacular non-motile coccoid bacterium *Staphylococcus aureus* is an important community and nosocomial acquired pathogen. It is a Gram positive bacterium, non-motile, Sporing and non-capsulated; the cocci are typically arranged in grape like clusters. The pathogen can cause wide variety of infections such as wound infection, respiratory tract infection, sepsis and

life-threatening conditions such as endocarditis, osteomyelitis, pneumonia, brain abscesses, meningitis (Hoefnagels- Schuermans *et al.*, 1999; Sinha *et al.*, 1999). *Staphylococcus aureus* carries a wealth of pathogenic determinants which promote tissue colonization, tissue damage and distant diseases (Dziewanowska *et al*., 1999; Chambers and DeLeo, 2009; Gunasekar *et al*., 2018). *Staphylococcus aureus* is able to survive inside host cells and invade *in vitro* a variety of non-professional phagocytes including fibroblasts (Koneman *et al*., 1992), osteoblasts (Landau and Kaplan, 1980), endothelial (Jevon *et al*., 1999) and epithelial cells (Dinges *et al*., 2000). After internalization *Staphylococcus aureus* may either persist, escaping host defences and antibacterial agents or multiply and further disseminate. *Staphylococcus aureus* express number of virulence factors which help to establish infection by facilitating tissue attachment, tissue invasion and evading from host immune response. Most infection with *Staphylococcus aureus* are self-limiting and not life threatening, but many of these infections can rapidly become life threatening if not treated and managed appropriately, *Staphylococcus aureus* infection is most common cause of hospital acquired infections, certain strains of Staphylococci are the common cause of postoperative wound infection and they are usually resistant to Penicillin, Methicillin and other routinely used antibiotics. The ability to acquire resistance to multiple antibiotic classes makes *Staphylococcus aureus* a challenging pathogen to treat. *Staphylococcus aureus* strains that show resistance to Methicillin are known as Methicillin Resistant *Staphylococcus aureus* (MRSA). MRSA usually colonizes the broken skin and cause a wide range of local and systemic staphylococcal infection. These strains can cause wide range of infections such as pneumonia, endocarditis and bacteraemia. Understanding the functions and mechanism of action of Staphylococcal virulence factors is important for better prognosis of individuals suffering from Staphylococcal infections especially with hospitalized patients. Checking the prevalence of *Staphylococcus aureus* infections, speciation of *Staphylococcus* and analysis of their antibiotic pattern is important for both epidemiological purposes and formulating stewardship policy in a Health care set up. The aim of the present study was to compare the Staphylococcal virulence factor and biofilm formation with antibiotic susceptibility pattern among *Staphylococcus aureus* strains isolated from various clinical specimens.

Materials and Methods

This is a cross sectional study conducted at the Department of Microbiology, Saveetha Medical College and Hospital, Chennai, India. Continuous sampling method was used in the study. Samples received to Clinical Microbiology Laboratory during the period of 6 months (July- December 2021) were included in the study approved by SMCH- IEC- Institutional Review Board (IRB). The approval number is SMC/IEC/2021/07/010. 135 *Staphylococcus aureus* isolated samples were tested. The formula used is $n= Z^2 \times P \times (1-P)/d^2$, with 96% confidence level and 3.3% prevalence. All the *Staphylococcus aureus* isolates from clinical samples such as urine, pus, wound swab, blood and other body fluids were included in this study. Samples not yielding *Staphylococcus aureus* isolates were excluded. The samples not abiding universal precautions were also excluded. Sample processing, organism identification and speciation were done using conventional plating and biochemical test as already reported in many studies. Detection of Lecithinase enzyme, Staphylokinase activity, protease enzyme assay, Lipase enzyme and haemolysin production, Coagulase enzyme, clumping factor were detected based on Sharaf *et al*. (2014), El-baz *et al*. (2016) and Anacarso *et al*. (2013), respectively.

Tests for detection of biofilm formation was done by tissue culture plate method based on Gunasekar *et al*. (1999). Antibiotic susceptibility testing (Kirby Bauer disk diffusion method) was done according to CLSI 2021 (Central Laboratory Standards Institute) guidelines.

Table 1: Correlation between virulence factors and antibiotics

DRUGS		PROTEASE		LECITHINASE		HEAMOLYSIN		STAPHYLO KI-NASE		LIPASE	
		+	-	+	-	+	-	+	-	+	-
Gentamycin	Sensitive	51	39	57	31	65	35	25	64	72	26
	Resistance	40	5	34	13	20	15	16	30	31	6
	Chi- square	x^2= 14.17 P= .0001		x^2= 0.491 P=0.483		x^2=0.686 P= 0.407		x^2=0.642 P= 0.422		x^2= 1.58 P= 0.208	
Cotrimoxazole	Sensitive	57	25	51	28	60	32	27	75	58	17
	Resistance	34	19	39	15	25	18	14	19	45	15
	Chi-square	x^2= 0.421 P= 0.516		x^2= 0.861 P= 0.353		x^2= 0.629 P= 0.427		x^2= 3.000 P= 0.083		x^2= 0.100 P= 0.751	
Ciprofloxacin	Sensitive	21	20	18	9	6	16	10	21	23	15
	Resistance	70	24	72	34	79	34	41	73	80	17
	Chi-square	x^2= 7.023 P= 0.008		x^2= 0.015 P= 0.900		x^2= 14.356 P= 0.000		x^2= 0.146 P= 0.701		x^2= 7.272 P= 0.007	
Vancomycin	Sensitive	91	44	90	43	85	50	41	94	103	32
	Resistance	0	0	0	0	0	0	0	0	0	0
Penicillin	Sensitive	0	0	0	0	0	0	0	0	0	0
	Resistance	91	44	90	43	85	50	41	94	103	32
Lenezolid	Sensitive	89	41	79	41	79	39	39	84	96	30
	Resistance	2	3	11	2	6	11	2	10	7	2
	Chi- square	x^2= 1.775 P= 0.182		x^2= 1.891 P= 0.169		x^2= 6.385 P= 0.011		x^2= 1.169 P= 0.279		x^2= 0.011 P= 0.913	
Cloxacillin	Sensitive	38	27	40	25	45	25	26	39	44	21
	Resistance	53	17	54	16	43	27	15	55	59	11
	Chi- square	x^2=4.566 P= 0.032		x^2=3.880 P=0.0488		x^2=0.122 P=0.726		x^2=5.497 P=0.019		x^2=5.131 P=0.023	
Erythromycin	Sensitive	26	15	26	17	14	19	9	27	28	20
	Resistance	65	29	64	26	73	31	32	67	75	12
	Chi- square	x^2= 0.427 P= 0.513		x^2= 1.507 P=0.219		x^2= 1.373 P= 0.241		x^2= 0.669 P= 0.413		x^2= 13.28 P= 0.000	
Clindamycin	Sensitive	43	23	55	24	52	23	17	49	54	14
	Resistance	48	21	35	19	33	27	24	46	49	18
	Chi- square	x^2= 0.299 P= 0.584		x^2= 0.338 P= 0.560		x^2= 2.936 P= 0.086		x^2= 1.173 P= 0.278		x^2= 0.735 P= 0.391	

Results

Out of 135 isolates all the isolates showed coagulase and clumping factor positive and 41 (30%) isolates showed Staphylokinase positive and 94 (70%) isolates showed Staphylokinase negative, 85 (63%) isolates showed Haemolysin positive and 50 (37%) isolates showed Haemolysin negative, 103 (76%) isolates showed Lipase positive and 32 (24%) isolates showed Lipase negative, 91 (67%) isolates showed Protease positive and 44 (33%) isolates showed Protease negative, 82 (61%) isolates showed Lecithinase positive and 53 (39%) isolates showed Lecithinase negative. 70 isolates were MRSA and 65 isolates were MSSA. In antibiotic sensitivity test 48 (74%) isolates of MSSA showed sensitivity to

Table 2: Correlation between Biofilm formation and antibiotics

DRUGS		BIOFILM PRODUCTION	
		BIOFILM PRODUCER 82 ISOLATES	NON BIOFILM PRODUCER 53 ISOLATES
Gentamycin	Sensitive	58	32
	Resistance	24	21
		x^2= 1.553, p= 0.212	
Cotrimoxazole	Sensitive	53	32
	Resistance	29	21
		x^2= 0.2501, p= 0.616	
Ciprofloxacin	Sensitive Resistance	6	14
		76	39
		x^2= 9.3041, P= 0.002	
Vancomycin	Sensitive Resistance	82	53
		0	0
Penicillin	Sensitive Resistance	0	0
		82	53
Lenezolid	Sensitive Resistance	73	48
		9	5
		x^2=0.082, P= 0.774	
Cloxacillin	Sensitive Resistance	38	27
		44	26
	x^2= 0.262, P= 0.608		
Erythromycin	Sensitive Resistance	21	22
		61	31
		x^2=0.273, P= 0.601	
Clindamycin	Sensitive Resistance	45	24
		37	29
		x^2= 1.186,P= 0.833	

Gentamycin whereas 55 (79%) isolates of MRSA showed sensitivity to Gentamycin. 45 (69%) isolates of MSSA showed sensitivity to Cotrimoxazole whereas 46 (66%) isolates of MRSA showed sensitivity to Cotrimoxazole, 21 (32%) isolates of MSSA showed sensitivity to Ciprofloxacin whereas 23 (33%) isolates of MRSA showed sensitivity to Ciprofloxacin, 65 (100%) isolates of MSSA showed sensitivity to Vancomycin, in case of MRSA also 70 (100%) isolates were sensitive. 60 (92%) isolates of MSSA showed sensitivity to Linezolid whereas 60 (86%) isolates of MRSA showed sensitivity to Linezolid. 65 (100%) isolates of MSSA showed sensitivity to Cloxacillin. 70 (100%) isolates of MRSA showed resistance to Cloxacillin, 28 (43%) isolates of MSSA showed sensitivity to Erythromycin whereas 27 (39%) isolates of MRSA showed sensitivity to Erythromycin, 42 (65%) isolates of MSSA showed sensitivity to Clindamycin whereas 40 (57%) showed sensitivity to Erythromycin. 27 (39%) isolates of MRSA showed sensitivity to Erythromycin, 42 (65%) isolates of MSSA showed sensitive to Clindamycin whereas 40 (57%) isolates of MRSA showed sensitivity to Clindamycin. 65 (100%) isolates of MSSA showed resistance to Penicillin similarly in case of MSSA 70 (100%) isolates MRSA showed resistance to

Penicillin. Out of 70 isolates from MRSA, 25% of isolates showed no biofilm production, 10% of isolates showed weak biofilm production, 42% of isolates showed moderate biofilm production and 23% of isolates showed strong biofilm production. Out of 65 isolates of MSSA, 46.1% of isolates were non-biofilm producers, 7.7% of isolates were weak biofilm producers, 30.7% of isolates were Moderate biofilm producers and 15.4% isolates were Strong biofilm producers. Tables 1 and 2 illustrate the correlation between virulence factors vs antibiotic resistance and Biofilm production vs Antibiotic resistance, respectively.

Discussion

In our study Staphylokinase was produced by 30% isolates. A different result was reported in the study conducted by Wieckowska-Szakiel *et al*. (2007) who have reported a higher percentage (75.6%) of Staphylokinase producing isolates. Another different result was reported by El-baz *et al*. (2016) where Staphylokinase was produced by 41.15% of tested isolates. In the present study, about 76% of isolates showed lipolytic activity, this is in accordance with Rodrigues *et al*. (2014) and Saising *et al*. (2012) where 71% of isolates showed lipase activity. El-baz *et al*. (2016) reported that 71.1% of isolates presented a lipolytic activity.

68% of isolates showed Lecithinase activity. A different result was reported in the study conducted by Anacarso *et al*. (2013) where only 20.5% of the isolates showed Lecithinase activity. El-baz *et al*. (2016) reported that 56.4% of tested isolates were Lecithinase producers. In this study, 67% of the isolates showed protease activity, this result is higher than the result reported by Karlsson (2002), while Saising *et al*. (2012) recorded 97% of isolates showed protease activity. El-baz *et ai.* (2016) reported that 54.1% of isolates showed Proteolytic activity.

Biofilm formation is an important factor of pathogenesis of *Staphylococcus aureus*. In our study, 61% of total isolates showed biofilm formation. A different result was reported in the study conducted by Khan *et al*. (2011) where only 14.5% of isolates showed biofilm formation. El-baz *et al*. (2016) noticed 82.3% of total isolates which showed Biofilm formation.

Conclusion

The result of this study will be useful to update the antibiotic policy in our hospital setup. It provides a better understanding of prevalence of each *Staphylococcus aureus* virulence factor. Considering the high prevalence of *Staphylococcus aureus* infections, it is important to know the different mechanisms of virulence of antimicrobial resistance by this pathogen to succeed during colonization and the interaction between them. Genetic studies are also needed for more detailed information about what drives the drug resistance and virulence factor in *Staphylococcus aureus* infection. This could allow the most effective control tools to be implemented to help reduce the impact of both community acquired and health care associated infections.

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