DOI: 10.7860/JCDR/2023/60256.17558

Original Article

Microbiology Section

Isolation of Methicillin-Resistant *Staphylococcus aureus* from Wound Samples during the COVID-19 Pandemic: A Retrospective Study

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ABSTRACT

Introduction: On March 11th, 2020, the World Health Organisation (WHO) declared the outbreak of the novel coronavirus disease caused by the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) as a pandemic. This recently discovered β-coronavirus spread instantaneously across mainland China due to human-to-human transmission and crossed international borders aided by intercontinental travel. In most nations, the logarithmic growth of the cases very quickly overwhelmed the healthcare system which led to the overcrowding of the hospitals and led to a sudden surge in Hospital-Acquired Infections (HAIs). Implementation of contact precautions was implemented to control cross-infection.

Aim: To determine the effect of Coronavirus Disease-2019 (COVID-19) on the prevalence of HAIs with special emphasis on *Staphylococcus aureus* (*S. aureus*).

Materials and Methods: This three year retrospective study (September 2018 to August 2021) was undertaken at Department of Microbiology, Sher-i-Kashmir Institute of Medical Sciences, Kashmir, an apex tertiary care institute in Northern India. A total of 2548 wound swabs samples were collected and processed in the laboratory for the presence of aerobic bacterial isolates. *S. aureus* was identified using conventional methods and antimicrobial sensitivity was performed by the Kirby-Bauer disc diffusion method. Data was entered in Microsoft excel and later analysed in International Business Machines (IBM) Statistical Package for the Social Sciences (SPSS) version 22.0.

Results: A steady increase in the isolation of Methicillin-resistant *Staphylococcus aureus* (MRSA) was noted during the study period (60.5% in 2018 to 78.1% in 2021). A statistically significant increase was noted in the detection of MRSA after the onset of the COVID-19 pandemic (p=0.018) despite the reduced number of surgeries conducted in the institution and rigorous execution of contact precautions.

Conclusion: There was an increase in the rate of MRSA isolation during the study period. The increase was significantly affected by the onset of COVID-19. To contain the spread of MRSA, novel methods including preoperative screening of patients undergoing elective surgeries and periodic screening of hospital staff need to be implemented along with standard infection control precautions at all times.

Keywords: Contact precautions, Hospital-acquired infections, Hospital infection control, Pandemic, Severe acute respiratory syndrome coronavirus-2

INTRODUCTION

Staphylococcus aureus is a human pathogen with a wide range of disease spectrum which encompasses mild skin and soft tissue infections to severe life-threatening sepsis. With the advent of methicillin-resistant strains, especially in the hospital environment, treatment options have become limited. These strains, being Multidrug Resistant (MDR), lead to higher morbidity and mortality [1]. World Health Organisation (WHO) describes Hospital Acquired Infections (HAIs) as an emerging health hazard having a major economic impact both on the community and the individual. Although, progress has been made in the control and prevention of HAIs over the last decade, the resilient nature of the organism makes its eradication difficult and continues to be a major cause of the increased cost of care [2]. The bacterial flora of hospitals comprises MDR species which vary with time and location inside the hospital. Working knowledge of the antibiogram of the hospital is of vital importance in the treatment of such infections. Additionally, diagnostic tests have to be performed without delay to identify the aetiologic agents associated with HAIs to guide the choice of antibiotics [3].

Staphylococcus aureus is well known for boasting several drug resistant mechanisms, the most menacing being methicillin resistance. MRSA arises due to the transfer of novel Deoxyribonucleic Acid (DNA) which induces the production of a new Penicillin-binding Protein-(PBP2a). PBP2a has a low affinity for methicillin and other β-lactams [4]. In an institution, the prevalence of Hospital Acquired-MRSA (HA-MRSA) is indicative of the overall infection rate and is influenced by various factors such as the inflow of patients, magnitude of overcrowding in the wards and nursing load. One of the main sources of transmission of MRSA within hospitals is via the hands of Healthcare Workers (HCWs) [5]. The main reservoirs of HA-MRSA are the infected and colonised patients. The colonisation of patients is proportional to the length of hospital stay, nutritional status of the patient, recurrent or recent antibiotic treatment, and presence of wound and/or invasive devices [6]. Wound infections are defined as the discharge of pus from the wound, or a clinical suspicion of wound infection, based on inflammatory signs such as raised temperature, redness and tenderness of the wound. Wound infections caused by HA-MRSA are associated with high morbidity and mortality [7].

The COVID-19 pandemic further increased the burden on infection prevention practices which had been prevalent in healthcare settings [8]. Due to the contagious nature of the pathogen, unprecedented measures were introduced to prevent the spread of the disease among the patients admitted to the hospitals [9]. Prior to the advent of the pandemic, these measures were implemented only in high-risk units due to concerns about cost-effectiveness [10]. Although, these measures led to the control of the pandemic at various levels, other areas of patient care and control programmes for other HAIs suffered as a consequence of all the efforts being diverted to COVID-19 mitigation [11]. During the outbreak of SARS-CoV-2 in 2003, various reports suggested increased rates of MRSA in healthcare settings [12]. Given the above, continuous surveillance of microorganisms and a regular update of their antibiotic resistance pattern is essential to maintain good infection control practices in the hospital. Keeping this perspective in view, authors designed this retrospective study to estimate the prevalence of the major aerobic bacterial isolates especially MRSA from wound samples of patients. Also, the trend for the past three years was studied with special emphasis on the later part of the study period which coincided with the emergence of the COVID-19 pandemic. Baseline information was recorded for further detailed and large epidemiological and drug resistance investigations in an attempt to develop a comprehensive treatment protocol.

MATERIALS AND METHODS

This retrospective study was carried out in the Department of Microbiology, Sher-i-Kashmir Institute of Medical Sciences, a 1200 bedded apex tertiary care centre of the valley of Srinagar, Jammu and Kashmir, India. The study protocol was approved by the Institutional Ethics Committee (IEC-SKIMS/2022-365).

Inclusion criteria: All wound swab samples sent to the bacteriology laboratory during the study period of three years (September 2018 to August 2021) were included. The data were subsequently analysed (August 2022 to September 2022).

Exclusion criteria: Improperly labelled samples and any repeat isolate from the same patient received on more than one occasion were excluded from the study.

Study Procedure

Specimen collection: Specimens were collected on the day when patients reported clinical evidence of infection (purulent drainage from wound site). Sterile cotton wool swabs were used for sample collection. Before sample collection, the wound was thoroughly cleansed with sterile normal saline or sterile water. The swab was rotated over a 1 cm^2 area of viable tissue for five seconds using sufficient pressure to extract fluid from the wound tissue (Levine method) [13]. It was ensured that the sample was collected from viable tissue and not necrotic slough, purulent material or eschar that was heavily contaminated with colonising bacteria. A total of two swabs were collected and immediately transported to the laboratory for processing.

Sample processing: Samples were processed within two hours after receipt in the laboratory.

Direct microscopy: One swab was used for making smears which was stained and screened for pus cells and the presence/absence of bacteria, their gram reaction along with the morphology and arrangement.

Culture for aerobic organisms: The second swab was inoculated onto plates of sheep blood agar containing blood agar base (HiMedia) and 5% sheep blood and MacConkey agar (HiMedia) by rolling the swab over the agar and streaking. Also, a backup was put on Robertson's Cooked Meat (RCM). The plates were incubated overnight at 35°C±2 in bacteriological incubators. After performing preliminary identification tests such as gram stain, oxidase and catalase from the isolated colonies, identification and susceptibility testing were done. All isolates were identified by conventional biochemical tests (carbohydrate fermentation patterns and activity of amino acid decarboxylases and other enzymes). Antimicrobial susceptibility testing was performed by the Kirby-Bauer disc diffusion method on Mueller-Hinton agar according to Clinical and Laboratory Standards Institute (CLSI) guidelines [14]. *S. aureus* was reported as methicillin resistant if the disk diffusion zone for 30 μg cefoxitin disc was <22 mm.

Test control organisms: American Type of Culture Collection (ATCC) strains of *S. aureus* (25923), *E. coli* (25922), and *P. aeruginosa* (27853) were used as test control organisms (Hi-Media Laboratories Pvt., Ltd., Maharashtra, India).

STATISTICAL ANALYSIS

Data was entered in Microsoft excel and later analysed in International Business Machines (IBM) Statistical Package for the Social Sciences (SPSS) version 22.0. Statistical analysis was performed by using a Chi-square test. Statistical significance was defined for $p<0.05$. Test results are presented both graphically and in tabular form.

RESULTS

A total of 2548 samples were received and analysed in the laboratory during the study period. The mean age of the studied population was 33.83 years (1-85 years). The positivity of the swab culture was 97.8%. Out of the culture positive samples, 1843 (73.9%) were gram negative organisms, while 648 (26.1%) were gram positive bacteria. The most common organism isolated was *Escherichia coli* (n=504) among the gram negative organisms whereas, amongst the gram positive organisms, the most frequently isolated was *S. aureus* (n=310) [Table/Fig-1]. Confirmation of *S. aureus* was done by gram staining, catalase test and a tube coagulase test. Methicillin resistance was confirmed by the 30 μg disk diffusion method, as per CLSI guidelines [Table/Fig-2]. The majority of the *S. aureus* samples were from the Outpatient Department (OPD) section while in the Inpatient Department (IPD) section, the majority of samples were from the Department of Plastic Surgery [Table/Fig-3]. The percentage of MRSA isolated during the study period was 66.1%. The incidence of gram positive and gram negative organisms changed little over the three years. However, there was a constant and significant increase in the incidence of MRSA from 60.5% during the first year of study to 78.1% during the third year of study [Table/Fig-4]. The mean age of patients with MRSA was 28 years as opposed to those with Methicillin-sensitive *Staphylococcus aureus* (MSSA) (42 years). There was no significant association between various demographic factors except COVID-19, wherein a statistically significant rise in MRSA was noted between the prepandemic and postpandemic period [Table/Fig-5]. MSSA strains showed considerable sensitivity to co-trimoxazole (80%), erythromycin (32.3%) and clindamycin (84%) [Table/Fig-6]. As *S. aureus* showed high resistance to standard antibiotics, the second line of drugs, were also tested [Table/Fig-7].

Gram reaction	Organism	n	Total
Gram negative organisms	*Escherichia coli*	504	1843
	Pseudomonas aeruginosa	429	
	Klebsiella pneumoniae	385	
	Acinetobacter baumannii	325	
	Proteus mirabilis	102	
	Acinetobacter lwoffii	34	
	Proteus vulgaris	29	
	Klebsiella oxytoca	20	
	Citrobacter freundii	5	
	Providencia spp.	4	
	Citrobacter koseri	2	
	Morganella morganii	2	
	Enterobacter aerogenes	1	
	Salmonella typhi	1	
Gram positive organisms	***Staphylococcus aureus***	**310**	648
	Coagulase negative *Staphylococcus*	241	
	Enterococcus	96	
	Streptococcus viridans	1	
Sterile		57	
Total		**2548**	

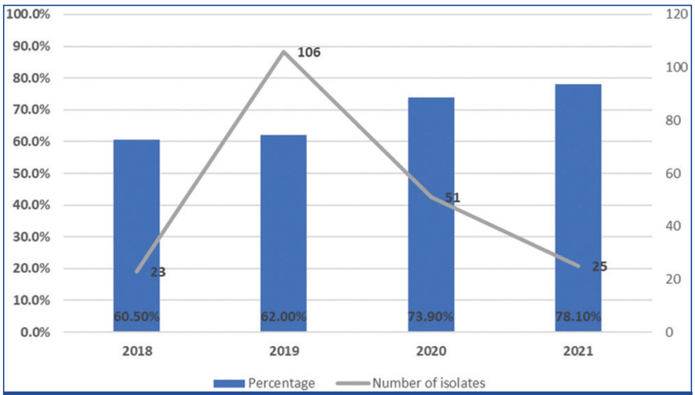
[Table/Fig-1]: Distribution of microorganisms isolated from the studied population.

Total samples (n=2548)	Sterile (n=57)			
	Gram negative (n=1843)			
	Gram positive (n=648)	Catalase negative (n=97)		
		Catalase positive (n=551)	Coagulase negative (n=241)	
			Coagulase positive (n=310)	Methicillin-sensitive *Staphylococcus aureus* (n=105)
				Methicillin-resistant *Staphylococcus aureus* (n=205)

[Table/Fig-2]: Breakdown of samples processed during the study period.

Variable		Speciality	Frequency (n)	Percent (%)	Percent (%)
IPD	Emergency	Accident emergency	2	0.6	1.0
		Paediatric emergency	1	0.3	
	Medicine and allied	Endocrinology	10	3.2	14.5
		Medical observation	10	3.2	
		General medicine	8	2.6	
		Radiation oncology	8	2.6	
		Haematology	5	1.6	
		Neonatology	2	0.6	
		Nephrology	2	0.6	
	Surgery and allied	Plastic surgery	63	20.3	34.8
		Paediatric surgery	12	3.9	
		Neurosurgery	9	2.9	
		Postoperative ward	6	1.9	
		Urology	6	1.9	
		General surgery	5	1.6	
		Kidney transplant unit	3	1	
		Colorectal surgery	2	0.6	
		Surgical observation	2	0.6	
	Intensive care units	Surgical intensive care unit	11	3.5	3.9
		Neonatal intensive care unit	1	0.3	
OPD	OPD	Outpatient department	142	45.8	45.8
		Total	**310**	**100**	**100**

[Table/Fig-3]: Distribution of *S. aureus* in the studied population according to the admitting speciality.



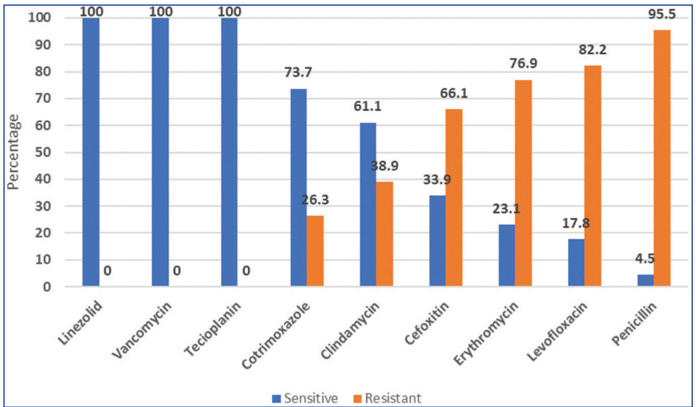
[Table/Fig-4]: Distribution of MRSA isolated according to year.

Variable			MSSA	MRSA	p-value
Gender	Female (40.6%)	n	40	86	0.513
		%	(31.7)	(68.3)	
	Male (59.4%)	n	65	119	
		%	(35.3)	(64.7)	
Speciality	Intensive care units (3.9%)	n	2	10	0.623
		%	(16.7)	(83.3)	
	Medicine and allied (15.5%)	n	17	31	
		%	(35.4)	(64.6)	
	Outpatient department (45.8%)	n	50	92	
		%	(35.2)	(64.8)	
	Surgery and allied (34.8%)	n	36	72	
		%	(33.3)	(66.7)	
Location	Inpatient department (54.2%)	n	55	113	0.647
		%	(32.7)	(67.3)	
	Outpatient department (45.8%)	n	50	92	
		%	(35.2)	(64.8)	
COVID-19	Post COVID-19 pandemic (32.6%)	n	25	76	0.018*
		%	(24.8)	(75.2)	
	Prior to COVID-19 pandemic (67.4%)	n	80	129	
		%	(38.3)	(61.7)	

[Table/Fig-5]: Distribution of MRSA amongst the studied population according to demographic variables.

Antibiotic		MSSA		MRSA		p-value
		n	%	n	%	
Clindamycin	Resistant	16	16.0	95	51.4	p<0.00*
	Sensitive	84	84.0	90	48.6	
Erythromycin	Resistant	67	67.7	160	81.6	0.007*
	Sensitive	32	32.3	36	18.4	
Cotrimoxazole	Resistant	1	20.0	4	28.6	0.709
	Sensitive	4	80.0	10	71.4	

[Table/Fig-6]: Antibiotic susceptibility of MRSA and MSSA strains (in percentage).



[Table/Fig-7]: Sensitivity pattern of *Staphylococcus aureus* isolates.

DISCUSSION

Ever since MRSA was isolated in 1961, it has established itself as an endemic pathogen in healthcare facilities throughout the world. According to reports, more than half of the strains of *S. aureus* isolated from the Asia-Pacific region show methicillin resistance; mainly associated with skin and soft tissue infections. The increased virulence of MRSA strains makes them more virulent and leads to higher morbidity, mortality and healthcare costs. Due to its significant impact, WHO has included MRSA in the high-priority list of drug resistant bacteria for the targeted development of novel antibiotics [15]. As the incidence of HAI associated with MRSA increases, the detection of such strains has become imperative for treatment and epidemiological purposes [3].

In the present study, a total of 2548 samples were received during the study period. A high culture positivity of 2491 (97.8%) was observed in patients with wound infections. *S. aureus* was isolated in 310 (12.17%) of the samples. A study conducted by Vidhani S et al., also reported similar rates (17.6%) of staphylococcal wound infection [16]. Among the 310 *S. aureus* strains isolated, 205 were MRSA while the remaining 105 were MSSA. A high proportion of MRSA (66.1%) was identified in the study. A lesser rate of 28.5% and 27.3% have been reported by Manian FA et al., and Cerveira JJ et al., respectively [17,18]. The hospital is a referral hospital that caters to the entire population of the division. This might explain the high incidence of MRSA in the hospital. The epidemiology of MRSA displays wide geographic variation. Care has to be taken when data from different regions were compared as different definitions may be used in defining data collection, study population and surveillance methods. Nonetheless, such data is of vital importance in benchmarking and monitoring the effectiveness of control strategies [15]. The increase in the rate of isolation of MRSA strains in hospitalised patients requires rapid and reliable characterisation. Efforts should be made to formulate guidelines to prevent the spread of such strains. Despite numerous eradication measures implemented over the past decade, MRSA continues to be a major nosocomial pathogen worldwide [7]. The mean age of patients infected with MRSA (28 years) was less than that of MSSA (42 years). Several reports have mentioned that increasing age is a risk factor for MRSA colonisation [19,20]. A multicentric study done in the United States found that persons older than 65 years, women, diabetics and those admitted to long-term care in the past year had a higher risk for MRSA colonisation [21]. Present study comprised of a younger subset population (mean age=33.83 years), which might explain the lower age of colonisation by MRSA.

On further analysis, an upward trend was noted in the isolation of MRSA from the hospital; 60.5% in 2018 to 78.1% in 2021. Previous studies conducted in this institute reiterate the fact that MRSA isolation has been on the rise and this worrisome trend has further increased post-COVID-19 [22-24]. United States reported a steadily increasing rate of MRSA from 1998 up to 2005, when it reached 53% of *S. aureus* clinical isolates [25]. Rigorous infection control practices implemented thereafter led to a 17% annual reduction [26]. Similar trend was noted across Europe [27]. This downward trend was mainly attributed to a multimodal strategy involving universal MRSA screening, contact precautions, and the promotion of hand hygiene. Similar practices may be instituted across all high prevalence areas of the hospital [15].

The MRSA strains were found to be resistant to many antibiotics in this study while MSSA strains showed considerable sensitivity to cotrimoxazole (80%), erythromycin (32.3%), and clindamycin (84%). A statistical significance was observed between sensitivities of clindamycin and erythromycin for MRSA and MSSA strains, while cotrimoxazole showed no such significance. Similar findings have been reported elsewhere. In a study conducted by Kothari A et al., all MRSA isolates showed 72% for macrolides and cotrimoxazole, whereas the MSSA isolates showed a lower rate of resistance (55%) [28].

In the United States, HAI affects 1 out of 31 hospitalised patients. MRSA is a major contributing factor to HAI, especially in patients with immunocompromising conditions. Centre for Disease Control and Prevention (CDC) has advised the implementation of contact precautions to control and prevent cross-transmission between patients [29]. There have been no standard recommendations for routine screening of HCWs for MRSA carriage. The available guidelines recommend screening only during outbreaks. The current hospital guidelines suggest that all MRSA carriers be referred to a physician for decolonisation therapy with a follow-up resampling 10 days after the therapy has been completed. Healthcare facilities may consider pre-employment screening and periodic cross-sectional screening of HCWs in between outbreaks [30]. In addition, MRSA carriers need to be informed regarding the risk that they carry of developing postoperative MRSA Surgical Site Infections (SSI). Studies have reported a 2.5 times higher risk in MRSA carriers and the consequences of such a complication are severe [23]. During the postoperative period, these patients need to be monitored closely for signs of wound infection so that prompt treatment may be started. Also, the high morbidity in such cases suggests that elective surgeries may be delayed until either MRSA status is known or the patient is sent for decolonisation therapy. In case of emergency surgeries, measures such as prophylactic use of antibiotics active against MRSA have been shown to reduce postoperative MRSA wound infection [31]. MRSA SSI is very troublesome to eradicate once set in and such prophylactic antibiotics have a definite advantage.

Several innovative measures need to be implemented to prevent MRSA SSIs in a high load hospital. Universal screening for MRSA in dated patients, along with improved staff and patient education, timely and regular screening of HCWs, implementation of hand hygiene before and after interacting with patients, and a search and destroy policy may help in reducing the spread and reduce cross-contamination and overall MRSA burden [32].

At the start of 2020, when the first patients infected with COVID-19 were admitted to the hospital, several measures were initiated to avoid transmission among patients and HCWs. These included having a dedicated infectious disease block, rigorous use of Personal Protective Equipment (PPE), and extensive use of hand sanitisers. Hand sanitiser use in the hospital increased many folds during the pandemic. However, the incidence of nosocomial MRSA was unaffected and showed a continual upward trend [33].

The COVID-19 pandemic spread rapidly and hospitals around the world have been overwhelmed by patients. During the initial phase of the pandemic, antibiotics were massively prescribed because of a lack of knowledge and guidelines for management. As many as 95% of the patients were prescribed antibiotics during the first few months of the pandemic [34]. Apart from rare cases of bacterial coinfection antibiotics had no documented role in the treatment of such cases. Moreover, antibiotics were prescribed without samples being sent for microbiological testing [35]. There was no clear benefit that was documented due to excessive use of antibiotic use and if any was counterbalanced by the magnitude of side-effects especially the increase in antibiotic resistance rates [36].

Before the advent of COVID-19 pandemic, WHO had targeted its efforts to reduce and prevent antimicrobial resistance. COVID-19 highlighted several issues regarding the healthcare setup and along with it, brought new threats to the forefront including excessive use of antibiotics even without evidence of their utility [35]. Several studies have indicated that antibiotic use is high among patients hospitalised for COVID-19 [37]. This highlights the importance of having a proactive antibiotic stewardship committee. While uncertainty regarding the management of COVID-19 led to the widespread use of antibiotics, several studies suggested the restrictive use of antibiotics. Antibiotics may be withheld in milder cases. In addition, the prescribing of antibiotics in the “Watch”, “Reserve” and “Not recommended” groups of the WHO’s AWaRe classification system need to be restricted. An ecological study in England found that broad-spectrum antibiotics were being prescribed in larger proportions despite an overall decrease in antibiotic use in the community [38]. The increase in MRSA isolation rate was in spite of the lower admission rates in hospitals across the valley and the lesser number of procedures done during the COVID-19 pandemic [39,40].

Limitation(s)

This study had several limitations, including the fact that it was a monocentric study and retrospective in nature. Another limitation was the small sample size. Also, the potential role of HCW with the most patient contact was not examined. The COVID-19 history of the subjects was not known. It was presumed that antibiotic usage had increased and it was extrapolated to the study subjects. Nevertheless, this study is one of the first to focus on the misuse of antibiotics against COVID-19, and the increase in MRSA prevalence in a tertiary care centre. This study could be complemented by an analysis of the risk factors that led to the continued surge of MRSA rates despite the rigorous implementation of contact precautions following the COVID-19 pandemic.

CONCLUSION(S)

The MRSA is a major cause of morbidity and mortality. MRSA isolation rates have continued to increase in the hospital although the magnitude of elective surgeries done during the pandemic was low as compared to earlier years. Clinicians should be cautious about antibiotic prescriptions in the absence of strong evidence of infection especially patients with COVID-19, as their prevalence is not high, a certain diagnosis is not accessible, and the benefit/risk ratio is not clear. Novel methods should be implemented in the hospital to contain the spread of MRSA, an epidemic of MRSA may propagate during the COVID-19 pandemic.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]

- Plagiarism X-checker: Sep 20, 2022
- Manual Googling: Dec 29, 2022
- iThenticate Software: Jan 02, 2023 (10%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:

- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Sep 16, 2022**
Date of Peer Review: **Nov 26, 2022**
Date of Acceptance: **Jan 04, 2023**
Date of Publishing: **Mar 01, 2023**