

Frequency and Antibiotic Susceptibility Pattern of Community-associated Methicillin-resistant *Staphylococcus Aureus* (CA-MRSA) in Uncomplicated Skin and Soft Tissue Infections

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ABSTRACT

Objective: To determine the frequency and antibiotic susceptibility pattern of CA-MRSA in patients with uncomplicated skin and soft tissue infections reporting to the dermatology outpatient of a tertiary health care hospital.

Study Design: A descriptive study.

Place and Duration of Study: Dermatology outpatient of a tertiary care hospital in Punjab province of Pakistan, from September 2020 to August 2021.

Methodology: Patients of all age groups and both genders reporting during the study period with community-associated uncomplicated bacterial skin and soft tissue infections were enrolled in the study. Samples were collected from skin lesions and cultured on blood agar and MacConkey agar plates. Antimicrobial susceptibility testing using the modified Kirby Baur disc diffusion technique was performed.

Results: A total of 157 patients were included in the study. Impetigo was most common infection (n=80, 51%), followed by Furunculosis (n=47, 29.9%). The frequency of MRSA isolates was 54.1% (n=85). MRSA was significantly more frequently isolated from patients with furunculosis, carbuncle and cutaneous abscesses as compared to impetigo. All MRSA isolates were sensitive to linezolid, teicoplanin, and vancomycin. 97.6%, 84.7%, and 72.9% of MRSA isolates were sensitive to rifampicin, minocycline, and fusidic acid respectively. 89.4% of MRSA were sensitive to amikacin and clindamycin. 63.5% were sensitive to doxycycline and 58.8% were sensitive to co-trimoxazole. Only 20% of MRSA were sensitive to ciprofloxacin.

Conclusion: The antibiotics active against CA-MRSA including rifampicin, minocycline, amikacin, and clindamycin may be used empirically in patients with furunculosis, cutaneous abscess, and carbuncles. Linezolid, teicoplanin, and vancomycin should be reserved for severe infections.

Key Words: Uncomplicated skin and soft tissue infections, Community-associated Methicillin-resistant *staphylococcus aureus* (CA-MRSA), Antibiotic susceptibility pattern.

How to cite this article: Habib A, Qadir A. Frequency and Antibiotic Susceptibility Pattern of Community-associated Methicillin-resistant *Staphylococcus Aureus* (CA-MRSA) in Uncomplicated Skin and Soft Tissue Infections. *J Coll Physicians Surg Pak* 2022; **32(11)**:1398-1403.

INTRODUCTION

Skin and soft tissue infections (SSTIs) are the second largest group of skin disorders reported in approximately 28 to 32% of patients attending the Dermatology outpatient department, out of which bacterial infections account for approximately 5.7% to 8.4%.¹

SSTIs are classified as simple (uncomplicated) or complicated (necrotizing). Simple or uncomplicated SSTIs are confined to the skin and underlying superficial soft tissues. Common simple SSTIs include cellulitis, erysipelas, impetigo, ecthyma, folliculitis, furuncles, carbuncles, and abscesses.²⁻⁴

The infections are further grouped as either nonpurulent or purulent. Nonpurulent infections include impetigo, ecthyma, folliculitis, erysipelas, and cellulitis (in the absence of drainable abscess). Purulent infections include furuncle, carbuncles, abscesses and cellulitis (associated with drainable abscesses).²⁻⁴

Staphylococcus aureus (*S. aureus*) is the main pathogenic species that cause skin infections.^{3,5,6} It is further classified as MSSA (Methicillin-sensitive *Staphylococcus aureus*) or MRSA

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Received: June 25, 2022; Revised: September 27, 2022;
Accepted: October 18, 2022
DOI: <https://doi.org/10.29271/jcpsp.2022.11.1398>

(Methicillin-resistant *Staphylococcus aureus*). Nearly half of isolated *S. aureus* are Methicillin-resistant *S. aureus* (MRSA).^{5,6} MRSA is a common cause of nosocomial infections.⁵ More recently, MRSA has become a common cause of infections in healthy patient populations that lack traditional risk factors for MRSA exposure.⁷ The MRSA strains responsible for these infections are found to be epidemiologically and genetically unique from nosocomial MRSA strains and are now designated as community-acquired or community-associated MRSA (CA-MRSA).^{8,9} The Center for Disease Control and Prevention (CDC) defines CA-MRSA as an MRSA strain isolated in an outpatient setting or isolated from patients within 48 h of hospital admission.⁸ CA-MRSA has emerged worldwide and its prevalence is rapidly increasing.¹⁰

Furthermore, it has been reported that there is a correlation between the types of *S. aureus* strains and the kinds of skin infections.¹¹ The resistance to various antibiotics is more frequent amongst organisms isolated from furuncle than those isolated from impetigo.¹¹

Knowledge of antimicrobial susceptibility patterns of common pathogens is important for clinicians and helps in the selection of empirical antibiotic therapy.¹²

The frequency and antibiotic susceptibility pattern of CA-MRSA have been studied in different populations,^{3,12-18} but there is a paucity of such studies in the Pakistani population.^{3,11,19-21}

This paucity of studies has led to the injudicious use of antibiotics, which in turn produces economical loss to a developing country and also results in the emergence of highly resistant bacterial pathogens.^{3,12}

The aim of the present study was to determine the frequency and antibiotic susceptibility pattern of CA-MRSA in patients with uncomplicated bacterial skin and soft tissue infections reporting to the dermatology outpatient of a tertiary health care hospital in the Punjab province of Pakistan.

METHODOLOGY

This descriptive cross-sectional study was conducted at the Dermatology Outpatient of Combined Military Hospital, Kharian, from September 2020 to August 2021. The study was approved by the Ethical Committee of the hospital (Ref no: 17, dated 20 August 2020). The sample size was calculated by using the WHO sample size calculator. Non-probability consecutive sampling technique was used to gather the required sample size for this study.

Patients of all age groups and both genders reporting to dermatology outpatient during the study period with community-acquired common bacterial skin infections reporting for the first time for treatment were enrolled in the study after obtaining informed consent. The bacterial infections included impetigo, ecthyma, paronychia infection, erysipelas, cellulitis, folliculitis, furunculosis, and carbuncle. Only those patients with culture-positive *S. aureus* skin infections were not included in the study. The patients with prior history of healthcare exposure, i.e. use of

any topical or systemic antibiotics, hospitalisation, surgery, permanent devices, or hemodialysis during the last week were excluded from the study.

A total of 157 patients were included in the study. Skin lesions were examined by a dermatologist to diagnose the clinical type of infection. History of a visit to any medical facility or antibiotic intake during the last week was obtained from each patient. The infections were grouped as either non-purulent or purulent. Non-purulent skin and soft tissue infections included impetigo, ecthyma, folliculitis, erysipelas, and cellulitis (in the absence of drainable abscess). Purulent infections included acute paronychia, furuncle, carbuncles, abscess, and cellulitis (associated with drainable abscess).

Samples were collected from the skin lesions from patients included in the study using sterile swabs. The samples were cultured on blood agar and MacConkey agar plates and were incubated at 35°C + 2°C aerobically for 24-48 hours. The isolates of *S. aureus* were identified using colony morphology, gram staining, and biochemical testing including catalase, coagulase, and DNase tests. Antimicrobial susceptibility testing using the Modified Kirby Baur disc diffusion technique was applied using CLSI-M100, 30th Ed, 2020 guidelines.²² Antimicrobial discs (Oxoid, UK) for Penicillin (10 units), Amikacin (30ug), amoxycillin-clavulanate (20/10ug), ceftazidime (30ug), ciprofloxacin (5ug), clindamycin (2ug), doxycycline (30ug), erythromycin (15ug), fusidic acid (10ug), gentamicin (10ug), linezolid (30ug), meropenem (10ug), minocycline (30ug), piperacillin-tazobactam (100/10ug), rifampicin (5ug), teicoplanin (30ug), and trimethoprim-sulfamethoxazole (1.25/23.75ug) were applied. For vancomycin susceptibility, Minimum inhibitory concentrations (MICs) using E-strip were performed. MIC of <2 µg/mL was detected as vancomycin sensitive. For the detection of MRSA, ceftazidime (30ug) disc (as a surrogate test for oxacillin) was applied. A zone size of <21 mm was detected as resistant (MRSA) and >22 mm as sensitive to methicillin/methicillin/oxacillin. For further confirmation, MICs using oxacillin E strip (oxoid, UK) were used. MIC of <2 µg/mL was detected as sensitive (MSSA) and MIC of >4 µg/mL was detected as resistant to methicillin/oxacillin (MRSA). Methicillin/oxacillin susceptible isolates were also considered susceptible to Beta-lactam combinations, 1st, 2nd, 3rd & 4th generation cephalosporins and carbapenems as per CLSI guidelines. *S. aureus* ATCC 29213 was used as a control strain.

Data collected were analysed by SPSS version 16.0 and descriptive statistics (mean, percentages, and frequency distribution) were used to evaluate the results. Pearson's chi-square (χ^2) was used to evaluate the relationship between antimicrobial resistance and specific variables. The p-value <0.05 was considered statistically significant.

RESULTS

A total of 157 patients were included in the study. There were 114 (72.6%) male patients and 43 (27.4%) female patients. The male-to-female ratio was 2.65:1. The mean age of the patients was 30.30±15.181 years with a range of 1 to 74 years. The majority of the patients (n = 97, 61.78%) were between the age of 20 to 40 years (Table I).

Table I: Distribution of bacterial skin infections with age.

Age group (Years)	Type of infection							Total
	Non purulent infections Erysipelas/ Cellulitis	Ecthyma	Folliculitis	Impetigo	Purulent infections Abscess	Carbuncle	Acute Paronychia	
<10	1 (12.5%)	0	1 (33.3%)	9 (11.2%)	0	0	0	14 (8.9%)
>10-20	0	1 (14.3%)	0	7 (8.8%)	1 (11.1%)	0	0	20 (12.7%)
>20-30	4 (50%)	4 (57.1%)	1 (33.3%)	29 (36.2%)	3 (33.3%)	0	2 (100%)	55 (35.0%)
>30-40	3 (37.5%)	0	1 (33.3%)	24 (30%)	2 (22.2%)	1 (100%)	0	42 (26.8%)
>40-50	0	1 (14.3%)	0	4 (5%)	3 (33.3%)	0	0	12 (7.6%)
>50-60	0	1 (14.3%)	0	3 (3.8%)	0	0	0	6 (3.8%)
>60-70	0	0	0	2 (2.5%)	0	0	0	5 (3.2%)
>70	0	0	0	2 (2.5%)	0	0	0	3 (1.9%)
Total	8 (5.1%)	7 (4.5%)	3 (1.9%)	80 (51%)	9 (5.7%)	1 (0.6%)	2 (1.3%)	157

Table II: Sensitivity pattern of Isolates from Non-purulent versus purulent infections.

Type of infection		Frequency	Frequency of Isolates		p-value for total of purulent vs total of non-purulent infections
			MSSA	MRSA	
Non-purulent bacterial infections	Ecthyma	7 (7.1%)	3 (42.9%)	4 (57.1%)	0.067
	Erysipelas/Cellulitis	8 (8.2%)	2 (25%)	6 (75%)	
	Folliculitis	3 (3.1%)	1 (33.3%)	2 (66.7%)	
	Impetigo	80 (81.6%)	44 (55%)	36 (45%)	
	Total	98 (62.42%)	50 (51%)	48 (49%)	
Purulent bacterial infections	Abscess	9 (15.3%)	4 (44.4%)	5 (55.6%)	
	Acute Paronychia	2 (3.4%)	2 (100%)	0	
	Carbuncle	1 (1.7%)	0	1 (100%)	
	Furuncle	47 (79.7%)	16 (34%)	31 (66%)	
	Total	59 (37.58%)	22 (37.3%)	37 (62.7%)	
uSSTIs	Grand Total	157	72 (45.9%)	85 (54.1%)	

Table III: Antibiotic susceptibility pattern of *S. aureus* isolates.

Antibiotic	MSSA			MRSA			Total		
	S [*]	R [*]	I [†]	S [*]	R [*]	I [†]	S [*]	R [*]	I [†]
Amikacin	72 (100%)	0	-	76 (89.4%)	3 (3.5%)	6 (7.1%)	148 (94.3%)	3 (1.9%)	6 (3.8%)
Ampicillin	7 (9.7%)	65 (90.3%)	-	0	85 (100%)	-	7 (4.5%)	150 (95.5%)	-
Amoxycillin-clavulanate	72 (100%)	0	-	0	85 (100%)	-	72 (45.9%)	85 (54.1%)	-
Clindamycin	63 (87.5%)	9 (12.5%)	-	76 (89.4%)	9 (10.6%)	-	139 (88.5%)	18 (11.5%)	-
Ciprofloxacin	49 (68%)	22 (30.4%)	1 (1.39%)	17 (20%)	68 (80%)	0	66 (42.04%)	90 (57.3%)	1 (0.64%)
Doxycycline	54 (75%)	17 (23.6%)	1 (1.39%)	54 (63.5%)	31 (36.5%)	-	108 (68.8%)	48 (30.5)	1 (0.6%)
Erythromycin	53 (73.6%)	18 (25%)	1 (1.39%)	26 (30.6%)	52 (61.2%)	7 (8.2%)	75 (50.3%)	70 (44.6%)	8 (5.1%)
Co-trimoxazole	59 (81.9%)	12 (16.7%)	1 (1.4%)	50 (58.8%)	32 (37.6%)	3 (3.5%)	109 (69.4%)	44 (28%)	4 (2.5%)
Fusidic Acid	56 (77.8%)	16 (22.2%)	-	62 (72.9%)	23 (27.1%)	-	118 (75.2%)	39 (24.1%)	-
Gentamycin	70 (97.2%)	1 (1.4%)	1 (1.4%)	58 (68.2%)	26 (30.6%)	1 (1.2%)	128 (81.5%)	27 (17.2%)	2 (1.3%)
Linezolid	72 (100%)	0	-	85 (100%)	0	-	157 (100%)	0	-
Minocycline	72 (100%)	0	-	72 (84.7%)	13 (15.3%)	-	13 (8.3%)	144 (91.7%)	-
Rifampicin	71 (98.6%)	1 (1.4%)	-	83 (97.6%)	2 (2.4%)	-	154 (98.1%)	3 (1.9%)	-
Sulbactam-Cefoperazone	72 (100%)	0	-	2 (2.4%)	83 (97.6%)	-	74 (47.1%)	83 (52.9%)	-
Teicoplanin	72 (100%)	0	-	85 (100%)	0	-	157 (100%)	0	-
Vancomycin	72 (100%)	0	-	85 (100%)	0	-	157 (100%)	0	-

S^{*} = Sensitive, R^{*} = Resistant, I[†] = Intermediate.

The number of patients with non-purulent infections was 98 (62.42%) and the number of patients with purulent infections was 59 (37.58%). Impetigo was the most common infection, followed by furunculosis, abscess, erysipelas/ cellulitis, ecthyma, folliculitis, acute paronychia, and carbuncle in descending order of frequency (Figure 1).

Overall, MRSA caused 54.1% (n=85) of infections. MRSA was isolated more frequently in patients with purulent infections, p-value was 0.067 (>0.05) (Table II). This means that there is no significant difference in the frequency of MRSA in isolates from purulent infections and non-purulent infections. In case of furuncles/carbuncles and cutaneous abscess frequency of MRSA isolates was 64.9% (n=37). This was significantly more when compared with the frequency of MRSA isolates in patients with impetigo (Table II). The p-value was 0.016 (<0.05).

Antibiotic susceptibility pattern of *S. aureus* isolates to linezolid, teicoplanin, vancomycin, rifampicin, minocycline, fusidic acid, amikacin, clindamycin, doxycycline, co-trimoxazole, and ciprofloxacin is shown in Table III. Of particular interest was very high resistance to ciprofloxacin amongst MRSA isolates (80%).

All the methicillin / oxacillin susceptible isolates (MSSA) were also considered susceptible to beta-lactam combinations, first to fourth generation cephalosporins and carbapenems. All the MSSA isolates were susceptible to amikacin, amoxycillin-clavulanate, cephadrine, ceftazidime, linezolid, minocycline, vancomycin, and teicoplanin. Majority of MSSA were sensitive to rifampicin, gentamycin, clindamycin, and co-trimoxazole (Table III). Resistance amongst MSSA to ampicillin was found in 90.3% of isolates. Resistance amongst MSSA to fusidic acid, erythromycin, and ciprofloxacin was found in 22.2%, 25% and 30% of isolates respectively (Table III).

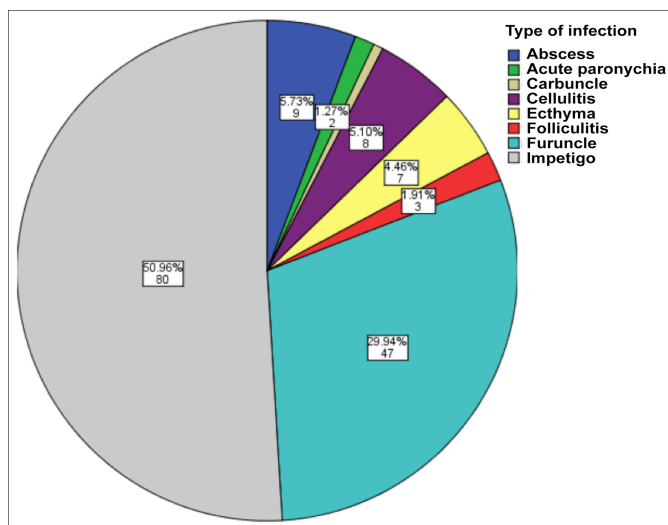


Figure 1: Frequency of Bacterial skin infections

DISCUSSION

S. aureus, the main pathogenic organism causing skin infections is notorious for its remarkable ability to develop antibiotic resistance rapidly.^{3,4,23,24} Methicillin-resistant *S. aureus* (MRSA) is a strain of *S. aureus* that has acquired resistance to β -lactam antibiotics. Methicillin resistance in MRSA develops as a result of alterations in penicillin-binding proteins (PBPs). Genes on the bacterial chromosome called *mecA* genes encode these altered PBPs and the resistance can be transferred on a mobile genetic element. Initially, infections due to MRSA occurred as outbreaks in hospital settings but over the next two to three decades Hospital-acquired MRSA (HA-MRSA) clones spread worldwide.^{3,4,23,24} Currently, more than half of the hospital isolates are methicillin-resistant in the United States.³

By the last decade of the twentieth century, reports of community outbreaks of skin and soft tissue infections (SSTIs) due to MRSA among otherwise healthy individuals with no hospital exposure began appearing. These community-acquired or more precisely community-associated methicillin-resistant *S. aureus* (CA-MRSA) strains were subsequently found to be genetically and phenotypically distinct strains from HA-MRSA. These strains rapidly spread worldwide first in the community and later in healthcare facilities.^{8,9} The most frequent disease manifestation associated with CA-MRSA is an infection of the skin and soft tissues.⁸ CA-MRSA SSTI often occur in otherwise healthy individuals.³ In general, CA-MRSA is more virulent compared to HA-MRSA due to the presence of various virulence factors.^{8,10}

CA-MRSA isolates frequently have a different antibiotic susceptibility profile than HA-MRSA but local patterns may be quite variable.^{3-4,8-10,24} Recognising infections caused by resistant pathogens can guide the appropriate selection of antibiotic therapy.³

The present study was designed to determine the frequency of uncomplicated SSTIs in patients reporting to Dermatology outdoor and to know the frequency of Methicillin-resistant *S. aureus* (MRSA) and Methicillin-sensitive *S. aureus* (MSSA) in uncomplicated skin soft tissue infections and their antibiotic sensitivity to different antibiotics.

The frequency of different purulent and non-purulent infections, was similar to Hanif *et al.* who reported impetigo/ecthyma in 60.7% of their patients, folliculitis/furunculosis in 28.2%, and cellulitis in 11.1%.¹² The present findings were little different from Mir *et al.* who reported furunculosis (45.7%) to be the most common skin infection in their patients followed by impetigo 22.3%, cellulitis 15.0%, ecthyma 8.5%, and erysipelas.⁴

Previously, Hanif *et al.* reported methicillin resistance in 8.3% of *S. aureus* isolated from patients with skin soft tissue infections,¹² and Mir *et al.* reported it in 30.9% of their isolates.⁴ Forcade *et al.* reported methicillin resistance in 61% of their isolates.¹⁵ The present study found much higher frequency of MRSA (54.1%). Therefore, it can be assumed that the incidence of skin infections caused by CA-MRSA is increasing in local population. Similar findings have been reported previously.^{9,15}

The authors compared the frequency of MRSA isolation in patients with non-purulent infections (49%) with the frequency of MRSA isolation in patients with purulent infections (62.7%) without statistical significance. Although the frequency of MRSA isolates was higher, the difference was not significant (Table II). This means that there is no significant difference in the frequency of MRSA in isolates from purulent infections and non-purulent infections.

The frequency of MRSA isolates in patients with furuncles/carbuncles and abscesses (n=37, 64.9%) was higher than the frequency of MRSA isolates in patients with impetigo (n=36, 45%, p=0.016), the difference was significant. Previously, Dekio *et al.* reported that the frequency of MRSA in isolates from furuncles was significantly more likely as compared to isolates from Impetigo. The findings of this study were similar to those of Dekio *et al.*¹¹

Resistance amongst MSSA isolates to ampicillin was found in 90.3% of isolates. All the MSSA isolates were susceptible to amikacin, amoxicillin-clavulanate, linezolid, minocycline, Vancomycin, and teicoplanin. Seventy-one (98.6%) of MSSA were sensitive to Rifampicin, 97.2% were sensitive to gentamycin, 87.5% were sensitive to clindamycin, and 81.9% were sensitive to co-trimoxazole. Resistance amongst MSSA to fusidic acid, erythromycin, and ciprofloxacin was found in 22.2%, 26.4%, and 32% of isolates respectively (Table III).

Previously it was reported that all CA-MRSA and CA-MSSA isolates were susceptible to linezolid, teicoplanin, and

vancomycin.¹⁴⁻¹⁸ The present findings were in concordance with previous studies.¹⁴⁻¹⁸

Community-acquired MRSA isolates are often susceptible to several non-beta-lactam drug classes including trimethoprim-sulfamethoxazole, clindamycin, doxycycline, or minocycline, and fluoroquinolones.^{5,7-9,17} Similar findings were observed in these patients (Table III). All the isolates (CA-MRSA and CA-MSSA) were 100% susceptible to linezolid, teicoplanin, and vancomycin. This was in concordance with previous studies.¹⁴⁻¹⁸ However, these antibiotics are relatively costly. Patients with uSSTIs are usually treated in the outdoor which makes the use of vancomycin impractical. Therefore, the utility of these agents in patients with uSSTIs is limited.

The sensitivity of CA-MRSA to rifampicin and minocycline in the studied patients were similar to previous reports.^{5,7-9,11,12,15-19} However, the resistance to fusidic acid was high as compared to previous reports.^{4,12} Mir et al. reported 79.8% sensitivity to Fusidic acids amongst MRSA isolates.⁴ Hanif et al. reported 100% susceptibility to vancomycin and fusidic acid.¹² 63.5% of isolates were sensitive to doxycycline which is less as compared to previous reports. Similarly, the frequency of resistance to co-trimoxazole and ciprofloxacin (Table III) was very high as compared to previous studies.^{5,7-9,11,12,15-19} Resistance to fusidic acid, doxycycline, co-trimoxazole, and ciprofloxacin has markedly increased. In the case of fusidic acid, this may be due to the widespread use of its topical formulation. Similarly, doxycycline, co-trimoxazole, and ciprofloxacin are very widely used in general practice in Pakistan.

Based on the susceptibility pattern of MRSA isolates, rifampicin, minocycline, amikacin, and clindamycin may be used empirically whenever infection with CA-MRSA is suspected as in the case of furunculosis, carbuncles, and cutaneous abscess. Beta-lactam antibiotics are to be used empirically only when infection with MSSA is suspected as in the case of impetigo and ecthyma.

The study has clearly pointed out the frequency and antibiotic susceptibility pattern of CA-MRSA in patients with uSSTIs. A limited number of patients from a single-centre were recruited in the study which has been the main limitation of this study.

CONCLUSION

Impetigo and furunculosis are commonly reported uncomplicated skin and soft tissue infections. The frequency of CA-MRSA isolates is significantly more frequent in cases of furunculosis, carbuncles, and cutaneous abscesses. Therefore, antibiotics active against CA-MRSA which include rifampicin, minocycline, amikacin, and clindamycin may be used empirically in patients with these infections. Linezolid, teicoplanin, and vancomycin should be reserved for severe infections.

ETHICAL APPROVAL:

The study was approved by the Ethical Committee of the hospital (Ref no: 17, dated 20 Aug 2020).

PATIENTS' CONSENT:

Written informed consent were obtained from all the patients.

COMPETING INTEREST:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The authors have no conflicts of interest to declare that are relevant to the content of this article.

AUTHORS' CONTRIBUTION:

AH: Corresponding author, conceptualisation, methodology, data curation, validation, investigation, visualisation, Writing-original draft, writing, reviewing, and editing.

AQ: Data curation, visualisation, investigation, writing, reviewing, and editing.

Both authors have approved the final version of the manuscript to be published.

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