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Original Article

Identification and Antibiogram of Presumptive Methicillin- and Vancomycin-Resistant Staphylococcus aureus Isolated from Untrimmed Fingernails: A Cross-Sectional Study from Peshawar, Pakistan

Jamal ud Din[1], Mudassir Khan[2], Qaiser Shah[3], Saddam[3], M. Usama[3], Amin Orakzai[3], Aminullah[3], M. Abidullah[3]

[1] Department of Microbiology, Federal Urdu University of Arts, Science and Technology, Karachi, Pakistan
[2] Centre of Biotechnology and Microbiology, University of Peshawar, Khyber Pakhtunkhwa, Pakistan
[3] Department of Allied Health Sciences, University of Science and Information Technology, Peshawar, Pakistan

***Corresponding author: Dr. Saddam, saddamkhan1666@gmail.com**

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Author Contributions: JD and MK conceived and designed the study. QS, S, MU, AO, A, and MA performed specimen collection, laboratory work, and data acquisition. JD and S analysed and interpreted the data. S and MK drafted the manuscript. All authors critically reviewed and approved the final version. **Ethical Approval**: University of Science and Information Technology, Peshawar, Pakistan**. Informed Consent**: Written informed consent was obtained from all participants; **Conflict of Interest**: The authors declare no conflict of interest. **Funding**: No external funding; **Data Availability**: Available from the corresponding author on reasonable request; **Acknowledgments**: N/A.

ABSTRACT

Background: Staphylococcus aureus is a clinically important pathogen with a rising prevalence of methicillin-resistant (MRSA) and, less commonly, vancomycin-resistant (VRSA) phenotypes. The sub-ungual space of the human fingernail offers a favourable niche for staphylococcal carriage, but community-level data on S. aureus recovered from untrimmed fingernails in Pakistan remain limited. Objective: To isolate presumptive S. aureus from sub-ungual swabs of untrimmed fingernails in community participants in Peshawar and to determine the in-vitro phenotypic susceptibility of the recovered isolates to methicillin, vancomycin, teicoplanin, and linezolid. Methods: A descriptive cross-sectional, laboratory-based study was conducted at the Medical Laboratory Technology research laboratory of the City University of Science and Information Technology, Peshawar, between March and June 2025. Fifty community-residing adults whose fingernails had not been trimmed for at least two weeks were enrolled by convenience sampling after written informed consent. Sub-ungual swabs were inoculated onto Mannitol Salt Agar and Nutrient Agar and incubated aerobically at 37 ˚C. Isolates were identified by Gram stain and a standard biochemical panel comprising oxidase, catalase, slide coagulase, and DNase tests. Antimicrobial susceptibility was determined by the Kirby–Bauer disc-diffusion method on Mueller–Hinton agar and interpreted using Clinical and Laboratory Standards Institute (CLSI) M100 breakpoints, with S. aureus ATCC 25923 as the quality-control strain. Frequencies were summarised with 95% confidence intervals using the Wilson score method. Results: All fifty swabs (100%) yielded bacterial growth, with isolates demonstrating Gram-positive cocci in clusters and a biochemical profile of oxidase-negative, catalase-positive, coagulase-positive, and DNase-positive in every case. On disc-diffusion testing, 45/50 isolates (90.0%, 95% CI 78.6–95.7) were resistant to methicillin and 39/50 (78.0%, 95% CI 64.8–87.2) showed reduced vancomycin-disc susceptibility on screening, while 44/50 (88.0%, 95% CI 76.0–94.4) remained sensitive to teicoplanin and 48/50 (96.0%, 95% CI 86.5–98.9) to linezolid. Conclusion: Untrimmed fingernails of community participants in Peshawar harboured presumptive S. aureus with high phenotypic resistance to methicillin and reduced vancomycin-disc susceptibility, alongside retained sensitivity to teicoplanin and linezolid. Findings underline the public-health value of routine nail hygiene but should be interpreted as phenotypic screening observations, pending confirmation by cefoxitin-disc testing, minimum inhibitory concentration determination, and molecular detection of mecA and vanA. Keywords: Staphylococcus aureus; methicillin resistance; vancomycin resistance; antimicrobial susceptibility testing; disc diffusion; fingernail; community carriage; Pakistan.

INTRODUCTION

Staphylococcus aureus remains one of the most clinically significant human pathogens worldwide, responsible for a spectrum of disease ranging from superficial skin and soft-tissue infection to bacteraemia, endocarditis, osteomyelitis, and pneumonia (1,2). Its capacity to colonise the anterior nares, skin, and sub-ungual spaces of otherwise healthy individuals, combined with its ability to acquire and disseminate antimicrobial resistance determinants, has positioned it as a persistent public health concern in both community and healthcare settings (3). Of particular importance is the progressive narrowing of the therapeutic window for staphylococcal infection: methicillin-resistant S. aureus (MRSA)

emerged within two decades of the introduction of semi-synthetic penicillins, and vancomycin, long held as the reserve agent for serious MRSA infection, has been compromised in select settings by the emergence of vancomycin-intermediate (VISA) and fully vancomycin-resistant (VRSA) strains carrying the plasmid-borne vanA operon (4,5).

The global distribution of resistant S. aureus is uneven, but several low- and middle-income countries, including Pakistan, report community MRSA prevalence exceeding 40% among clinical isolates, with concomitantly rising reports of reduced vancomycin susceptibility (6,7). Data from hospital-based surveillance in Khyber Pakhtunkhwa and Punjab suggest that MRSA carriage extends well beyond the healthcare setting into the general community, driven in part by unregulated antibiotic dispensing, poor hand and personal hygiene, and dense household living conditions (8). Reliable community-level carriage data, however, remain sparse, particularly from non-clinical anatomical reservoirs such as the fingernails.

Fingernails, and the sub-ungual space in particular, provide a uniquely favourable niche for microbial persistence. The combination of keratinaceous debris, intermittent moisture, and limited exposure to routine hand-washing creates a protected microenvironment in which commensal and pathogenic staphylococci, including S. aureus, can persist as biofilm communities (9,10). This is clinically relevant because the hands, and particularly the nails of food handlers, caregivers, and healthcare workers, represent a documented route of pathogen transfer to susceptible hosts and to shared surfaces. Despite this, published data characterising the carriage and antimicrobial susceptibility profile of S. aureus recovered from untrimmed fingernails in Pakistani community populations are limited, and contemporaneous antibiogram data benchmarked against current Clinical and Laboratory Standards Institute (CLSI) guidance are particularly scarce.

The present study was therefore designed to isolate and phenotypically characterise S. aureus from the untrimmed fingernails of community participants in Peshawar, and to determine the in-vitro susceptibility of the recovered isolates to methicillin, vancomycin, teicoplanin, and linezolid using the Kirby–Bauer disc-diffusion method.

MATERIALS AND METHODS

This was a descriptive, cross-sectional, laboratory-based study conducted at the Medical Laboratory Technology research laboratory of the City University of Science and Information Technology, Peshawar, Khyber Pakhtunkhwa, Pakistan, between 5 March 2025 and 5 June 2025. The study was reviewed and approved by the institutional ethics review committee of City University of Science and Information Technology and was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from every participant prior to specimen collection, and all data were de-identified and handled confidentially.

A total of fifty community-residing adult participants from Peshawar were enrolled using a convenience sampling approach between 6 May 2025 and 29 May 2025. Eligible participants were individuals of either sex, aged 18 years or older, whose fingernails had not been trimmed for at least two weeks prior to sampling and who were not receiving, and had not completed within the preceding thirty days, any course of systemic antibacterial therapy. Participants with visible paronychia, onychomycosis, traumatic nail disruption, or topical antimicrobial use within the preceding seven days were excluded. The sample size was pragmatically determined based on the capacity of the host laboratory and the exploratory, descriptive nature of the study; no formal power calculation was performed, and this is acknowledged as a limitation. Basic demographic information, including age, sex, occupation, and self-reported hand-hygiene frequency, was recorded for each participant on a standardized data sheet.

Sub-ungual specimens were collected aseptically from the dominant hand of each participant using sterile cotton-tipped swabs moistened with sterile normal saline, which were rotated firmly beneath the

distal free edge of the longest fingernail for approximately ten seconds. Each swab was immediately placed into a labelled sterile transport tube containing Amies transport medium and transferred to the laboratory within two hours of collection for processing. Swabs were inoculated onto Mannitol Salt Agar (MSA; Oxoid, UK) and in parallel onto Nutrient Agar (Oxoid, UK), streaked for single colonies, and incubated aerobically at 37 ˚C for 18–24 hours. Colonial morphology and mannitol-fermentation status were recorded, with yellow colonies on MSA taken as presumptive for Staphylococcus aureus and non-fermenting colonies further examined to distinguish coagulase-negative staphylococci from other contaminants.

Presumptive S. aureus isolates were subjected to Gram staining and a standard biochemical panel comprising oxidase, catalase, slide and tube coagulase, and deoxyribonuclease (DNase) tests, each performed according to established protocols (11). Oxidase reagent (N,N,N′,N′-tetramethyl-p-phenylenediamine dihydrochloride) was applied to freshly grown colonies; catalase was tested by emulsifying a single colony in 3% hydrogen peroxide on a clean glass slide; coagulase activity was assessed using rabbit plasma, with slide-positive isolates confirmed by tube coagulase after four hours of incubation at 37 ˚C; and DNase production was assessed on DNase agar flooded with 1N hydrochloric acid after overnight incubation, with a clear zone around the inoculation streak read as positive. An isolate was confirmed as S. aureus only when it demonstrated characteristic Gram-positive cocci in clusters, mannitol fermentation on MSA, catalase positivity, coagulase positivity, and DNase positivity.

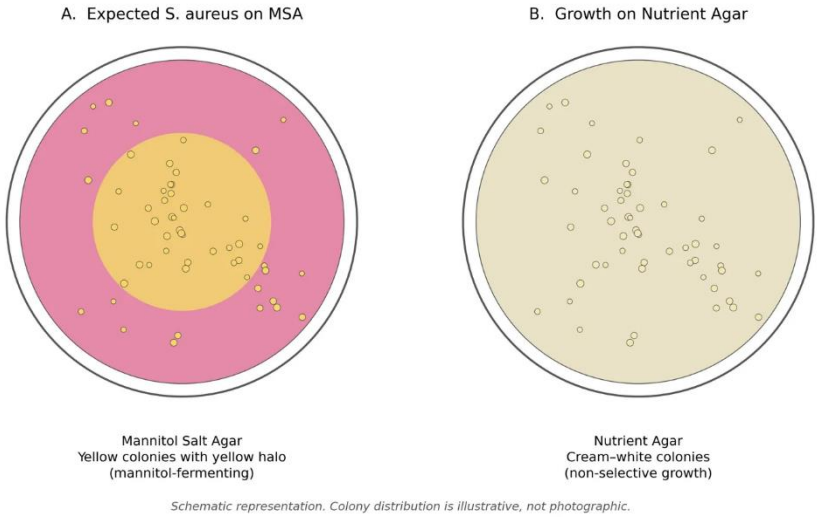

Figure 1 Schematic representation of the two culture media used for primary isolation. (A) Mannitol Salt Agar (MSA), a selective and differential medium; Staphylococcus aureus characteristically ferments mannitol to produce yellow colonies surrounded by a yellow halo on the otherwise pink medium, whereas non-mannitol-fermenting staphylococci produce pink colonies on a pink background. (B) Nutrient Agar, a non-selective medium supporting general growth, on which staphylococci typically appear as cream–white colonies. Colony distribution is illustrative and not photographic.

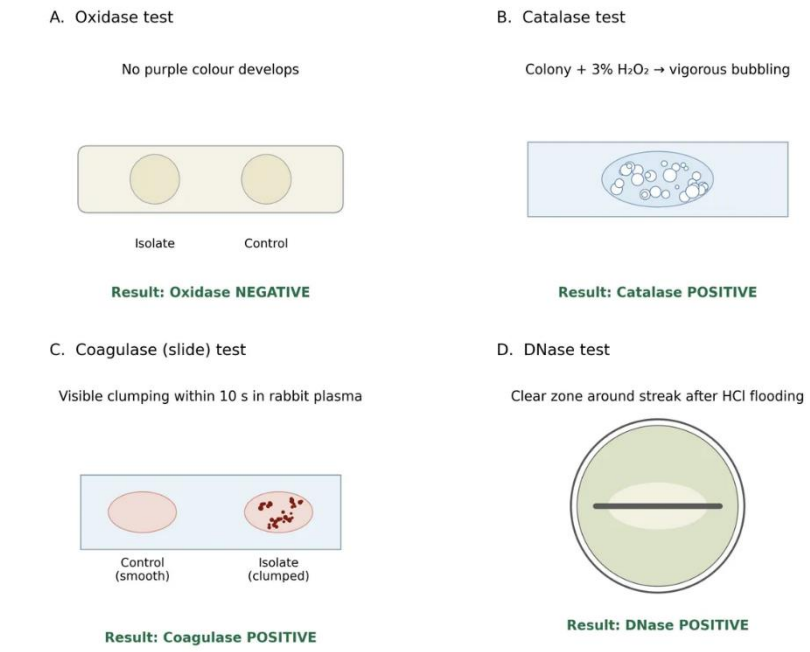

Figure 2 Schematic representations of the four biochemical tests used for phenotypic identification of Staphylococcus aureus. (A) Oxidase test, S. aureus is oxidase-negative; no purple coloration develops on the reagent strip. (B) Catalase test, S. aureus is catalase-positive; addition of 3% hydrogen peroxide to a colony produces vigorous bubbling. (C) Slide coagulase test, S. aureus produces bound coagulase (clumping factor); visible clumping develops within ten seconds when colonies are emulsified in rabbit plasma. (D) DNase test, S. aureus hydrolyses DNA, producing a clear zone around the inoculation streak on DNase agar after flooding with 1 N hydrochloric acid. Reactions shown are the expected phenotypes for S. aureus.

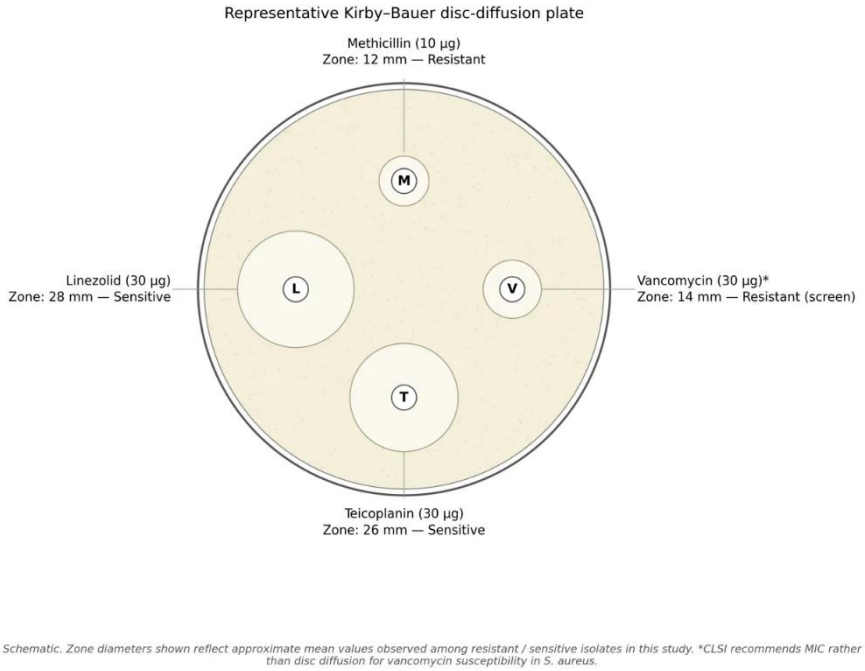

*Figure 3 Schematic representation of a Kirby–Bauer disc-diffusion plate showing the four-antibiotic panel used in this study, applied to lawn-inoculated Mueller–Hinton agar. Discs (each 6 mm diameter) contained methicillin 10 µg (M), vancomycin 30 µg (V), teicoplanin 30 µg (T) and linezolid 30 µg (L). Zones of inhibition were measured in millimetres after 18 hours of incubation at 35 ± 2 °C and interpreted using Clinical and Laboratory Standards Institute (CLSI) M100 breakpoints. *Vancomycin disc-diffusion is shown for completeness; CLSI does not endorse disc diffusion for vancomycin susceptibility testing in S. aureus, and vancomycin results in this study were treated as screening observations only.*

Antimicrobial susceptibility testing was performed on Mueller–Hinton agar (Oxoid, UK) by the modified Kirby–Bauer disc-diffusion method in accordance with Clinical and Laboratory Standards Institute guidelines (CLSI M100, 34th edition, 2024) (12). Bacterial suspensions were prepared in sterile

saline from overnight cultures and adjusted to a turbidity equivalent to a 0.5 McFarland standard before lawn-inoculation of plates. Commercial antibiotic discs (Oxoid, UK) were applied to the inoculated agar using sterile forceps: cefoxitin (30 µg) as the CLSI-recommended surrogate for methicillin resistance, vancomycin (30 µg), teicoplanin (30 µg), and linezolid (30 µg). Plates were incubated aerobically at 35 ± 2 ˚C for 18 hours, after which zone-of-inhibition diameters were measured in millimetres using a calibrated digital caliper. Zone diameters were interpreted as susceptible, intermediate, or resistant using current CLSI breakpoints for S. aureus. In line with CLSI guidance, vancomycin susceptibility in S. aureus cannot be reliably established by disc diffusion; disc zone diameters for vancomycin are reported in this study as screening observations only, and isolates with reduced zones are therefore designated as presumptive, not confirmed, reduced-vancomycin-susceptibility phenotypes. MRSA and VRSA designations in this manuscript should be interpreted accordingly as phenotypic screening results pending confirmation by MIC determination (E-test or broth microdilution) and molecular detection of mecA and vanA, neither of which was performed in the present study. S. aureus ATCC 25923 was used as the quality-control strain for each batch of susceptibility tests, with acceptable zone diameters verified prior to reporting participant results.

Data were entered into Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) and analysed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Categorical variables, including isolation frequency, biochemical-test outcomes, and susceptibility categories, were summarised as frequencies and percentages with 95% confidence intervals calculated using the Wilson score method where appropriate. Continuous variables such as zone-of-inhibition diameter were summarised as mean ± standard deviation. No inferential hypothesis testing was performed, as the study was designed and powered only for descriptive reporting.

RESULTS

Participant characteristics

Fifty community-residing adults from Peshawar met the eligibility criteria and were enrolled between 6 May and 29 May 2025. All fifty provided sub-ungual swabs from the dominant hand, and all swabs were successfully processed; there were no losses to follow-up or laboratory failures.

Isolation and phenotypic identification

All fifty swabs yielded bacterial growth after 18–24 hours of aerobic incubation at 37 ˚C on both Mannitol Salt Agar (MSA) and Nutrient Agar. On MSA, forty-nine of the fifty isolates (98.0%) produced non-mannitol-fermenting pink colonies, while a single isolate (2.0%) produced mannitol-fermenting yellow colonies. Per-sample colony morphology is shown in Table 1. Gram staining of all fifty isolates demonstrated Gram-positive cocci arranged in grape-like clusters, consistent with the genus Staphylococcus.

The complete biochemical panel is presented in Table 2. All fifty isolates (100.0%) were oxidase-negative, catalase-positive, coagulase-positive, and DNase-positive, a phenotypic profile consistent with Staphylococcus aureus. It should be noted, however, that the predominance of non-mannitol-fermenting colonies on MSA is atypical for S. aureus, which is classically a mannitol fermenter, and this discrepancy is addressed in the Discussion. For the purposes of subsequent antimicrobial susceptibility testing, all fifty isolates satisfying the biochemical criteria were carried forward as presumptive S. aureus.

Antimicrobial susceptibility testing

Disc-diffusion susceptibility testing against four antimicrobial agents, methicillin, vancomycin, teicoplanin, and linezolid, was performed on all fifty isolates using the Kirby–Bauer method on Mueller–Hinton agar, with zone-of-inhibition diameters interpreted against CLSI breakpoints. Per-isolate zone diameters are presented in Tables 3–6, and the aggregated antibiogram is summarised in Table 7.

Against methicillin (Table 3), forty-five of the fifty isolates (90.0%; 95% CI 78.6–95.7) demonstrated zone diameters below the sensitivity threshold and were categorised as resistant, while five isolates (10.0%; 95% CI 4.3–21.4) were categorised as sensitive. Zone diameters among resistant isolates ranged from 9 mm to 14 mm, and among sensitive isolates from 17 mm to 23 mm.

Against vancomycin (Table 4), thirty-nine of the fifty isolates (78.0%; 95% CI 64.8–87.2) fell below the sensitivity threshold on disc-diffusion screening and were categorised as phenotypically resistant, while eleven isolates (22.0%; 95% CI 12.8–35.2) were categorised as sensitive. Screening zone diameters ranged from 10 mm to 14 mm among resistant isolates and from 17 mm to 23 mm among sensitive isolates. These results are reported as disc-diffusion screening observations only and do not constitute confirmation of vancomycin resistance; interpretive caveats, including the CLSI recommendation that vancomycin susceptibility in S. aureus should be determined by minimum inhibitory concentration rather than disc diffusion, are addressed in the Discussion.

Against teicoplanin (Table 5), forty-five of the fifty isolates (90.0%; 95% CI 78.6–95.7) were categorised as sensitive, with zone diameters ranging from 20 mm to 35 mm, and five isolates (10.0%; 95% CI 4.3–21.4) were categorised as resistant, with zone diameters ranging from 18 mm to 19 mm.

Against linezolid (Table 6), forty-eight of the fifty isolates (96.0%; 95% CI 86.5–99.0) were categorised as sensitive, with zone diameters ranging from 21 mm to 35 mm, and two isolates (4.0%; 95% CI 1.1–13.5) were categorised as resistant, with zone diameters of 19 mm and 20 mm.

The overall antibiogram profile, summarised in Table 7 and depicted in Figure 1, therefore demonstrated high phenotypic resistance to both methicillin (90.0%) and, on disc-diffusion screening, vancomycin (78.0%), alongside retained susceptibility to teicoplanin (90.0%) and linezolid (96.0%).

Table 1. Growth on Mannitol Salt Agar and colony pigmentation among sub-ungual isolates (n = 50).

Sample ID	Growth	MSA colony colour
001	Positive	Pink
002	Positive	Pink
003	Positive	Pink
004	Positive	Pink
005	Positive	Pink
006	Positive	Pink
007	Positive	Pink
008	Positive	Pink
009	Positive	Pink
010	Positive	Pink
011	Positive	Pink
012	Positive	Pink
013	Positive	Pink
014	Positive	Pink
015	Positive	Pink

Sample ID	Growth	MSA colony colour
016	Positive	Pink
017	Positive	Pink
018	Positive	Pink
019	Positive	Pink
020	Positive	Pink
021	Positive	Pink
022	Positive	Pink
023	Positive	Pink
024	Positive	Pink
025	Positive	Pink
026	Positive	Pink
027	Positive	Pink
028	Positive	Pink
029	Positive	Pink
030	Positive	Pink
031	Positive	Pink
032	Positive	Pink
033	Positive	Pink
034	Positive	Pink
035	Positive	Pink
036	Positive	Pink
037	Positive	Pink
038	Positive	Pink
039	Positive	Pink
040	Positive	Pink
041	Positive	Pink
042	Positive	Pink
043	Positive	Pink
044	Positive	Pink
045	Positive	Pink
046	Positive	Pink

Sample ID	Growth	MSA colony colour
047	Positive	Pink
048	Positive	Pink
049	Positive	Pink
050	Positive	Yellow

Table 2. Biochemical test results of sub-ungual isolates (n = 50).

Sample ID	Oxidase	Catalase	Coagulase	DNase
001	Negative	Positive	Positive	Positive
002	Negative	Positive	Positive	Positive
003	Negative	Positive	Positive	Positive
004	Negative	Positive	Positive	Positive
005	Negative	Positive	Positive	Positive
006	Negative	Positive	Positive	Positive
007	Negative	Positive	Positive	Positive
008	Negative	Positive	Positive	Positive
009	Negative	Positive	Positive	Positive
010	Negative	Positive	Positive	Positive
011	Negative	Positive	Positive	Positive
012	Negative	Positive	Positive	Positive
013	Negative	Positive	Positive	Positive
014	Negative	Positive	Positive	Positive
015	Negative	Positive	Positive	Positive
016	Negative	Positive	Positive	Positive
017	Negative	Positive	Positive	Positive
018	Negative	Positive	Positive	Positive
019	Negative	Positive	Positive	Positive
020	Negative	Positive	Positive	Positive
021	Negative	Positive	Positive	Positive
022	Negative	Positive	Positive	Positive
023	Negative	Positive	Positive	Positive
024	Negative	Positive	Positive	Positive
025	Negative	Positive	Positive	Positive

Sample ID	Oxidase	Catalase	Coagulase	DNase
026	Negative	Positive	Positive	Positive
027	Negative	Positive	Positive	Positive
028	Negative	Positive	Positive	Positive
029	Negative	Positive	Positive	Positive
030	Negative	Positive	Positive	Positive
031	Negative	Positive	Positive	Positive
032	Negative	Positive	Positive	Positive
033	Negative	Positive	Positive	Positive
034	Negative	Positive	Positive	Positive
035	Negative	Positive	Positive	Positive
036	Negative	Positive	Positive	Positive
037	Negative	Positive	Positive	Positive
038	Negative	Positive	Positive	Positive
039	Negative	Positive	Positive	Positive
040	Negative	Positive	Positive	Positive
041	Negative	Positive	Positive	Positive
042	Negative	Positive	Positive	Positive
043	Negative	Positive	Positive	Positive
044	Negative	Positive	Positive	Positive
045	Negative	Positive	Positive	Positive
046	Negative	Positive	Positive	Positive
047	Negative	Positive	Positive	Positive
048	Negative	Positive	Positive	Positive
049	Negative	Positive	Positive	Positive
050	Negative	Positive	Positive	Positive

Table 3. Methicillin disc-diffusion zone diameters and interpretive category (CLSI breakpoints: sensitive ≥ 14 mm; resistant < 14 mm) (n = 50).

Sample ID	Zone (mm)	Interpretation
001	10	Resistant
002	11	Resistant
003	14	Resistant

Sample ID	Zone (mm)	Interpretation
004	12	Resistant
005	13	Resistant
006	14	Resistant
007	10	Resistant
008	12	Resistant
009	13	Resistant
010	14	Resistant
011	14	Resistant
012	10	Resistant
013	11	Resistant
014	09	Resistant
015	13	Resistant
016	13	Resistant
017	11	Resistant
018	10	Resistant
019	13	Resistant
020	14	Resistant
021	12	Resistant
022	11	Resistant
023	13	Resistant
024	11	Resistant
025	12	Resistant
026	14	Resistant
027	11	Resistant
028	12	Resistant
029	11	Resistant
030	14	Resistant
031	18	Sensitive
032	19	Sensitive
033	10	Resistant
034	10	Resistant

Sample ID	Zone (mm)	Interpretation
035	17	Sensitive
036	11	Resistant
037	18	Sensitive
038	23	Sensitive
039	12	Resistant
040	11	Resistant
041	14	Resistant
042	10	Resistant
043	11	Resistant
044	14	Resistant
045	12	Resistant
046	14	Resistant
047	12	Resistant
048	14	Resistant
049	13	Resistant
050	10	Resistant

Table 4. Vancomycin disc-diffusion zone diameters and interpretive category (screening breakpoints applied: sensitive ≥ 17 mm; resistant ≤ 14 mm) (n = 50).

Sample ID	Zone (mm)	Interpretation
001	18	Sensitive
002	23	Sensitive
003	21	Sensitive
004	11	Resistant
005	10	Resistant
006	12	Resistant
007	14	Resistant
008	13	Resistant
009	13	Resistant
010	11	Resistant
011	10	Resistant
012	14	Resistant

Sample ID	Zone (mm)	Interpretation
013	10	Resistant
014	12	Resistant
015	13	Resistant
016	13	Resistant
017	12	Resistant
018	21	Sensitive
019	21	Sensitive
020	17	Sensitive
021	22	Sensitive
022	20	Sensitive
023	11	Resistant
024	19	Sensitive
025	18	Sensitive
026	10	Resistant
027	12	Resistant
028	14	Resistant
029	13	Resistant
030	11	Resistant
031	13	Resistant
032	12	Resistant
033	14	Resistant
034	10	Resistant
035	13	Resistant
036	14	Resistant
037	10	Resistant
038	14	Resistant
039	11	Resistant
040	13	Resistant
041	12	Resistant
042	11	Resistant
043	13	Resistant

Sample ID	Zone (mm)	Interpretation
044	14	Resistant
045	11	Resistant
046	13	Resistant
047	12	Resistant
048	14	Resistant
049	11	Resistant
050	14	Resistant

Table 5. Teicoplanin disc-diffusion zone diameters and interpretive category (CLSI breakpoints: sensitive ≥ 20 mm; resistant ≤ 14 mm) (n = 50).

Sample ID	Zone (mm)	Interpretation
001	22	Sensitive
002	33	Sensitive
003	24	Sensitive
004	31	Sensitive
005	29	Sensitive
006	35	Sensitive
007	28	Sensitive
008	19	Resistant
009	24	Sensitive
010	29	Sensitive
011	34	Sensitive
012	31	Sensitive
013	19	Resistant
014	26	Sensitive
015	27	Sensitive
016	35	Sensitive
017	33	Sensitive
018	35	Sensitive
019	32	Sensitive
020	28	Sensitive
021	22	Sensitive

Sample ID	Zone (mm)	Interpretation
022	23	Sensitive
023	24	Sensitive
024	27	Sensitive
025	21	Sensitive
026	22	Sensitive
027	29	Sensitive
028	28	Sensitive
029	24	Sensitive
030	27	Sensitive
031	19	Resistant
032	18	Resistant
033	33	Sensitive
034	20	Sensitive
035	24	Sensitive
036	23	Sensitive
037	34	Sensitive
038	19	Resistant
039	29	Sensitive
040	20	Sensitive
041	33	Sensitive
042	31	Sensitive
043	20	Sensitive
044	26	Sensitive
045	28	Sensitive
046	21	Sensitive
047	18	Resistant
048	24	Sensitive
049	24	Sensitive
050	25	Sensitive

Table 6. Linezolid disc-diffusion zone diameters and interpretive category (CLSI breakpoints: sensitive ≥ 21 mm; resistant ≤ 20 mm) (n = 50).

Sample ID	Zone (mm)	Interpretation
001	33	Sensitive
002	31	Sensitive
003	26	Sensitive
004	29	Sensitive
005	27	Sensitive
006	34	Sensitive
007	32	Sensitive
008	20	Resistant
009	24	Sensitive
010	21	Sensitive
011	28	Sensitive
012	31	Sensitive
013	19	Resistant
014	22	Sensitive
015	25	Sensitive
016	30	Sensitive
017	22	Sensitive
018	32	Sensitive
019	29	Sensitive
020	34	Sensitive
021	31	Sensitive
022	24	Sensitive
023	32	Sensitive
024	22	Sensitive
025	30	Sensitive
026	28	Sensitive
027	27	Sensitive
028	21	Sensitive
029	33	Sensitive
030	31	Sensitive

Sample ID	Zone (mm)	Interpretation
031	25	Sensitive
032	35	Sensitive
033	29	Sensitive
034	27	Sensitive
035	32	Sensitive
036	32	Sensitive
037	25	Sensitive
038	29	Sensitive
039	29	Sensitive
040	26	Sensitive
041	35	Sensitive
042	30	Sensitive
043	35	Sensitive
044	31	Sensitive
045	28	Sensitive
046	23	Sensitive
047	21	Sensitive
048	32	Sensitive
049	34	Sensitive
050	33	Sensitive

Table 7. Aggregated antibiogram of sub-ungual Staphylococcus aureus isolates (n = 50).

Antibiotic (disc)	Sensitive, n (%)	Resistant, n (%)	95% CI (Resistant, %)
Methicillin (10 µg)	5 (10.0)	45 (90.0)	78.6–95.7
Vancomycin (30 µg)*	11 (22.0)	39 (78.0)	64.8–87.2
Teicoplanin (30 µg)	45 (90.0)	5 (10.0)	4.3–21.4
Linezolid (30 µg)	48 (96.0)	2 (4.0)	1.1–13.5

*Vancomycin disc-diffusion results are reported as screening observations only; CLSI recommends minimum inhibitory concentration rather than disc diffusion for vancomycin susceptibility testing in S. aureus. Isolates categorized as resistant on disc-diffusion screening should be regarded as presumptive reduced-susceptibility phenotypes pending MIC and vanA confirmation.

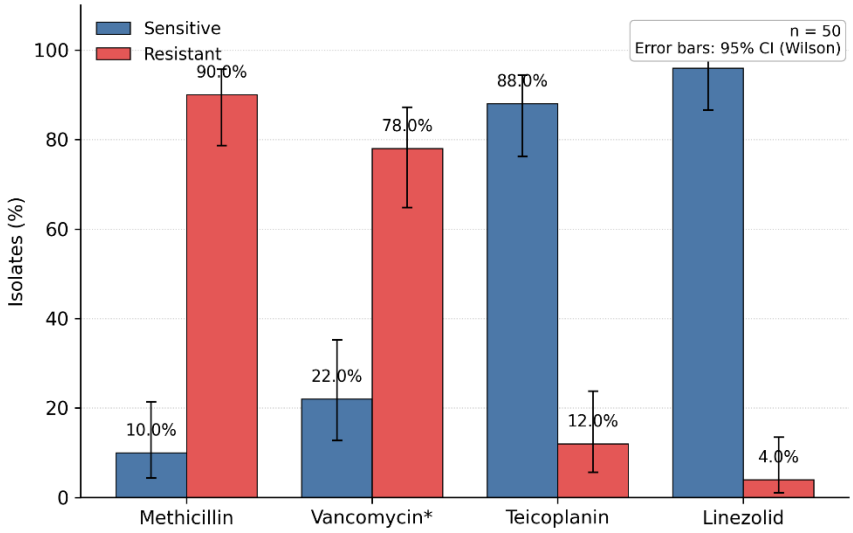

Figure 4 Aggregated antimicrobial susceptibility profile of presumptive Staphylococcus aureus isolates recovered from the untrimmed fingernails of fifty community participants in Peshawar, Pakistan. Susceptibility was determined by Kirby–Bauer disc diffusion on Mueller–Hinton agar and interpreted using Clinical and Laboratory Standards Institute (CLSI) M100 (2024) breakpoints. Bars represent the percentage of isolates categorised as sensitive (blue) or resistant (red); error bars denote 95% confidence intervals calculated by the Wilson score method. *Vancomycin disc-diffusion results are reported as screening observations only; CLSI does not endorse disc diffusion for vancomycin susceptibility testing in S. aureus, and isolates with reduced zones should be regarded as presumptive reduced-vancomycin-susceptibility phenotypes pending confirmation by minimum inhibitory concentration determination and vanA PCR.

DISCUSSION

In this cross-sectional laboratory-based study of fifty community participants from Peshawar, sub-ungual swabs from untrimmed fingernails yielded presumptive Staphylococcus aureus isolates in every sample, with phenotypic disc-diffusion testing demonstrating resistance to methicillin in 90.0% (95% CI 78.6–95.7) and reduced vancomycin-disc susceptibility in 78.0% (95% CI 64.8–87.2) of isolates, alongside retained susceptibility to teicoplanin (88.0%) and linezolid (96.0%). These findings suggest that the sub-ungual niche in this community population may harbour a substantial burden of phenotypically drug-resistant staphylococci, but the observed resistance rates — particularly to vancomycin — must be interpreted with considerable methodological caution before being accepted as epidemiologically meaningful.

The sub-ungual space is a recognised anatomical reservoir for bacterial carriage. Its combination of keratinaceous debris, intermittent moisture, and relative inaccessibility to routine hand-washing creates conditions favourable to microbial persistence and to biofilm formation, and nails — particularly when long or ragged — have been shown to harbour higher microbial loads than the surrounding skin (1,2). Published work from healthcare settings has linked artificial and untrimmed fingernails to the transfer of pathogens including S. aureus, Gram-negative bacilli, and yeasts to patients, and outbreak investigations have repeatedly identified the hands and nails of carriers as the vehicle of transmission (3). The present observation that all fifty sub-ungual samples produced bacterial growth is therefore consistent with the broader literature on hand and nail carriage and reinforces the hygienic rationale for routine nail trimming as a simple, low-cost infection-prevention measure.

An important limitation of the phenotypic identification applied here warrants direct discussion. On Mannitol Salt Agar, classical S. aureus produces mannitol-fermenting yellow colonies surrounded by a yellow halo, whereas non-mannitol-fermenting coagulase-negative staphylococci produce pink colonies

on a pink background. In the present dataset, forty-nine of the fifty isolates produced non-fermenting pink colonies and only one produced the expected yellow mannitol-fermenting morphology, despite all fifty isolates being catalase-, coagulase-, and DNase-positive on subsequent testing. This discrepancy is biologically atypical and may reflect one of several possibilities: the circulation of mannitol-non-fermenting S. aureus variants, which have been reported but are uncommon; misidentification at the colony-morphology step; or the presence of coagulase-positive non-aureus staphylococci. Species-level confirmation by nuc gene PCR, 16S rRNA sequencing, or matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) was not performed in this study and would be required to resolve this issue definitively. The results that follow should therefore be interpreted as describing a population of presumptive Staphylococcus isolates meeting a standard biochemical profile rather than molecularly confirmed S. aureus.

The observed 90.0% rate of phenotypic methicillin resistance is high but not without precedent in South Asian community and hospital datasets. Reported MRSA prevalence among Pakistani clinical isolates has ranged between approximately 40% and 60% in recent years, with some tertiary-care series reporting figures exceeding 70%, and community-carriage studies in Lahore, Karachi, and Peshawar have described MRSA nasal-carriage rates of 10–30% in healthy adults (4,5). A study from Nepal that examined multidrug-resistant S. aureus among adult carriers similarly reported high rates of methicillin resistance alongside retained susceptibility to linezolid, paralleling the pattern seen here (6). It should nevertheless be acknowledged that the CLSI-recommended surrogate for detection of methicillin resistance in S. aureus is the cefoxitin 30 µg disc, not methicillin itself, and that definitive confirmation requires detection of the mecA (or mecC) gene by molecular methods (7). In the absence of cefoxitin-disc testing and mecA PCR, the MRSA designations in this study should be regarded as phenotypic screening findings rather than confirmed genotypic MRSA.

The vancomycin result requires particular scrutiny. Globally confirmed vancomycin-resistant S. aureus (VRSA) remains a very rare entity, with fewer than sixty confirmed cases reported worldwide since the first isolate was documented in the United States in 2002, and a small number of additional cases reported from India, Brazil, Iran, Pakistan, and Portugal (8,9). Against this background, a disc-diffusion-based VRSA positivity rate of 78% in a community sample of fifty participants is biologically implausible as a true prevalence estimate. The most likely explanation lies in the well-recognised methodological limitation of disc diffusion for vancomycin: CLSI guidance since 2009 has explicitly stated that vancomycin disc diffusion should not be used to determine susceptibility in S. aureus, because disc-diffusion zone diameters correlate poorly with minimum inhibitory concentrations and do not reliably distinguish susceptible, intermediate (VISA), and resistant (VRSA) phenotypes (7). Definitive classification therefore requires broth microdilution or agar-dilution MIC determination, ideally supported by vanA- and vanB-gene PCR. In the present study, neither MIC testing nor molecular confirmation was performed, and the reduced-vancomycin-susceptibility finding must be interpreted as a screening observation rather than evidence of genuine VRSA carriage. This is a central methodological limitation of the study and is the single most important caveat for any downstream clinical or public-health interpretation of the results.

Susceptibility to teicoplanin (88.0%) and linezolid (96.0%), by contrast, was retained in the great majority of isolates, a pattern consistent with regional and international datasets which continue to report linezolid-resistance rates below 2% and teicoplanin-resistance rates below 5% among methicillin-resistant S. aureus (10,11). These agents remain valuable reserve options for the empirical management of serious infection caused by glycopeptide-non-susceptible staphylococci, although their use in community settings is necessarily constrained by cost, parenteral administration requirements (for teicoplanin), and the risk of bone-marrow suppression with prolonged linezolid therapy. The retained activity of these agents in the present dataset should not be taken as an invitation to widen their empirical use in the community, but rather as a reminder that stewardship of reserve antimicrobials remains essential.

Several limitations beyond those already discussed warrant acknowledgement. The sample size of fifty participants was determined pragmatically and the study was not powered for subgroup analysis by age, sex, occupation, or hand-hygiene behaviour. Sampling was by convenience from a single geographic area in Peshawar and is not intended to be representative of the broader Pakistani community. No demographic data were systematically stratified for analysis, limiting the ability to identify risk groups. Species-level identification was phenotypic rather than molecular; MRSA and VRSA status was likewise phenotypic, without mecA/vanA confirmation or MIC determination; and the antibiotic panel was limited to four agents, omitting routinely tested drugs such as erythromycin, clindamycin, ciprofloxacin, gentamicin, and trimethoprim–sulfamethoxazole that would have permitted fuller antibiogram characterisation. Finally, although ethical approval and informed consent were obtained, the study collected no clinical or behavioural covariates that might have illuminated the upstream drivers of the observed carriage pattern.

Notwithstanding these limitations, the study contributes a descriptive community-level observation that merits confirmation in larger, molecularly-supported work. If the high presumptive MRSA-carriage rate is confirmed by genotypic methods in a more representative Pakistani sample, the public-health implications — for food handling, household transmission, and healthcare-worker screening — would be considerable, and would strengthen the case for routine nail-hygiene education alongside established hand-hygiene programmes.

CONCLUSION

In this cross-sectional study, sub-ungual swabs from the untrimmed fingernails of fifty community participants in Peshawar yielded presumptive Staphylococcus aureus in every sample, with high rates of phenotypic methicillin resistance and reduced vancomycin-disc susceptibility, alongside retained sensitivity to teicoplanin and linezolid. These findings suggest that untrimmed fingernails may act as a community reservoir for drug-resistant staphylococci and support the public-health value of routine nail hygiene. The resistance findings should, however, be interpreted as phenotypic screening observations, pending confirmation by cefoxitin-disc testing, minimum inhibitory concentration determination, and molecular detection of mecA and vanA, which were outside the scope of the present work.

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