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PREVALENCE, BIOFILM-FORMING ABILITY, AND ANTIMICROBIAL RESISTANCE PROFILE OF STAPHYLOCOCCUS AUREUS ISOLATED FROM HOSPITAL FOMITES IN A TERTIARY CARE HOSPITAL, ISLAMABAD, PAKISTAN

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Abstract

Background: *Hospital fomites act as reservoirs for pathogenic microorganisms and contribute substantially to the transmission of healthcare-associated infections (HAIs). Staphylococcus aureus, including methicillin-resistant strains (MRSA), is among the most clinically significant organisms recovered from such surfaces because of its capacity for biofilm formation and antimicrobial resistance. This study determined the prevalence, biofilm-forming potential, and antimicrobial resistance profile of S. aureus isolated from high-touch fomites at a tertiary care hospital in Islamabad, Pakistan.*

Methods: *A hospital-based, descriptive cross-sectional study was conducted at the Federal General Hospital, Islamabad. A total of 120 swab samples were collected by convenience sampling from high-touch surfaces and equipment across the Emergency, Medical, Gynecology/Labor, Pediatrics and Operation Theatre/Intensive Care Unit departments. Isolates were cultured on blood agar and identified as S. aureus by Gram staining, catalase and coagulase reactions, mannitol fermentation on Mannitol Salt Agar, and DNase activity. Biofilm formation was assessed qualitatively by the tube adherence method, and antimicrobial susceptibility was determined by the Kirby–Bauer disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) criteria.*

Results: *S. aureus recovered from 69 of 120 samples (57.5%), of which (32.0%) were cefoxitin-resistant and therefore classified as MRSA. Contamination was highest in the Medical and Emergency departments and on mobile phones, side tables, oxygen masks/nebulizers, door handles, and the hands of medical personnel. Resistance was most frequent to ciprofloxacin (54%), followed by trimethoprim sulfamethoxazole (41%) and clindamycin (37%). No phenotypic resistance to vancomycin was detected by disk diffusion, although MIC-based confirmation was not performed and cannot therefore exclude vancomycin-intermediate or heteroresistant isolates. Most isolates were moderate (38.7%) or strong (28%) biofilm producers, and only (4.7%) were biofilm negative.*

Conclusion: *Hospital fomites at this facility harbor a high burden of biofilm-forming, multidrug-resistant S. aureus, including MRSA. These findings support*

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the need for targeted environmental surveillance, expanded disinfection protocols for frequently handled items such as mobile phones and respiratory equipment, and reinforced antimicrobial stewardship to limit the risk of nosocomial transmission.

1. INTRODUCTION

Hospital-acquired infections (HAIs) remain one of the most persistent challenges to patient safety worldwide. Inanimate objects and surfaces within the clinical environment, collectively referred to as fomites, are increasingly recognized as important reservoirs and vehicles for the transmission of pathogenic microorganisms between patients, healthcare workers, and visitors [1]. Bed rails, mobile phones, identification badges, stethoscopes, and door handles provide continuous points of contact between staff and patients, and contamination of these surfaces has been directly implicated in outbreaks of nosocomial infection. Among the bacterial species most frequently isolated from such surfaces, Staphylococcus aureus occupies a prominent position because of its ability to survive for prolonged periods on dry, inanimate surfaces, its capacity to form protective biofilms, and its well-documented propensity to acquire resistance to multiple classes of antimicrobial agents [2].

S. aureus is a Gram-positive, catalase- and coagulase-positive coccus that colonizes the anterior nares, skin, and upper respiratory tract of a substantial proportion of the healthy population, while also acting as one of the most versatile opportunistic human pathogens, capable of causing disease ranging from superficial skin infections to bacteremia, endocarditis, and toxic shock syndrome [2]. The organism was among the first bacterial pathogens to demonstrate rapid antimicrobial adaptation: methicillin, the semisynthetic penicillin developed specifically to overcome penicillinase-mediated resistance, entered clinical use in 1959, and the first methicillin-resistant isolates were reported in 1961, approximately two years later, giving rise to methicillin-resistant *S. aureus* (MRSA), a lineage that continues to dominate global discussions of antimicrobial resistance (AMR) [3,4].

Bacterial AMR has been formally recognized by the World Health Organization as one of the top global public health threats of the present era and has been estimated to be directly associated with approximately 4.71 million deaths globally, with around 1.14 million deaths directly attributable to resistant bacterial infections [5,6]. *S. aureus*, and MRSA in particular, is consistently listed among the pathogen–drug combinations contributing most heavily to this global burden, and its presence in the hospital environment, including on fomites, therefore represents a tangible contributor to the wider AMR crisis rather than a mere microbiological curiosity [5,6].

A fomite is broadly defined as any inanimate object or surface capable of carrying and transferring infectious organisms from one host to another [1]. Although the microbial burden on any single fomite is generally lower than that found on the skin of a colonized or infected patient, the high virulence and low infectious dose of certain nosocomial pathogens mean that even modest surface contamination can be clinically significant, particularly given that MRSA has been documented to survive on dry hospital surfaces for up to nine days or longer [7]. Environmental surveillance studies from a range of healthcare settings worldwide have repeatedly confirmed the presence of *S. aureus* and MRSA on hospital surfaces, with heaviest contamination typically reported on bedrails, tabletops, occupied beds, and shared communication devices [8,11].

A biofilm is a structured, surface-associated community of bacterial cells encased within a self-produced extracellular polymeric substance (EPS) matrix. Biofilm formation in *S. aureus* proceeds through reversible attachment, irreversible attachment and microcolony formation, biofilm maturation, and finally dispersal [12,13]. The clinical importance of this process lies chiefly in the protection it affords against host immune defenses and antimicrobial agents: cells embedded within a biofilm matrix are shielded from phagocytosis, exhibit reduced metabolic activity that limits antibiotic efficacy, and benefit from

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restricted diffusion of antimicrobial molecules through the dense matrix [12]. Specifically, biofilm formation additionally enhances adherence to abiotic surfaces such as plastic, metal, and glass, prolonging environmental persistence and increasing the likelihood of transmission [12,14]. A growing body of evidence links biofilm-forming capacity with elevated antimicrobial resistance in *S. aureus*, with several Pakistani studies reporting that resistance to ciprofloxacin, levofloxacin, erythromycin, and trimethoprim–sulfamethoxazole is significantly more prevalent among biofilm-forming isolates than among non-producers [15,16].

Resistance in *S. aureus* is mediated through several overlapping mechanisms, most notably acquisition of the *mecA* (and less commonly *mecC*) gene carried on the staphylococcal cassette chromosome *mec* (SCC*mec*) element, which confers resistance to the entire beta-lactam class. Reduced susceptibility, and more rarely full resistance, to vancomycin has also been documented, giving rise to vancomycin-intermediate and vancomycin-resistant phenotypes [3]. Pakistan has consistently been identified as a region of high MRSA prevalence relative to global averages, with multicenter surveillance reporting an overall MRSA prevalence of 42% among clinical *S. aureus* isolates and 46% specifically in the Rawalpindi/Islamabad region, while single-center studies in Rawalpindi have reported clinical MRSA prevalence as high as 88% [17–22]. The environmental and zoonotic dimension of MRSA in the twin cities has also been documented outside the clinical setting, with the majority of *S. aureus* isolates from slaughterhouse surfaces in Rawalpindi and Islamabad found to be phenotypically cefoxitin-resistant [23].

Taken together, the available literature establishes that hospital fomites are a well-documented reservoir for *S. aureus* and MRSA worldwide, that biofilm-forming capacity is closely associated with enhanced antimicrobial resistance and environmental persistence, and that Pakistan, and the Rawalpindi–Islamabad region in particular, already carries a disproportionately high burden of clinical MRSA infection. What remains comparatively underexplored is the intersection of these three factors within a single, methodologically integrated investigation of the fomite reservoir itself. Despite the recognized role of hospital fomites in nosocomial transmission, there is a paucity of locally generated data characterizing *S. aureus* isolated specifically from fomites within tertiary care hospitals in Islamabad; in the absence of such data, infection prevention and control strategies and empirical antimicrobial choices in this setting rely largely on clinical isolate data or on evidence extrapolated from other regions, which may not accurately reflect the local environmental reservoir.

This study was therefore designed to determine (i) the prevalence of *S. aureus* among bacterial isolates recovered from selected hospital fomites in a tertiary care hospital in Islamabad; (ii) the biofilm-forming ability of the recovered isolates using phenotypic methods; (iii) the antimicrobial susceptibility and resistance profile, including methicillin resistance, of the isolates; and (iv) the association between biofilm-forming ability and antimicrobial resistance patterns among the isolates.

2. MATERIALS AND METHODS

2.1 Study Design, Setting, and Duration

This was a hospital-based, descriptive cross-sectional study designed to determine the prevalence, biofilm-forming ability, and antimicrobial resistance profile, including methicillin resistance, of *S. aureus* isolated from fomites within a tertiary care hospital setting. Environmental samples were collected from the Federal General Hospital (FGH), Islamabad, Pakistan, and processed in the Department of Microbiology. Sample collection, laboratory processing, and analysis were carried out over a period of 6 months, from September 2025 to March 2026.

2.2 Study Population and Sampling

The study population comprised inanimate, frequently touched hospital surfaces and objects located within various clinical areas of the hospital, including bed rails, over-bed tables, door handles, call buttons, stethoscopes, blood pressure

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cuffs, computer keyboards, mobile phones, and staff identification badges, consistent with items previously implicated in the nosocomial transmission of *S. aureus* [2,7]. A non-probability convenience sampling technique was employed; accessible, high-touch fomites across representative clinical areas, including wards, intensive care units, operation theatres, and outpatient areas, were selected sequentially as they became available for sampling during scheduled visits. Inanimate, frequently touched items accessible to patients, healthcare workers, or visitors at the time of sample collection were included, while items that had undergone terminal disinfection or sterilization immediately prior to sampling, discarded single-use disposable items, and surfaces inaccessible for swabbing were excluded.

2.3 Sample Size

The minimum required sample size was first calculated using the standard Cochran formula for estimating a single population proportion in an infinite population:

$$n_0 = \frac{Z^2 pq}{e^2}$$

where Z = 1.96 (95% confidence level), p = 0.5 (the most conservative estimate in the absence of a locally established prevalence figure for *S. aureus* contamination of hospital fomites in Islamabad), q = 1 − p, and e = 0.05 (5% margin of error). This yielded $n_0 = (1.96)^2 \times 0.5 \times 0.5 / (0.05)^2 = 384.16 \approx 385$.

Since the total accessible population of eligible fomite sites within the study setting during the sampling period was finite and estimated at N = 173, Cochran's finite population correction formula was applied to adjust the initial estimate:

$$n = \frac{n_0}{1 + \frac{n_0 - 1}{N}}$$

Substituting $n_0 = 385$ and N = 173:

$$n = \frac{385}{1 + \frac{384}{173}} = \frac{385}{1 + 2.220} = \frac{385}{3.220} \approx 120$$

The finite population-corrected minimum required sample size was therefore approximately **120 samples**. Accordingly, a total of 120 fomite samples were collected and processed for this study, meeting the statistically required sample size at a 95% confidence level and 5% margin of error for the defined finite population of 173 eligible fomite sites.

2.4 Sample Collection

Samples were collected using sterile cotton swabs pre-moistened with sterile normal saline (0.85% NaCl). Each swab was rolled firmly over a defined surface area of the selected fomite in multiple directions to maximize recovery of adherent organisms. Following collection, swabs were immediately placed into sterile transport tubes and transported to the microbiology laboratory for prompt processing to minimize loss of viability of fastidious or environmentally stressed organisms.

2.5 Culture, Isolation, and Identification

Swabs were inoculated onto blood agar and incubated aerobically at 37 °C for 18–24 hours. Colonies displaying morphology suggestive of staphylococci (round, smooth, opaque, and variably pigmented) were sub-cultured onto Mannitol Salt Agar (MSA) and DNase agar for further identification. Presumptive identification of *S. aureus* was based on the following combination of criteria: (i) colony morphology small, golden-yellow, mannitol-fermenting colonies on MSA, indicated by a color change of the medium from red to yellow; (ii) Gram staining Gram-positive cocci arranged in characteristic grape-like clusters under oil-immersion microscopy; (iii) catalase test positive, distinguishing staphylococci from streptococci; (iv) coagulase test positive, confirming *S. aureus* and differentiating it from coagulase-negative staphylococci; and (v) DNase test a clear zone of hydrolysis around growth on DNase agar following flooding with 1N hydrochloric acid. Isolates fulfilling all of the above criteria were confirmed as *S. aureus* and retained for downstream antimicrobial susceptibility and biofilm assays.

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2.6 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed by the modified Kirby Bauer disk diffusion method on Mueller–Hinton agar, in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines. A standardized inoculum, adjusted to 0.5 McFarland turbidity, was used to uniformly lawn the plates prior to disk placement. The following antimicrobial disks were tested: cefoxitin (FOX, 30 µg), gentamicin (CN, 10 µg), trimethoprim–sulfamethoxazole (SXT, 25 µg), clindamycin (DA, 2 µg), ciprofloxacin (CIP, 5 µg), and vancomycin (VA, 30 µg). Plates were incubated at 37 °C for 18–24 hours, and zones of inhibition were measured in millimeters and interpreted as susceptible, intermediate, or resistant according to CLSI breakpoints. Resistance to cefoxitin was used as a phenotypic surrogate marker for methicillin resistance (MRSA), consistent with standard laboratory practice.

Two methodological caveats apply to this panel and are stated here so that the corresponding results are interpreted appropriately. First, CLSI guidance is explicit that disk diffusion is not a reliable method for determining vancomycin susceptibility in *S. aureus*, because zone diameters do not dependably separate susceptible isolates from vancomycin-intermediate (VISA) or heterogeneous vancomycin-intermediate (hVISA) strains; a minimum inhibitory concentration (MIC) based method such as broth microdilution, agar dilution, or gradient diffusion is required for definitive determination. MIC testing was not performed in the present study. Vancomycin findings are therefore reported throughout this manuscript strictly as the absence of detectable phenotypic resistance by disk diffusion, and not as confirmed susceptibility. Second, erythromycin was not included in the disk panel, and a double-disk diffusion (D-zone) test for inducible clindamycin resistance (inducible MLS$_B$ phenotype) could therefore not be performed. Isolates carrying inducible resistance would have been recorded as clindamycin-susceptible by disk diffusion alone, so the clindamycin resistance rate reported here may underestimate the burden of clinically relevant clindamycin resistance. Both limitations are discussed further in Section 4.6.

2.7 Detection of Biofilm Formation

Biofilm-forming ability was assessed phenotypically using the qualitative tube adherence method. Isolates were inoculated into tubes containing Trypticase Soy Broth supplemented with 1% glucose and incubated at 37 °C for 24 hours. Tubes were then decanted, gently washed with phosphate-buffered saline to remove non-adherent planktonic cells, and stained with 0.1% crystal violet. Excess stains were rinsed off with distilled water, and tubes were air-dried in an inverted position. Biofilm formation was assessed visually by observing the presence and thickness of a stained adherent layer on the inner wall and bottom of the tube, and isolates were graded as strong, moderate, weak, or negative biofilm producers according to established criteria [28,31].

2.8 Data Analysis

Data generated from sample source distribution, morphological identification, antimicrobial susceptibility, and biofilm formation assays were entered and analyzed using Microsoft Excel. Descriptive statistics, including frequencies and percentages, were used to summarize the distribution of positive samples by location and fomite type, the antimicrobial resistance profile, and the distribution of biofilm-forming categories. Unless otherwise stated, percentages were calculated using the total number of confirmed *S. aureus* isolates (n = 69) as the denominator and are reported to one decimal place. Results are represented graphically using pie charts and bar graphs.

An association between biofilm-forming ability and antimicrobial resistance phenotype was originally planned using the chi-square test, with $p < 0.05$ as the threshold for statistical significance. Given the sample size achieved (n = 69) and the absence of stratified resistance data by biofilm category, this test was not carried out as a confirmatory analysis. The relationship between the two variables is instead described descriptively

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in Section 3.5 and interpreted as a hypothesis-generating observation in Section 4.5.

2.9 Ethical Considerations

Ethical approval for this study was obtained from the Ethical Review Committee of Federal General Hospital Islamabad, prior to commencement of sample collection. As this study involved only the collection of samples from inanimate hospital surfaces and objects, and did not involve human participants, patient-identifiable data, or clinical specimens, individual patient consent was not required; however, permission for environmental sampling was obtained from the relevant hospital administration.

3. RESULTS

3.1 Sample Distribution and Overall Prevalence

A total of 120 fomite swab samples were collected and processed during the study period. Of these, 69 samples (57.5%) yielded positive bacterial growth on culture, while the remaining 51 samples (42.5%) showed no growth. Among the 69 positive isolates, (16/69(32%) were confirmed as Methicillin-Resistant Staphylococcus aureus (MRSA). These findings indicate that fomites within the hospital environment represent a substantial reservoir for bacterial contamination, with MRSA constituting a notable proportion of the recovered isolates.

3.2 Distribution of Positive Results by Fomite Type

Analysis of the 69 positive samples by fomite source revealed that mobile phones were the most frequently contaminated items, accounting for 10 isolates (14.4%), followed by oxygen masks and nebulizers with 8 isolates (11.5%), and side tables with 9 isolates (13.0%). Door handles and the hands of medical personnel each contributed 7 isolates (10.1%). Bed rails and wash basins each accounted for 6 isolates (8.6%), while machine display panels yielded 5 isolates (7.2%). Fewer isolates were recovered from bed sheets (3, 4.3%), medicine trays (3, 4.3%), pillow covers (2, 2.9%), and BP machine cuffs (2, 2.9%). The ultrasound probe yielded a single positive isolate (1.4%). Notably, electric switches showed no bacterial growth (0%), suggesting these surfaces may be subject to more frequent cleaning or lower contact contamination. Overall, high-touch items handled directly by patients and staff, such as mobile phones, side tables, and door handles, emerged as the most significant fomite reservoirs.

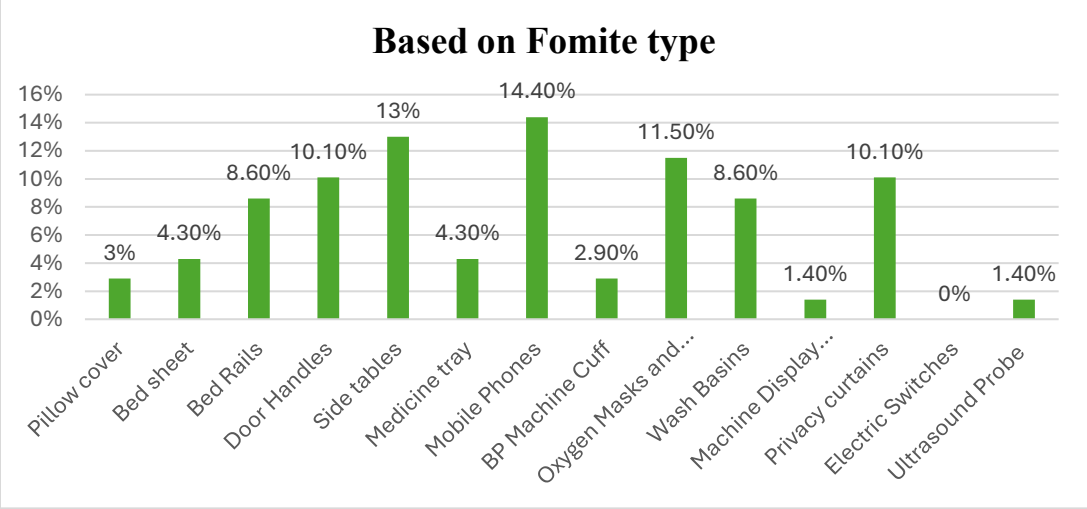


Figure 1a. Distribution of *S. aureus*–positive samples by hospital department.

3.3 Distribution of Positive Results by Hospital Ward

The distribution of culture-positive samples varied considerably across hospital wards. The Medical ward accounted for the highest proportion of positive isolates (33%), followed by the Emergency department (23%) and the Operation Theatre and Intensive Care Unit (OT and ICU) combined

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(19%). The Gynaecology ward contributed 15% of positive samples, while the Paediatric ward (Peads) recorded the lowest proportion at 10%. This pattern suggests that wards with higher patient turnover and more frequent equipment handling, such as the Medical and Emergency departments, may be at greater risk of fomite contamination.

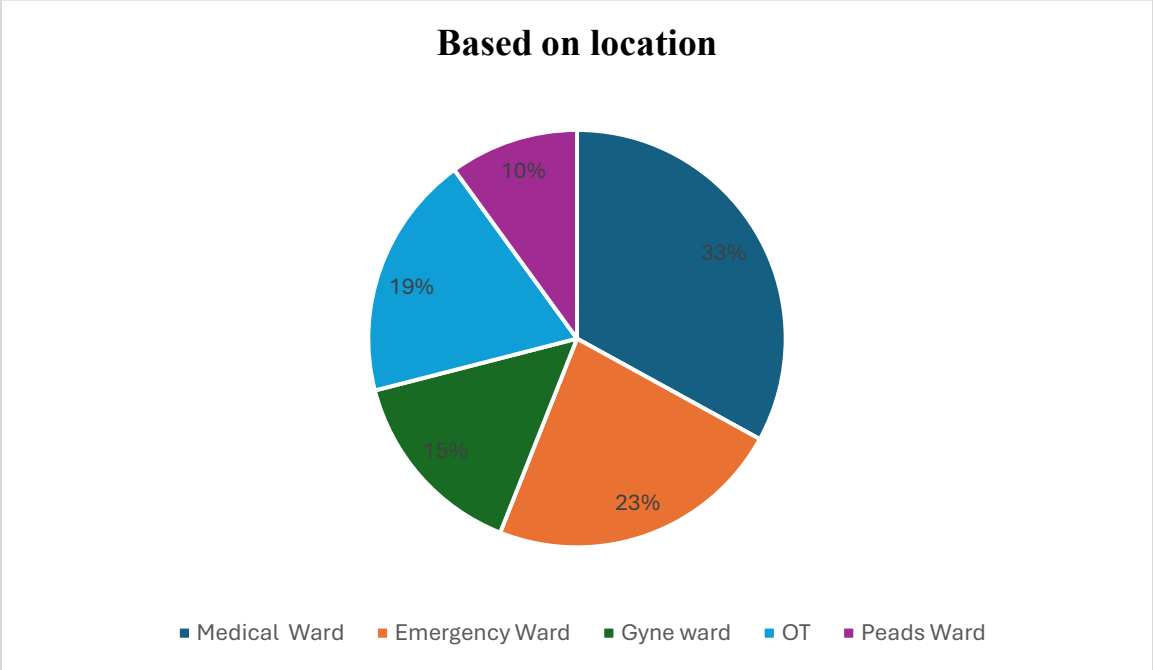


Figure 1b. Distribution of S. aureus positive samples by fomite type.

3.4 Morphological and Biochemical Identification

All 69 isolates included in downstream analysis satisfied the full combination of colony morphology, Gram-stain, catalase, coagulase, and DNase criteria described in Section 2.5.

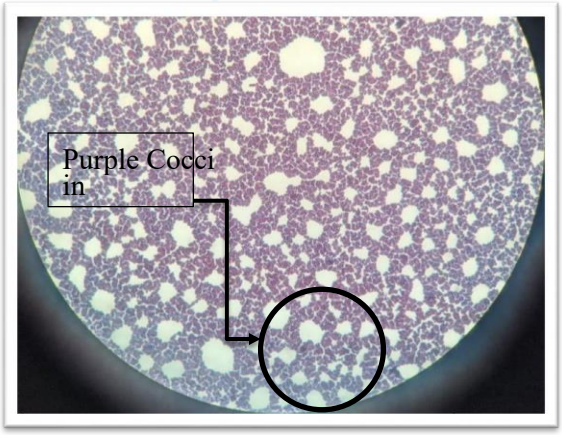


Figure 2. Gram-stained micrograph of *S. aureus* showing Gram-positive cocci in grape-like clusters (oil-immersion, 1000×).

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3.5 Antimicrobial Susceptibility Profile

Antimicrobial susceptibility testing by the Kirby Bauer disk diffusion method showed that the 69 confirmed *S. aureus* isolates were most frequently resistant to ciprofloxacin 54%, followed by trimethoprim sulfamethoxazole 41% and clindamycin 37%. And 32.0% were resistant to cefoxitin and were therefore classified as MRSA. "No isolate showed phenotypic resistance to vancomycin by disk diffusion (0.0%). As set out in Section 2.6, MIC testing was not performed, so this finding indicates the absence of detectable disk-diffusion resistance rather than confirmed vancomycin susceptibility.". No isolate fell within the intermediate interpretive category for any of the agents reported (Table 1).

Table 1. Antimicrobial resistance profile of *S. aureus* isolates recovered from hospital fomites .

Antimicrobial agent	Class	Resistant (%)	Susceptibility (%)
Ciprofloxacin (CIP, 5 µg)	Fluoroquinolone	54%	46%
Trimethoprim–sulfamethoxazole (SXT, 25 µg)	Folate-pathway inhibitor	41%	59%
Clindamycin (DA, 2 µg)	Lincosamide	37%	63%
Cefoxitin (FOX, 30 µg) — MRSA marker	Beta-lactam (surrogate)	32%	68%
Gentamicin (CN, 10 µg)	Aminoglycoside	3.0%	97%
Vancomycin (VA, 30 µg)†	Glycopeptide	0.0%	100%

3.6 Biofilm Forming Ability

Biofilm formation was detected in the majority of the 69 isolates. Moderate biofilm producers formed the largest category (27/69; 38.7%), followed by weak (20/69; 28.6%) and strong (19/69; 28%) producers, while only (3/69; 4.7%) were classified as biofilm-negative. (Table 2).

Table 2. Biofilm-forming ability of *S. aureus* isolates assessed by the tube adherence method (n = 69).

Biofilm category	Isolates, n	Percentage (%)
Strong producer	19	28
Moderate producer	27	38.7
Weak producer	20	28.6
Negative (non-producer)	3	4.7
Total	69	100

Percentages are calculated as a proportion of the 69 confirmed S. aureus isolates and rounded to one decimal place.

4. DISCUSSION

This study set out to determine the prevalence, biofilm-forming ability, and antimicrobial resistance profile of *S. aureus* isolated from fomites in a tertiary care hospital in Islamabad, Pakistan. Of 120 fomite samples processed, 69 (57.5%) yielded confirmed *S. aureus*; methicillin resistance, inferred from cefoxitin resistance, was detected in of (16/69; 32.0%); resistance was most pronounced against ciprofloxacin; and the majority of isolates demonstrated at least moderate biofilm-forming capacity. These findings

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are discussed below in relation to comparable local and international literature, with attention to their microbiological, clinical, and infection-control implications.

4.1 Overall Prevalence of S. aureus on Hospital Fomites

The overall recovery rate of *S. aureus* from hospital fomites in this study (57.5%) is markedly higher than figures reported from several other institutional environmental surveys. A six-hospital surface survey in the Buea Health District, Cameroon recovered *S. aureus* from only 4.6% of high-touch surfaces such as bedrails, tabletops, and door handles, while environmental screening of secondary schools in Guangzhou, China identified combined MRSA and MRSE contamination in under 7% of samples [8,9]. This disparity most plausibly reflects differences in sampling strategy rather than a genuinely higher underlying contamination burden: studies reporting low prevalence typically sampled a broad, indiscriminate range of surfaces, whereas the present study specifically targeted fomites with high manual contact frequency, such as mobile phones, side tables, oxygen delivery equipment, door handles, and the hands of medical personnel, which would be expected to carry a disproportionately higher bacterial burden. The identification criteria used here colony morphology, Gram staining, mannitol fermentation, catalase and coagulase reactions, and DNase activity, without molecular confirmation may also have contributed to a higher apparent yield than studies employing *nuc*- or *mecA*-based confirmatory protocols.

4.2 Departmental and Fomite Specific Contamination Patterns

Contamination was heaviest in the Medical Department (33%), followed by the Emergency Department (23%) and the OT/ICU complex (19%), with the Gynecology/Labor (15%) and Pediatrics (10%) departments recording the lowest contamination, a pattern broadly consistent with reports from other institutional settings in which high patient turnover, frequent equipment handling, and close, repeated contact between staff, patients, and shared surfaces have been associated with increased environmental bioburden. Surveillance of frequently touched sites in South African public hospitals similarly found the highest MRSA recovery on occupied beds, patient files, and nurses' stations, supporting the general principle that contamination intensity tracks closely with the frequency and diversity of human contact rather than with any single fixed department characteristic [10].

Mobile phones emerged as the single most heavily contaminated fomite type in this study (14.4% of positive isolates), consistent with a substantial body of international literature identifying healthcare workers' mobile phones as important, frequently overlooked reservoirs of *S. aureus*. A systematic review and meta-analysis of mobile phone contamination among African healthcare workers reported a high pooled bacterial contamination prevalence, with *S. aureus* accounting for the second largest share of isolates after coagulase-negative staphylococci, while a study from public hospitals in Ghana found that 70.9% of healthcare workers' mobile phones were colonized with *S. aureus*, of which 28% were confirmed as MRSA [24,25]. The comparatively lower absolute contamination rate recorded for mobile phones in the present study relative to some of these reports may reflect differences in swabbing technique or culture medium, but the consistent ranking of mobile phones among the most heavily contaminated items across settings strengthens the case for their inclusion as a standard target in hospital infection-control and hand-hygiene interventions [26]. The comparable contamination rates observed for side tables (13%), oxygen masks/nebulizers (11.5%), door handles (10.1%), and the Privacy curtains (10.1%) are consistent with the well-established role of carrier linking patient, environment, and fomite, a shared-source model previously demonstrated in Chongqing, China, where mobile phone contamination correlated significantly with hand contamination among the same healthcare workers [27]. The absence of *S. aureus* recovery from electric switches, despite frequent manual contact, may reflect the smooth, non-porous, low-moisture nature of these surfaces, which is

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generally less conducive to prolonged bacterial survival than fabric, rubber, or textured plastic items.

4.3 Antimicrobial Susceptibility Profile

The cefoxitin-based MRSA prevalence identified among fomite isolates in this study (16/69; 32.0%) is considerably lower than figures reported from clinical *S. aureus* isolates in Pakistan. A ten-month, multicenter surveillance study spanning several major cities reported an overall clinical MRSA prevalence of 42%, with a rate of 46% recorded specifically in the Rawalpindi/Islamabad region, while a characterization of clinical isolates from a tertiary care hospital in Rawalpindi reported an MRSA prevalence as high as 88% [17,18]. This distinction is plausible on biological and epidemiological grounds: clinical isolates are drawn disproportionately from patients with established infection, a population enriched for resistant, hospital-adapted strains under ongoing antibiotic selection pressure, whereas fomite isolates represent a broader environmental sampling frame capturing both hospital-adapted and more generalist, community-type *S. aureus* strains introduced via staff, patients, and visitors alike. This is reassuring in one respect, in that it suggests the environmental reservoir sampled here is not dominated by highly resistant hospital-endemic clones, but it does not eliminate the infection-control concern posed by the 32.0% of isolates that were methicillin-resistant, particularly given the demonstrated capacity of MRSA to survive on fomites for up to nine days or longer.

Resistance was most frequent to ciprofloxacin (54%), mirroring clinical resistance data from Pakistan; a recent cross-sectional study of MRSA from several hospitals in Rawalpindi and Islamabad similarly reported high resistance to levofloxacin (78.1%) and ciprofloxacin (75%), attributed to the extensive, often unregulated use of fluoroquinolones in both hospital and community settings [19–22]. The comparatively high resistance to trimethoprim sulfamethoxazole (40.6%) and clindamycin (37.7%) observed in this study is similarly consistent with the broader pattern of empirical, frequently over-the-counter antibiotic use documented across Pakistani healthcare settings. It should be noted, however, that because erythromycin was not tested and no D-zone test was performed, isolates with an inducible MLSB phenotype would have been classified as clindamycin-susceptible; the true burden of clinically relevant clindamycin resistance in this collection may therefore be higher than 37%.

The absence of any phenotypic resistance to vancomycin among all 69 isolates is a reassuring observation, particularly given prior reports from Pakistani hospitals documenting appreciable proportions of vancomycin-intermediate and vancomycin-resistant *S. aureus* among clinical isolates. This finding should nonetheless be interpreted conservatively. Vancomycin susceptibility was assessed by disk diffusion alone, and CLSI guidance is explicit that zone diameters do not reliably separate susceptible isolates from VISA or hVISA strains; MIC-based confirmation, which was not undertaken here, would be required before concluding that vancomycin retains full activity against the environmental reservoir sampled at this facility. The appropriate reading of these data is therefore that no vancomycin resistance was detectable by the method used, not that vancomycin resistance has been excluded.

4.4 Biofilm Forming Ability

Biofilm formation was detected in the majority of isolates, with strong (19/69; 28%) and moderate (27/69; 38.7%) producers together accounting for two-thirds of the collection and only 3 isolates (4.7%) classified as biofilm-negative. This pattern is broadly consistent with biofilm surveys of clinical *S. aureus* isolates in Pakistan; a study of isolates from Faisalabad, Punjab found that all tested isolates produced biofilm, with 63.6% classified as strong and 36.4% as moderate producers, while a more recent multicenter Pakistani study of MRSA isolates using Congo red agar classified 28% of isolates as strong and 40% as moderate biofilm producers, figures that align closely with the proportions recorded in the present fomite-derived collection [15,16]. The tube method used in this study, while simple, inexpensive, and widely used for phenotypic

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biofilm screening, is known to have comparatively lower sensitivity than the tissue culture plate (TCP) method, which is generally regarded as the reference standard; comparative evaluations have reported tube-method sensitivities of 47–82% relative to TCP, with strong producers reliably identified but weak producers less consistently discriminated from true non-producers [28-31]. This methodological characteristic means that the 4.7% biofilm-negative rate reported here may represent a conservative underestimate of true non-producers, a possibility discussed further as a study limitation below. The high overall proportion of biofilm-competent isolates recovered from fomites carries direct infection-control significance, since biofilm formation on abiotic surfaces such as plastic mobile phone casings, metal bed rails, and rubberized oxygen mask components confers protection against desiccation, routine surface disinfectants, and mechanical removal by wiping, thereby prolonging environmental persistence.

4.5 Relationship Between Biofilm Formation and Antimicrobial Resistance

Although the present study did not formally stratify antimicrobial resistance rates by biofilm category, the co-occurrence of a high proportion of biofilm-competent isolates (46/69; 66.7%) moderate-to-strong producers) alongside appreciable resistance to ciprofloxacin, trimethoprim sulfamethoxazole, clindamycin, and cefoxitin is consistent with the broader literature linking biofilm-forming capacity to elevated antimicrobial resistance in *S. aureus*. A study of clinical isolates from Faisalabad found that increasing biofilm-forming capacity was associated with greater resistance across most antimicrobial classes tested, while a multicenter Pakistani MRSA study similarly reported that resistance to ciprofloxacin, levofloxacin, erythromycin, trimethoprim sulfamethoxazole, and fosfomycin was significantly more prevalent among biofilm-forming isolates than among non-producers [15,16]. In the same vein, a tube-method comparison of biofilm formation among 150 clinical *S. aureus* isolates found that a substantially greater proportion of MRSA strains than methicillin-susceptible strains produced biofilm (53/65, 81.5% versus 41/85, 48.2%), lending indirect support to the hypothesis that the biofilm-competent, cefoxitin-resistant subset identified in the present fomite collection may represent a particularly persistent and difficult-to-eradicate component of the environmental reservoir [29]. This interpretation should, however, be regarded as hypothesis-generating rather than confirmatory, since the present study's design does not permit a formal statistical test of that association.

4.6 Strengths and Limitations

This study benefits from a systematic, department-stratified sampling frame targeting fomites of high plausible epidemiological relevance and resistance, and biofilm-forming capacity within a single integrated dataset, an approach that remains comparatively rare in the published Pakistani literature on hospital fomites specifically. Several limitations should nonetheless be considered when interpreting these findings.

First, the cross-sectional, single-institution design captures a snapshot of contamination at one point in time and cannot account for temporal, seasonal, or ward-occupancy-related fluctuations in fomite bioburden. Second, methicillin resistance was inferred phenotypically from cefoxitin disk diffusion rather than confirmed by *mecA* gene amplification, and species identification relied on conventional biochemical criteria rather than molecular confirmation, both of which are recognized gold-standard approaches in contemporary staphylococcal research. Third, vancomycin susceptibility was assessed by disk diffusion alone. Because CLSI guidance states that disk diffusion cannot reliably identify VISA or hVISA isolates, and no MIC-based method (broth microdilution, agar dilution, or gradient diffusion) was performed, the reported absence of vancomycin resistance cannot be treated as confirmation of full vancomycin susceptibility; MIC-based testing is a priority for any follow-up work. Fourth, biofilm formation was assessed using the qualitative tube method alone, which has documented limitations in sensitivity and inter-observer reproducibility relative to the quantitative tissue culture plate assay and lacks the

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genotypic corroboration that *ica* operon (*icaA*/*icaD*) detection would provide. Fifth, the absence of molecular typing (for example, *spa* typing or multilocus sequence typing) precludes any assessment of clonal relatedness between isolates recovered from different fomites, departments, or, potentially, from colonized patients or staff, limiting the ability to draw firm conclusions about transmission chains within the hospital. Finally, the achieved sample size (120 fomites, yielding 69 confirmed isolates) was smaller than the 385 samples calculated to be required for ±5% precision at 95% confidence, which widens the achieved margin of error to approximately ±9% and constrains the statistical power available for subgroup comparisons, including the association between biofilm category and resistance phenotype discussed in Section 4.5. Future studies incorporating molecular confirmation, MIC-based vancomycin testing, D-zone testing for inducible clindamycin resistance, quantitative biofilm assays, larger multicenter sample sizes, and longitudinal sampling would substantially strengthen the generalizability of these findings.

5. CONCLUSION

This study demonstrates that hospital fomites at a tertiary care hospital in Islamabad, Pakistan, harbor a high burden of *S. aureus* (69/120 samples; 57.5%), including a clinically relevant proportion of MRSA (16/69;32.0%), with pronounced resistance to ciprofloxacin, trimethoprim sulfamethoxazole, and clindamycin. No phenotypic resistance to vancomycin was detected by disk diffusion, although MIC-based confirmation would be required to exclude vancomycin-intermediate or heteroresistant isolates. The majority of isolates (46/69; 66.7%) were moderate-to-strong biofilm producers, a phenotype associated with enhanced environmental persistence and reduced susceptibility to disinfection and antimicrobial therapy. Contamination by fomite types Mobile phones 14.4%, side tables 13%, oxygen delivery equipment11.5% , door handles 10.1%, and the Privacy curtains 10.1% were identified as the fomites of greatest concern. These findings support the need for targeted environmental surveillance, expanded and enforced disinfection protocols for frequently handled items, and reinforced antimicrobial stewardship to limit the risk of nosocomial transmission of biofilm-forming, multidrug-resistant *S. aureus* within this and comparable healthcare settings. Future work incorporating molecular confirmation of resistance and biofilm-associated genes, MIC-based vancomycin testing, quantitative biofilm assays, and larger, multicenter sample sizes is recommended to build on and validate these findings.

Ethical Approval

This study was approved by the Ethical Review Committee of Federal General Hospital Islamabad. As the study involved sampling of inanimate hospital surfaces only, individual patient consent was not required; institutional permission for environmental sampling was obtained from the relevant hospital administration.

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