

Article

Disinfectant Resistance and Biofilm Formation among Multidrug-Resistant Bacteria Isolated from Hospital Surfaces

Fatima Hakim Obaid^{1*}, Kazem Mehdi Kazem², Mustafa Mohammed Al-Sultany³

1. Department of Medical Microbiology, Hammurabi College of Medicine, University of Babylon, Babylon, Hilla 51002, Iraq

2. Department of Medical Microbiology, Hammurabi College of Medicine, University of Babylon, Babylon, Hilla 51002, Iraq

3. Department of Medical Microbiology, Hammurabi College of Medicine, University of Babylon, Babylon, Hilla 51002, Iraq

*Correspondence: ham526.fatema.hakim@uobabylon.edu.iq

Citation: Obaid, F. H., Kazem, K. M., & Al-Sultany, M. M. Disinfectant Resistance and Biofilm Formation among Multidrug-Resistant Bacteria Isolated from Hospital Surfaces. American Journal of Biology and Natural Sciences 2026, 3(10), 36-57.

Received: 05th Jul 2026

Revised: 07th Aug 2026

Accepted: 05th Sep 2026

Published: 04th Oct 2026



Copyright: © 2026 by the authors. Submitted for open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>)

Abstract: Background: Routine cleaning of high-touch surfaces in the hospital may leave healthcare-associated bacteria. Resistance might be increased if antimicrobial resistance is combined with the ability to form biofilms and withstand killing by standard disinfectants. In this study, the association of multidrug resistance, quantitative biofilm formation and disinfectant tolerance was assessed in the bacteria isolated from the hospital surfaces. Study design: Analytical cross sectional environmental study with controlled laboratory study was carried out in Imam Al-Sadiq Teaching Hospital and Al-Hilla Surgical Teaching Hospital of Hilla, Iraq between January and June 2026. Six categories of clinical units were sampled for a total of 360 high-touch surface swabs, using a stratified approach. Conventional and Vitek 2 Compact were used for identification of bacteria. Explanation of antimicrobial susceptibility was done according to the CLSI M100 36th edition. The crystal-violet microtiter-plate assay was used to quantify the biomass of biofilm. MIC and MBC testing, neutralizer-validated time-kill testing and a mature-biofilm challenge were used to evaluate formulations of sodium hypochlorite, benzalkonium chloride and hydrogen peroxide. Results: Culture was positive in 200/360 samples (55.6%; 95% CI 50.4-60.6), yielding 246 isolates; 171 (69.5%) were Gram-negative. MDR and XDR phenotypes occurred in 158/246 (64.2%) and 22/246 (8.9%), respectively. Biofilm production was detected in 193/246 (78.5%), including 148/246 (60.2%) moderate or strong producers. The median log₁₀ reductions at the 5-minute contact time under dirty conditions were 5.28, 4.55 and 5.50 for sodium hypochlorite, benzalkonium chloride and hydrogen peroxide, respectively. A total of 301/738 (40.8%) pairs of isolates and products were operationally tolerant. Each 0.10 increase in biofilm OD was associated with higher adjusted odds of tolerance (aOR 1.39, 95% CI 1.27-1.51), as was MDR (aOR 2.50, 95% CI 1.49-4.20). Conclusion: Surface contamination was still prevalent and had a strong correlation with the pre-cleaning sampling. Operational killing was affected by MDR, biofilm biomass, organism group and disinfectant formulation. Routine environmental-cleaning audits should be followed by product verification at validated in-use conditions, correct dilution, removal of organic soil and maintenance of full wet contact time.

Keywords: Hospital environment, high-touch surfaces, multidrug-resistant bacteria, biofilm, disinfectant tolerance, minimum bactericidal concentration, time-kill assay, and infection prevention and control.

Introduction

Healthcare-associated infections (HAIs) continue to be a significant patient safety issue and infection prevention and control is a main strategy to prevent avoidable harm and curtail antimicrobial resistance. Environmental cleaning is a fundamental IPC function as identified by the World Health Organization and is particularly important when infrastructure, staffing, training and supply constraints may impact the ability to implement environmental cleaning consistently[1][2]. High-touch surfaces are especially important as these surfaces can spread organisms from the environment to healthcare providers and patients and from patients to other surfaces and providers, particularly when touched many times during the day[3][4]. Healthcare-associated bacteria can contaminate bed rails, bedside tables, door and faucet handles, switches, monitor and infusion-pump controls, treatment trolleys, keyboards and reusable non-critical equipment. Resistant Gram-negative and Gram-positive organisms have been found on the surfaces and equipment of various hospitals in a number of environmental investigations, including in Iraq[5][6][7][8][9]. Culture positivity does not confirm transmission or infection but rather an environmental reservoir which is important for cleaning performance and local IPC risk assessment. Persistence may be mediated by two factors: (1) antimicrobial resistance and (2) biofilm formation. If an environmental strain subsequently infects, then MDR is clinically significant as it reduces treatment options. Biofilms form communities in which the cells are organized into a structured community held together within an extracellular matrix, which results in the development of physiological heterogeneity and decreases penetration or activity of antimicrobial agents. Recent studies have shown high biofilm-forming ability of hospital organisms that are resistant but the relationship between MDR and biofilm is different depending on the species, origin of the specimen, and the assay conditions used[10]. Do not interpret results of disinfectant performance the same as antibiotic susceptibility. The clinical antibiotic breakpoints are not applicable to surface disinfectants. The efficacy of the product is dependent on the entire formulation, active ingredient level, organic soil, surface material, application method, temperature and wet contact time. MIC/MBC can be used to compare phenotype, but not wipe/carrier conditions. Testing at the labelled in-use concentration, therefore, is more relevant to operational performance, when neutralizer is used for validation[11][12][13][14]. The evidence from Iraq for the application of standardized environmental sampling methods and the current CLSI AST interpretation, quantitative biofilm classification, disinfectant MIC/MBC, in-use time-kill performance and direct mature-biofilm challenge is limited. In the present study, the above gap was filled by taking an all-phenotypic approach and two teaching hospitals in Hilla City. The aim of this study to identify the association between multidrug resistance, quantitative biofilm formation and tolerance to disinfectant formulations routinely used in hospitals among the bacteria isolated from surfaces in the hospital that are touched frequently.

Materials and Methods

The design and setting of the study and the reporting are studied

A two-hospital analytical cross sectional environmental microbiology study with a controlled experimental laboratory study. Environmental sampling was done for six months from 1 January to 30 June 2026 in Imam Al-Sadiq Teaching Hospital and Al-Hilla Surgical Teaching Hospital in Hilla, Babylon Province, Iraq. All the experiments of culture, identification, AST, biofilm and disinfectant were conducted in the private specialized microbiology laboratory in Hilla City. The observational part adhered to the reporting guidelines of the STROBE statement[15].

The sampling frame is the list of all the elements in the population

A target of 360 environmental swabs were targeted. The single-proportion formula $n = Z^2p(1-p)/d^2$ was used to calculate a sample size of 355.2, which was rounded to 360 to facilitate balanced stratification, assuming a 50% contamination rate, a two-sided 95% confidence level and an absolute precision of 5.2%. All the hospitals provided 180 swabs. From each hospital, 30 swabs were collected from six specific units – intensive care, emergency, operating theater, medical ward, surgical ward and dialysis. In each hospital-unit stratum, sampling was evenly distributed across the six classes of surfaces sampled and the six-month sampling period, and was balanced between pre and post cleaning periods.

Surfaces eligible and definitions

Eligible surfaces were those that are touched frequently by patients or staff, and were cleanable: bed rails, bedside tables, door handles, shared equipment controls, trolley/IV-pole handles and faucet handles. Samples were only considered post-cleaning if they had been documented as having undergone a routine cleaning and the elapsed time had been documented. Cleaning status was a non-randomized exposure. The visible soil/surface material, regular sanitizer/disinfectant, dilution, application technique, and labeled wet contact time were documented.

Environmental sample collection

For each surface a sterile flocked swab pre-moistened with neutralizing transport medium was used. A 10 x 10 cm template, which was sterile, was used to determine 100 cm² on flat surfaces. Ten horizontal, ten vertical and ten diagonal strokes were made in each area with the swab being rotated. Handles, and irregular objects, the whole of the accessible surface was sampled and estimated as to area. Different gloves were used for each sample. Field blanks were opened at sampling sites with no contact to the surface for 18 field blanks. The swabs were put in 10 mL of the neutralizing medium which was kept at 2-8°C and processed within 6 hours.

The culture, enumeration and selection of isolates.

Tubes were vortexed for 60 sec. Plates were made with undiluted eluate and ten-fold serial dilutions on blood agar and MacConkey agar, mannitol- Salt and cetrimide agar as indicated. A parallel tryptic-soy-broth enrichment enhanced the detection at low burden but enrichment-only growth was not included in the CFU counts. Plates were incubated aerobically at 35-37°C for 24 and 48 h and examined. The recoverable burden was determined as the number of colonies of CFU/100 cm², which is the number of colonies times the dilution factor times the volume recovered by the volume plated times 100 times the area sampled. One isolate was purified from each of the different colony morphotypes and isolates with materially different morphology or AST were kept as separate phenotypes associated with the parent swab but classified as the same species.

Bacterial identification

Colony morphology, Gram stain, catalase, coagulase, oxidase and biochemical reactions (conventional) were used for preliminary identification. For definitive identification, the VITEK 2 Compact system (bioMérieux, Marcy-l'Etoile, France) was used with GN or GP identification cards and manufacturer's recommended inoculum preparation procedure. Results with less than 100% confidence were repeated.

The definition of antimicrobial susceptibility and resistance.

AST was carried out by using VITEK 2 AST cards which are organism appropriate and standardized disk diffusion. Results were interpreted according to CLSI M100 36th edition, and the standardized procedures of CLSI M02 14th edition, M07 12th edition[16][17][18]. The intermediate results were not included in the calculation of non-susceptible for the MDR derivation but were shown separately in the drug level tables. MDR defined as being non-susceptible to at least one agent in three or more applicable antimicrobial categories; XDR defined as non-susceptible to at least one agent in all but 2 or fewer antimicrobial categories; and PDR defined as non-susceptible to all agents in all applicable categories[19]. Intrinsic resistance was not taken into account.

Quantitative biofilm assay

The biomass of the biofilm was measured by the 96-well microtiter-plate crystal-violet method. Overnight cultures were standardized to 0.5 McFarland, diluted 1:100 in tryptic soy broth with 1% glucose and then distributed 200 microliters per well in sterile flat bottom polystyrene plates. Three independent experiments were performed for each isolate with three replicate wells per experiment. Plates contained medium-only blanks, a positive procedural control of *Pseudomonas aeruginosa* ATCC 27853 and a low-biofilm comparator of *Escherichia coli* ATCC 25922. Wells were washed thrice with PBS, fixed with methanol for 15 minutes and stained with 0.1% crystal violet for 15 minutes and washed and solubilized in 95% ethanol after 24 hours at 37 C without agitation. OD was read at 570 nm. Within-isolate technical CVs >15% were repeated and controls failed were also repeated.

The cut-off for the run was OD_c = mean of the negative control OD + 3 standard deviations.

Isolates were considered as non-producers ($OD \leq OD_c$), weak producers ($OD_c < OD \leq 2OD_c$), moderate producers ($2OD_c < OD \leq 4OD_c$) and strong producers ($OD > 4OD_c$) and continuous blank-corrected OD was the major biofilm variable[20].

2.9 Disinfectant formulations

Three hospital-grade surface-disinfectant formulations were coded to help minimize observer expectation: D1, sodium hypochlorite (0.1% available chlorine, 5-minute contact time); D2, benzalkonium chloride (0.1%, 5-minute contact time); and D3, hydrogen peroxide (0.5%, 5-minute contact time). A commercial lot that was in date was used for each test and stored and diluted as per the label. Branding, manufacturing data, the laboratory source log, expiry data and data of preparation were kept and commercial names were masked in the anonymized manuscript to avoid endorsement.

Disinfectant MIC, MBC and neutralizer validation tests are performed.

Each complete formulation was serially diluted 2-fold in broth over the range of concentrations that encompass the in-use concentration. The plates were then inoculated and incubated at 37C for 18-24 hours. The lowest concentration that did not show any growth was MIC. The aliquots from wells at and above the MIC were immediately neutralized and were plated on a disinfectant-free agar. The lowest concentration that resulted in a 3 log₁₀ reduction in comparison with the starting inoculum was MBC. MIC and MBC were used as laboratory endpoints for comparison purposes as there are no disinfectant breakpoints in clinical use. Validation of the neutralization for each product/organism group was done, according to ASTM E1054-22[21] with efficacy and toxicity controls.

Suspension time-kill assay

The main disinfectant effect measured was the reduction in viable count in a 'time kill' assay modified from ASTM E2315-23[22]. The in-use concentration of the label was tested on an inoculum of about 107-108CFU/mL, in both clean and dirty conditions. Viability counts at baseline, at 1 minute and at 5 minutes. Aliquots were immediately placed in validated neutralizer, serially diluted and plated in duplicate. Three independent experiments were performed for each isolate/condition combination. Log₁₀ reduction was calculated as (log₁₀N₀ - log₁₀N_t). Tolerance was defined as failure to achieve 5-log₁₀ reduction at 5 minutes and this study endpoint does not represent a clinical breakpoint.

Mature-biofilm disinfectant challenge

A predetermined stratified random sample of 96 isolates was tested (48 MDR (including XDR) and 48 non-MDR). The mature (24 hours) 24-hour biofilms were grown in polystyrene wells, washed, and then exposed to in-use concentration of D1-D3 for 1 or 5 minutes. Adherent cells were immediately neutralized and recovered by scraping, 60 s vortex and 5 min bath sonication under a viability validated condition and enumerated by culture. The wells not treated were used to assess the baseline viable biofilm burden. Biofilm protection index was correlated with planktonic log₁₀ reduction – biofilm log₁₀ reduction and positive values were indicative of less killing in the biofilm state. The surface associated performance was interpreted based on the principles of EN 16615[23][24].

Quality assurance

Escherichia coli ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923 and 29213 were used as reference strains. The sterility and growth promoting properties of the media, the incubator temperature, the calibration of the pipettes and densitometers, the performance of the plate readers, the dilution records, and checks on the colony counters were documented. A second analyst blinded to the AST and biofilm phenotype results re-identified 10% of isolates and re-tested them for AST and biofilm phenotype. The raw plate count, OD, instrument output and reason for exclusion were preserved prior to derivation.

The outcomes and statistical analysis will be presented.

The main continuous outcome was log₁₀ reduction in viable count at the in-use concentration and 5-minute contact time and the main explanatory variable was continuous blank-corrected biofilm OD. Operating tolerances, MIC, MBC, MBC/MIC ratio, biofilm category, MDR/XDR status, surface culture positivity, CFU/100 cm² and paired biofilm protection index were secondary outcomes. The analysis used Python 3 with pandas 2.2.3, NumPy 2.3.5, SciPy 1.17.0, and scikit-learn 1.8.0. Parent-sample, ward and hospital linkages were maintained at both swab-level and isolate-level. Categorical

data were presented as n/N (Wilson 95% CIs) and skewed data were presented as median (IQR) and range. Surface positivity was tested by modified Poisson regression at the hospital ward level, and presented as adjusted prevalence ratios. Moderate/strong biofilm formation and operational tolerance were examined using multivariable logistic regression using cluster-robust variance by parent swab or isolate. The tolerance model comprised of the following factors: biofilm OD, MDR, organism group, product, hospital, ward risk, cleaning status and visible soil. With matched planktonic-biofilm differences, paired tests with 95% CIs were used. Two-sided $P < 0.05$ was considered significant and secondary comparisons were performed using Holm's control, when applicable. There were no missing primary laboratory outcomes and hence the method of complete-case analysis was used.

Ethics, administration and biosafety issues

Only inanimate surfaces of the environment and cultured isolates of bacteria were involved. It was done under the general conditions of scientific, administrative, biosafety and confidentiality of participating institutions. The sampling of the environment was approved by the proper hospital procedures. No patient specimens, contact with patients, or patient records were collected, nor was there any identifiable staff data collected; individual informed consent was therefore not applicable. Trained staff handled isolates with the following: Containment procedures, personal protective equipment, decontamination procedures, waste management procedures and incident-reporting procedures.

Results & Discussion

Bacterial recoverable contamination was common with 200 of 360 (55.6%, 95% CI: 50.4-60.6%) environmental swabs being culture-positive. There was no significant overall difference in positivity between the wards ($P=0.290$) or surface type ($P=0.303$) and the percentages of positivity for both Imam Al-Sadiq Teaching Hospital and Al-Hilla Surgical Teaching Hospital were almost identical (56.1% and 55.0%, respectively; $P=0.832$). Although there were descriptive differences, dialysis was the most positive ward (63.3%), and faucet handles were the most positive surface (66.7%) and should not be considered independent effects. The only clearly significant factor was cleaning (before and after) with the positivity decreasing from 72.8% before cleaning to 38.3% after cleaning ($P < 0.001$) and a lower burden before and after cleaning (129 vs 47 CFU/100 cm²), as shown in table (1), figure (1,2). However, the contamination of the environment has not been complete as evidenced by the presence of some positivity in over one-third of the swabs after cleaning.

Table 1. Sampling distribution, culture positivity and recoverable bacterial burden.

NO.	Domain	Category	Swabs	Positive	Culture positivity, % (95% CI)	CFU/100 cm ² , median (IQR)	P value
1.	Total	All samples	360	200	55.6 (50.4-60.6)	102 (47-194)	-
2.	Hospital	Imam Al-Sadiq Teaching Hospital	180	101	56.1 (48.8-63.2)	102 (47-170)	0.832
3.	Hospital	Al-Hilla Surgical Teaching Hospital	180	99	55.0 (47.7-62.1)	100 (50-228)	
4.	Ward	ICU	60	34	56.7 (44.1-68.4)	147 (76-281)	0.290
5.	Ward	Emergency	60	36	60.0 (47.4-71.4)	84 (38-143)	
6.	Ward	Operating theatre	60	26	43.3 (31.6-55.9)	102 (50-186)	
7.	Ward	Medical ward	60	31	51.7 (39.3-63.8)	94 (52-129)	
8.	Ward	Surgical ward	60	35	58.3 (45.7-69.9)	97 (39-153)	

NO.	Domain	Category	Swabs	Positive	Culture positivity, % (95% CI)	CFU/100 cm ² , median (IQR)	P value
9.	Ward	Dialysis	60	38	63.3 (50.7-74.4)	114 (41-195)	0.303
10.	Surface	Bed rail	60	32	53.3 (40.9-65.4)	107 (56-149)	
11.	Surface	Bedside table	60	35	58.3 (45.7-69.9)	91 (42-148)	
12.	Surface	Door handle	60	27	45.0 (33.1-57.5)	110 (48-216)	
13.	Surface	Shared equipment controls	60	33	55.0 (42.5-66.9)	118 (47-213)	
14.	Surface	Trolley/IV pole	60	33	55.0 (42.5-66.9)	96 (45-182)	<0.001
15.	Surface	Faucet handle	60	40	66.7 (54.1-77.3)	100 (50-186)	
16.	Cleaning status	Pre-cleaning	180	131	72.8 (65.9-78.8)	129 (79-224)	
17.	Cleaning status	Post-cleaning	180	69	38.3 (31.5-45.6)	47 (23-98)	

Note. Analysis unit: swab. CFU summarizes 190 quantified positive samples; 10 enrichment-only positives were excluded from burden estimates. P values are omnibus Pearson chi-square tests within each domain.

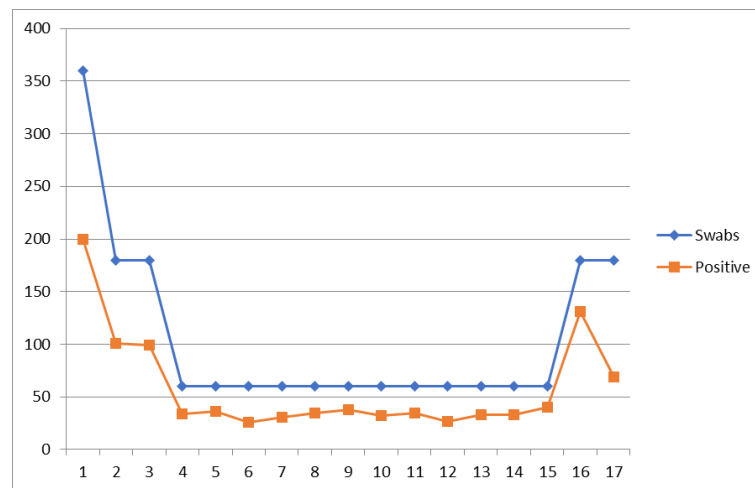


Figure 1. Shows the relationship between the number of swabs taken from 17 different locations, ranging between (60 - 360) swabs, and the number of positive swabs, ranging between (27 - 200).

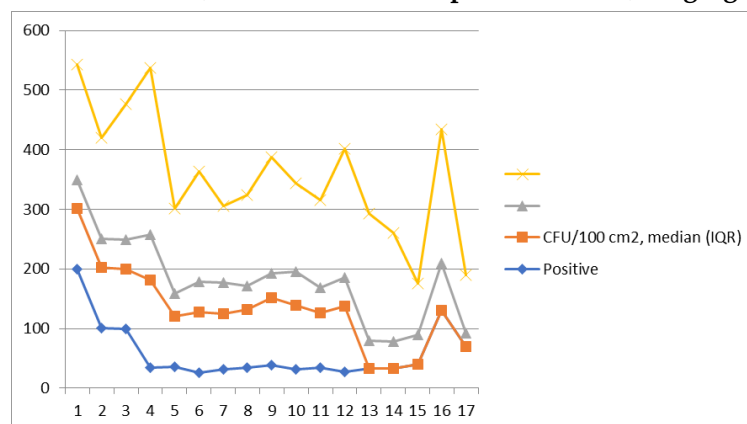


Figure 2. It shows the relationship between the level of microbial contamination on surfaces, measured in CFU/100 cm², and the number of positive swabs to determine the correlation between contamination and the number of positive swabs.

Of the 200 swabs that were positive, 246 non-duplicate isolates were recovered and 23.0% of the positive swabs had polymicrobial recovery. Gram-negative organisms were more prevalent (171/246; 69.5%) while Gram-positive were 30.5%. The common individual organisms were *Acinetobacter baumannii* (19.5%), *Staphylococcus aureus* (14.6%) and *Klebsiella pneumoniae* (13.4%). Totals were even (124 vs 122 isolates) but the number of species varied in a descriptive fashion between hospitals. In total, the results indicate that there is a variety of flora on the hospital surfaces, with dominantly clinically important Gram-negative bacilli. **Figure 3, table 2 below.**

Table 2. Bacterial isolates (excluding duplicates) by hospital.

Species/group	Isolates	Percent % of total	Imam Al-Sadiq	Al-Hilla Surgical
<i>Acinetobacter baumannii</i>	48	19.5	26	22
<i>Pseudomonas aeruginosa</i>	29	11.8	15	14
<i>Klebsiella pneumoniae</i>	33	13.4	18	15
<i>Escherichia coli</i>	27	11.0	11	16
Enterobacter spp.	25	10.2	10	15
<i>Staphylococcus aureus</i>	36	14.6	22	14
Coagulase-negative staphylococci	29	11.8	12	17
<i>Enterococcus</i> spp.	10	4.1	6	4
Other Gram-negative bacilli	9	3.7	4	5
Gram-negative subtotal	171	69.5	84	87
Gram-positive subtotal	75	30.5	40	35
All isolates	246	100.0	124	122

Note. Analysis unit: isolate. The 200 positive swabs yielded 246 isolates; polymicrobial recovery occurred in 46/200 positive swabs (23.0%).

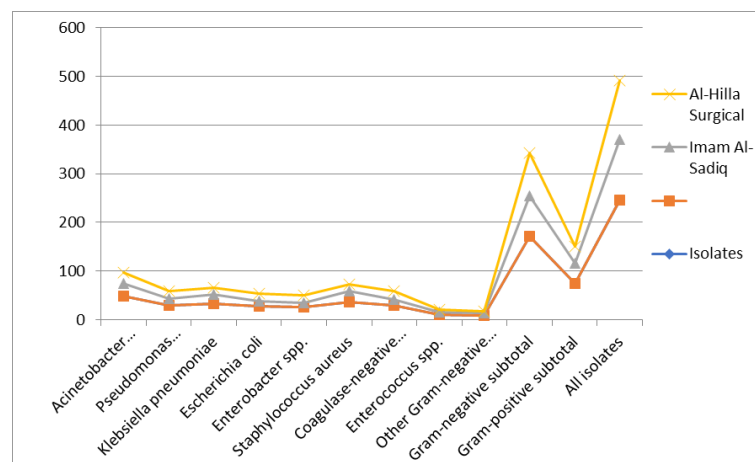


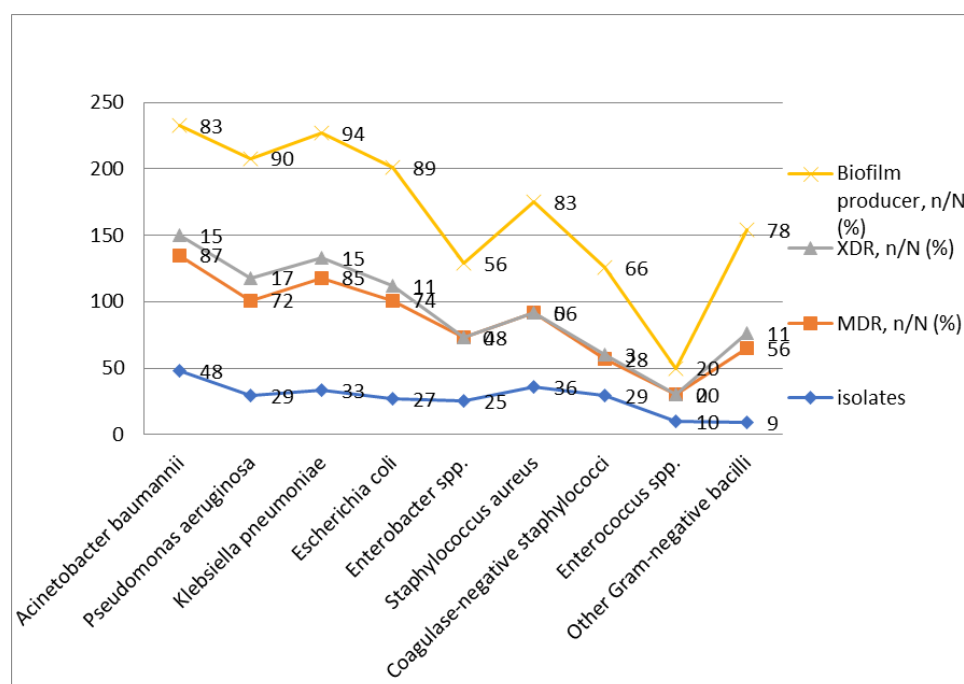
Figure 3. shows the relative distribution of bacterial isolates by type and bacterial group in the aforementioned hospitals.

MDR and XDR were commonly found (64.2% and 8.9% respectively) and biofilm was also commonly produced (78.5%) with no PDR isolate detected. The high prevalence of MDR was observed in *A. baumannii* (87.5%) and *K. pneumoniae* (84.8%) and the highest XDR was found in *P. aeruginosa* (17.2%). The highest proportion of biofilm producers (93.9%) were the *K. pneumoniae* followed by *P. aeruginosa* (89.7%) and *E. coli* (88.9%). These data indicate that there are several groups of Gram-negative bacteria that are reservoirs for multidrug resistance and biofilm formation. Illustrated by **Table 3, figure 4** below.

Table 3. The burden of non-susceptible categories (MDR/XDR phenotype) and biofilm production among bacterial groups.

Species/group	N	Non-susceptible categories, median (IQR)	MDR, n/N (%)	XDR, n/N (%)	Biofilm producer, n/N (%)
<i>Acinetobacter baumannii</i>	48	4.0 (3.0-5.0)	42/48 (87.5%)	7/48 (14.6%)	40/48 (83.3%)
<i>Pseudomonas aeruginosa</i>	29	4.0 (2.0-5.0)	21/29 (72.4%)	5/29 (17.2%)	26/29 (89.7%)
<i>Klebsiella pneumonia</i>	33	4.0 (3.0-5.0)	28/33 (84.8%)	5/33 (15.2%)	31/33 (93.9%)
<i>Escherichia coli</i>	27	4.0 (2.0-5.0)	20/27 (74.1%)	3/27 (11.1%)	24/27 (88.9%)
Enterobacter spp.	25	2.0 (1.0-4.0)	12/25 (48.0%)	0/25 (0.0%)	14/25 (56.0%)
<i>Staphylococcus aureus</i>	36	3.0 (1.0-3.0)	20/36 (55.6%)	0/36 (0.0%)	30/36 (83.3%)
Coagulase-negative staphylococci	29	2.0 (1.0-3.0)	8/29 (27.6%)	1/29 (3.4%)	19/29 (65.5%)
Enterococcus spp.	10	2.0 (1.0-2.0)	2/10 (20.0%)	0/10 (0.0%)	2/10 (20.0%)
Other Gram-negative bacilli	9	3.0 (2.0-5.0)	5/9 (55.6%)	1/9 (11.1%)	7/9 (77.8%)
All isolates	246	3.0 (2.0-4.0)	158/246 (64.2%)	22/246 (8.9%)	193/246 (78.5%)

Note. MDR includes XDR isolates. Intrinsic resistance was excluded from category counts. No PDR phenotype was detected. Drug-level S/I/R results are presented in Supplementary Tables S1 and S2.

**Figure 4. The distribution of bacterial isolates is shown according to antibiotic resistance (MDR and XDR) and the ability to produce a biofilm (Biofilm producer).**

There was a significant difference between the biofilm phenotype of the different bacterial groups ($P < 0.001$). Sixty percent of all the isolates (60.2%) produced moderate or strong biofilms and 29.7% of the

isolates were strong producers. Maximum proportions of strong-biofilm were observed with *P. aeruginosa* (44.8%), *A. baumannii* (43.8%) and *K. pneumoniae* (42.4%) while the least proportion of producers (80.0%) was observed with *Enterococcus* spp. So, the ability to form a biofilm was organism specific and was especially seen in the major Gram-negative hospital associated pathogens. explained by Table 4, figure 5.

Table 4. Quantitative biofilm phenotype (bacterial group).

Species/group	N	Biofilm OD, median (IQR)	Non	Weak	Moderate	Strong
<i>Acinetobacter baumannii</i>	48	0.424 (0.258-0.770)	8/48 (16.7%)	2/48 (4.2%)	17/48 (35.4%)	21/48 (43.8%)
<i>Pseudomonas aeruginosa</i>	29	0.423 (0.233-0.783)	3/29 (10.3%)	5/29 (17.2%)	8/29 (27.6%)	13/29 (44.8%)
<i>Klebsiella pneumoniae</i>	33	0.471 (0.246-0.681)	2/33 (6.1%)	6/33 (18.2%)	11/33 (33.3%)	14/33 (42.4%)
<i>Escherichia coli</i>	27	0.308 (0.190-0.433)	3/27 (11.1%)	6/27 (22.2%)	12/27 (44.4%)	6/27 (22.2%)
<i>Enterobacter</i> spp.	25	0.198 (0.093-0.366)	11/25 (44.0%)	3/25 (12.0%)	6/25 (24.0%)	5/25 (20.0%)
<i>Staphylococcus aureus</i>	36	0.331 (0.213-0.450)	6/36 (16.7%)	7/36 (19.4%)	15/36 (41.7%)	8/36 (22.2%)
Coagulase-negative staphylococci	29	0.184 (0.102-0.227)	10/29 (34.5%)	12/29 (41.4%)	5/29 (17.2%)	2/29 (6.9%)
<i>Enterococcus</i> spp.	10	0.067 (0.046-0.106)	8/10 (80.0%)	1/10 (10.0%)	0/10 (0.0%)	1/10 (10.0%)
Other Gram-negative bacilli	9	0.224 (0.147-0.720)	2/9 (22.2%)	3/9 (33.3%)	1/9 (11.1%)	3/9 (33.3%)
All isolates	246	0.317 (0.159-0.591)	53/246 (21.5%)	45/246 (18.3%)	75/246 (30.5%)	73/246 (29.7%)

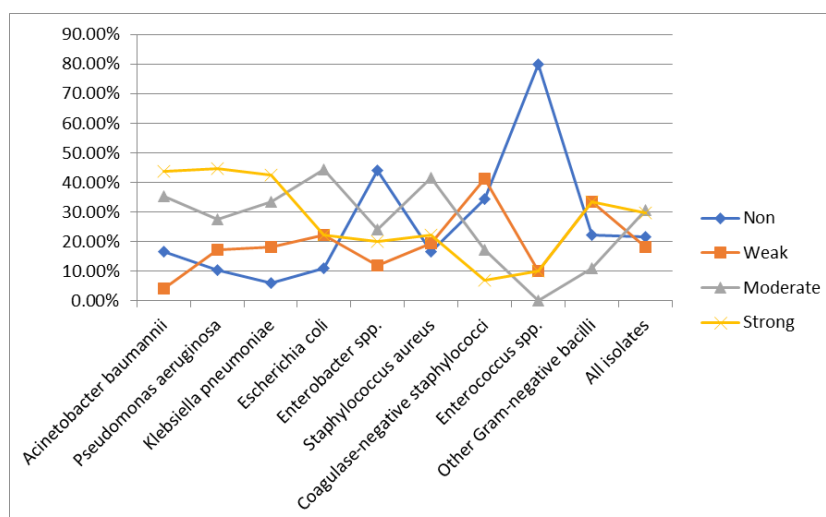


Figure 5. illustrates the distribution of bacterial isolates and their type according to their ability to produce a biofilm, classified as non-producing, weak, intermediate, and strong.

The resistance phenotype was correlated with biofilm formation, with a progressive increase in the ability to form a biofilm as the resistance increased (unadjusted $P < 0.001$). The percent of non-MDR, MDR non-XDR and XDR isolates with moderate/strong biofilm were 36.4%, 69.9% and 95.4%, respectively. MDR non-XDR isolates had 3.62-fold higher odds for moderate/strong biofilm formation

as compared to non-MDR isolates (95% CI: 2.00 – 6.56; $P < 0.001$), and XDR isolates had 31.50-fold higher odds (95% CI: 3.99 – 248.82; $P = 0.001$), after adjustment for Gram group and hospital. The broad XDR confidence interval is due to the small size of the XDR sample and should be interpreted with care, however the direction and size of the interval does lend itself to a strong association between resistance and biofilm.

Table 5. Relationship between resistance phenotype and biofilm category.

Resistance phenotype	N	Non	Weak	Moderate	Strong	Adjusted OR for moderate/strong biofilm (95% CI); P
Non-MDR	88	31/88 (35.2%)	25/88 (28.4%)	24/88 (27.3%)	8/88 (9.1%)	Reference
MDR (non-XDR)	136	21/136 (15.4%)	20/136 (14.7%)	42/136 (30.9%)	53/136 (39.0%)	3.62 (2.00-6.56); $P < 0.001$
XDR	22	1/22 (4.5%)	0/22 (0.0%)	9/22 (40.9%)	12/22 (54.5%)	31.50 (3.99-248.82); $P = 0.001$

Note. Unadjusted distribution: Pearson chi-square $P < 0.001$. Logistic model adjusted for Gram group and hospital; variance clustered by parent swab.

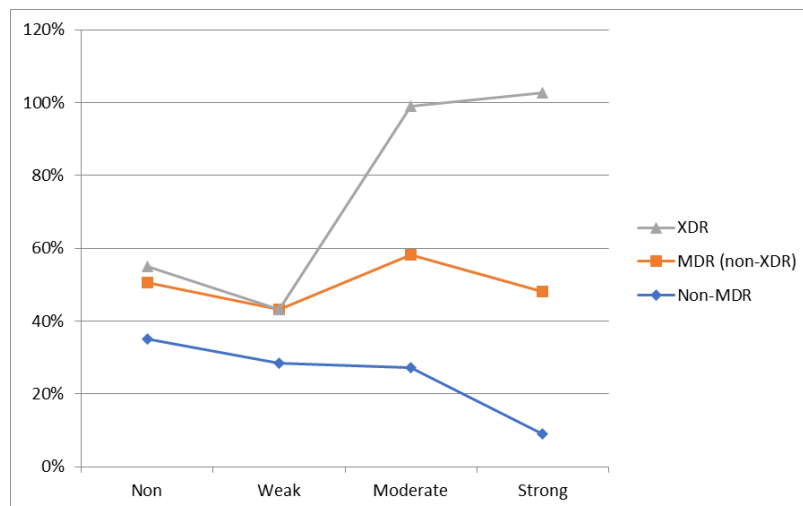


Figure 6. The distribution of bacterial isolates is shown according to their resistance phenotype and their ability to produce a biofilm, classified as non-producing, weak, intermediate, and strong.

The MICs for MDR strains were significantly higher compared to non-MDR strains for sodium hypochlorite, benzalkonium chloride and hydrogen peroxide (all $P < 0.001$). Median MBC values were also generally higher for the MDR isolates and the median MBC/MIC ratios were around 2 for all products and resistance groups. The percentages of active-ingredient concentrations are product-specific and should not be used to directly compare products, which explains why the percentages were higher for hydrogen peroxide for both the MIC and MBC. These distributions reflect decreased in vitro susceptibility and not clinical resistance, as there are no clinical disinfectant breakpoints.

Table 6. Compare the distribution of disinfectant MIC values and the distribution of disinfectant MBC values for MDR and non-MDR.

Disinfectant	Group	N	MIC, % active ingredient median (IQR); range	MBC, % active ingredient median (IQR); range	MBC/MIC, median (IQR)
D1: sodium hypochlorite	All	246	0.02500 (0.01250-0.02500); 0.00625-0.10000	0.05000 (0.02500-0.10000); 0.01250-0.20000	2.0 (2.0-4.0)
D1: sodium	MDR	158	0.02500 (0.02500-	0.05000 (0.05000-	2.0 (2.0-4.0)

Disinfectant	Group	N	MIC, % active ingredient median (IQR); range	MBC, % active ingredient median (IQR); range	MBC/MIC, median (IQR)
hypochlorite			0.02500); 0.00625-0.10000	0.10000); 0.02500-0.20000	
D1: sodium hypochlorite	Non-MDR	88	0.01250 (0.01250-0.02500); 0.00625-0.05000	0.02500 (0.02500-0.05000); 0.01250-0.20000	2.0 (2.0-4.0)
D2: benzalkonium chloride	All	246	0.02500 (0.01250-0.02500); 0.00625-0.05000	0.05000 (0.02500-0.10000); 0.01250-0.10000	2.0 (2.0-2.0)
D2: benzalkonium chloride	MDR	158	0.02500 (0.02500-0.04375); 0.00625-0.05000	0.05000 (0.05000-0.10000); 0.01250-0.10000	2.0 (2.0-2.0)
D2: benzalkonium chloride	Non-MDR	88	0.01250 (0.01250-0.02500); 0.00625-0.05000	0.05000 (0.02500-0.05000); 0.01250-0.10000	2.0 (2.0-2.0)
D3: hydrogen peroxide	All	246	0.25000 (0.12500-0.25000); 0.06250-0.50000	0.50000 (0.25000-0.50000); 0.12500-1.00000	2.0 (2.0-2.0)
D3: hydrogen peroxide	MDR	158	0.25000 (0.12500-0.25000); 0.06250-0.50000	0.50000 (0.50000-1.00000); 0.12500-1.00000	2.0 (2.0-2.0)
D3: hydrogen peroxide	Non-MDR	88	0.12500 (0.12500-0.25000); 0.06250-0.50000	0.25000 (0.25000-0.50000); 0.12500-1.00000	2.0 (2.0-2.0)

Note. Values refer to active-ingredient concentration and have no clinical breakpoint. MDR/non-MDR MIC comparisons by Mann-Whitney U: D1 $P < 0.001$; D2 $P < 0.001$; D3 $P < 0.001$.

The longer the disinfectant had contact with the surface (increased contact time), the more effective it was; and the dirtier the surface (compared against the clean surface), the less effective the disinfectant was. The sodium hypochlorite and benzalkonium chloride operational tolerance (OT) in clean conditions was 2.8% and 24.8%, respectively, at 5 minutes where the hydrogen peroxide OT was 0%. Tolerances rose to 10.6%, 28.5% and 83.3% respectively when it happened under dirty conditions at 5 minutes. The results reveal that the presence of organic soil material has a negative effect on the disinfectant activity and that benzalkonium chloride was the least effective disinfectant under the conditions tested.

Table 7. The suspension time-kill data at the labeled in-use concentrations.

Disinfectant	Soil condition	Exposure	Pairs	Log10 reduction, median (IQR); range	<5-log10 reduction, n/N (%)
D1: sodium hypochlorite	Clean	1 min	246	4.27 (3.92-4.55); 3.19-5.47	228/246 (92.7%)
D1: sodium hypochlorite	Clean	5 min	246	5.91 (5.57-6.24); 4.39-7.08	7/246 (2.8%)
D1: sodium hypochlorite	Dirty	1 min	246	3.68 (3.31-3.97); 2.25-4.81	246/246 (100.0%)
D1: sodium hypochlorite	Dirty	5 min	246	5.28 (4.95-5.56); 3.83-6.46	70/246 (28.5%)

Disinfectant	Soil condition	Exposure	Pairs	Log10 reduction, median (IQR); range	<5-log10 reduction, n/N (%)
D2: benzalkonium chloride	Clean	1 min	246	3.47 (3.14-3.84); 2.15-4.95	246/246 (100.0%)
D2: benzalkonium chloride	Clean	5 min	246	5.33 (5.00-5.68); 3.84-6.84	61/246 (24.8%)
D2: benzalkonium chloride	Dirty	1 min	246	2.72 (2.36-3.08); 1.15-3.99	246/246 (100.0%)
D2: benzalkonium chloride	Dirty	5 min	246	4.55 (4.22-4.84); 3.29-5.64	205/246 (83.3%)
D3: hydrogen peroxide	Clean	1 min	246	4.66 (4.36-5.07); 3.37-6.19	179/246 (72.8%)
D3: hydrogen peroxide	Clean	5 min	246	6.12 (5.81-6.41); 5.03-7.20	0/246 (0.0%)
D3: hydrogen peroxide	Dirty	1 min	246	4.08 (3.74-4.43); 2.71-5.31	242/246 (98.4%)
D3: hydrogen peroxide	Dirty	5 min	246	5.50 (5.23-5.83); 4.44-6.81	26/246 (10.6%)

Note. The prespecified operational tolerance endpoint was <5-log10 reduction at the labelled 5-minute contact time. The threshold is a study definition, not a clinical susceptibility breakpoint.

At both the contact times, killing was significantly lower when the biofilm was mature for all disinfectants (all paired comparisons $P < 0.001$). The mean $\log_{10}$ reduction varied between 1.73 and 2.31 indicating a significant protective effect of a biofilm state. The protection indices (PI) for benzalkonium chloride were the highest (2.30 at 1 min and 2.32 at 5 min), while those for hydrogen peroxide were the lowest, but still significant, reductions in activity. The absolute killing was enhanced after 5 min of exposure but the biofilm protection was not affected.

Table 8. Double disinfection action on planktonic and mature-biofilm.

Disinfectant	Exposure	Pairs	Planktonic LR, median (IQR)	Biofilm LR, median (IQR)	Mean paired difference (95% CI); P	Protection index, median (IQR)
D1: sodium hypochlorite	1 min	96	4.29 (3.91-4.57)	2.40 (1.96-2.77)	1.88 (1.82-1.95); $P < 0.001$	1.90 (1.64-2.11)
D1: sodium hypochlorite	5 min	96	5.97 (5.52-6.29)	4.06 (3.64-4.67)	1.83 (1.77-1.88); $P < 0.001$	1.85 (1.64-2.02)
D2: benzalkonium chloride	1 min	96	3.47 (3.14-3.89)	1.21 (0.74-1.64)	2.28 (2.22-2.34); $P < 0.001$	2.30 (2.07-2.47)
D2: benzalkonium chloride	5 min	96	5.35 (5.02-5.69)	3.03 (2.47-3.54)	2.31 (2.24-2.37); $P < 0.001$	2.32 (2.11-2.53)
D3: hydrogen peroxide	1 min	96	4.68 (4.40-5.12)	3.02 (2.60-3.45)	1.73 (1.66-1.79); $P < 0.001$	1.77 (1.52-1.89)
D3: hydrogen peroxide	5 min	96	6.21 (5.87-6.55)	4.42 (3.93-4.84)	1.77 (1.71-1.83); $P < 0.001$	1.82 (1.56-1.97)

Note. Subset: 96 isolates (48 MDR including XDR; 48 non-MDR). Positive protection index = lower killing in the mature-biofilm state.

An operational disinfectant tolerance at 5 minutes was independently associated with higher biofilm OD, MDR status, Gram-negative identity and disinfectant product. The odds were increased by 39% (adjusted OR 1.39; $P < 0.001$) for each 0.10 increase in OD, while the odds were increased by 2.50- and 2.65-fold for MDR and Gram-negative isolates, respectively. Benzalkonium chloride showed a significant increase in the odds (OR 30.00; $P < 0.001$) and hydrogen peroxide showed a significant decrease in the odds (OR 0.22; $P < 0.001$) when compared with sodium hypochlorite. After adjustment, hospital, ward risk, soil origin of cleaning and visible soil at the source were not significant. Model discrimination was good (AUC=0.910) although external validation would be necessary before applying the model for prediction.

Table 9. Multivariable logistic model for operational disinfectant tolerance that is cluster-robust.

Predictor	Contrast/unit	Adjusted OR	95% CI	P value
Biofilm OD per 0.10	Biofilm OD per 0.10	1.39	1.27-1.51	<0.001
MDR status	MDR vs non-MDR	2.50	1.49-4.20	<0.001
Organism group	Gram-negative vs Gram-positive	2.65	1.58-4.46	<0.001
Disinfectant	D2 vs D1	30.00	17.41-51.69	<0.001
Disinfectant	D3 vs D1	0.22	0.12-0.39	<0.001
Hospital	Al-Hilla Surgical vs Imam Al-Sadiq	1.02	0.66-1.58	0.920
Ward risk	High-risk vs another ward	1.00	0.65-1.54	0.994
Cleaning origin	Post-cleaning vs pre-cleaning origin	0.80	0.50-1.30	0.374
Visible soil at origin	Visible soil at origin	0.88	0.47-1.62	0.671

Note. Outcome: <5-log10 reduction under dirty conditions at 5 minutes. Observations=738; events=301; isolate clusters=246; complete cases=738; AUC=0.910; calibration slope=1.000.

An independent cleaning effect was seen as the adjusted prevalence of culture positivity of before-cleaning swabs was 1.88 times that of post-cleaning swabs (95% CI: 1.59–2.23; $P < 0.001$). Faucet handles also were independently associated with an increase in positivity compared with other surfaces (adjusted prevalence ratio 1.27; 95% CI: 1.07 – 1.51; $P=0.007$). After adjustment, hospital, ward, controls for shared equipment and visible soil were not statistically significant. Thus, the major independent factors that were included in this model were cleaning status and the location of the faucet-handle.

Table 10. The surface culture positivity was modelled using modified Poisson.

Predictor	Contrast	Adjusted prevalence ratio	95% CI	P value
Cleaning status	Pre-cleaning vs post-cleaning	1.88	1.59-2.23	<0.001
Hospital	Al-Hilla Surgical vs Imam Al-Sadiq	0.98	0.80-1.20	0.855
Ward	ICU vs dialysis	0.90	0.58-1.42	0.663
Ward	Emergency vs dialysis	0.96	0.57-1.61	0.867
Ward	Operating theatre vs dialysis	0.69	0.41-1.14	0.147
Ward	Medical ward vs dialysis	0.82	0.52-1.28	0.379
Ward	Surgical ward vs dialysis	0.93	0.59-1.46	0.740
Surface	Shared equipment controls vs other surfaces	1.04	0.84-1.30	0.708
Surface	Faucet handle vs other surfaces	1.27	1.07-1.51	0.007

Predictor	Contrast	Adjusted prevalence ratio	95% CI	P value
Visible soil	Visible soil	1.05	0.90-1.21	0.548

Note. Analysis unit: swab (N=360). Variance was clustered by 12 hospital-ward strata. All listed covariates were retained a priori.

All of the sampling and laboratory quality indicators were met that were prespecified. All field blanks were negative, transport times were within the 6-hour criterion, 97.2% of the isolates were accepted for the first run VITEK 2 identification and 96.8% were categorically accepted for the repeat AST with no very major discrepancy. Technical precision for biofilm assays was good and repeat classification (weighted kappa) was excellent (0.91) as were neutralization and time-kill repeatability criteria. Full primary-outcome data also help to alleviate worry of contamination, measurement instability or missing-data bias.

Table 11. Sampling and quality-assurance indicators in the laboratory.

Quality domain	Denominator	Result	Assessment
Field blanks	18 sampling-session blanks	0/18 culture-positive	Passed
Transport interval	All 360 swabs	Median 2.1 h (IQR 1.3-3.0); maximum 5.2 h	Passed ≤6 h rule
VITEK 2 identification	246 isolates	239/246 (97.2%) acceptable on first run; 7 resolved after repeat	Passed
Repeat AST	25 isolates	96.8% categorical agreement; no very-major discrepancy	Passed
Biofilm technical precision	246 isolates x 3 experiments	Median within-run CV 7.8% (IQR 5.2-10.6)	Passed <15% rule
Biofilm repeat classification	25 blinded repeats	23/25 exact category agreement; weighted kappa 0.91	Passed
Neutralizer efficacy/toxicity	18 product-organism-condition checks	18/18 met predefined ≤0.5-log recovery difference	Passed
Time-kill repeatability	25 isolate-product pairs	Median absolute between-run difference 0.22 log ₁₀	Passed
Primary outcome missingness	738 dirty-condition 5-min pairs	0/738 missing	Complete

Significant and organism- and drug-specific resistance was observed with gram-negative isolates. *A. baumannii* exhibited a high level of resistance to ceftazidime and cefepime (62.5% each), and around 48-50% resistance to the carbapenems. The highest resistance for *K. pneumoniae* was observed for ceftazidime (57.6%) and imipenem (51.5%) and for *E. coli* it was trimethoprim-sulfamethoxazole (59.3%) and ceftazidime (55.6%). The high resistance was observed for piperacillin-tazobactam (65.5%) and ceftazidime (58.6%) in *P. aeruginosa*. Generally, susceptibility to the carbapenems was higher in *Enterobacter* spp. but results for the small group of other Gram-negative (N=9) should be interpreted with caution.

Supplementary Table S1. Antimicrobial susceptibility of Gram-negative isolates (drug level).

Species	Antimicrobial	N	Susceptible	Intermediate	Resistant
<i>Acinetobacter baumannii</i>	Amikacin	48	25/48 (52.1%)	3/48 (6.2%)	20/48 (41.7%)
<i>Acinetobacter baumannii</i>	Gentamicin	48	19/48 (39.6%)	2/48 (4.2%)	27/48 (56.2%)
<i>Acinetobacter baumannii</i>	Ciprofloxacin	48	19/48 (39.6%)	3/48 (6.2%)	26/48 (54.2%)

Species	Antimicrobial	N	Susceptible	Intermediate	Resistant
<i>Acinetobacter baumannii</i>	Ceftazidime	48	13/48 (27.1%)	5/48 (10.4%)	30/48 (62.5%)
<i>Acinetobacter baumannii</i>	Cefepime	48	14/48 (29.2%)	4/48 (8.3%)	30/48 (62.5%)
<i>Acinetobacter baumannii</i>	Imipenem	48	22/48 (45.8%)	3/48 (6.2%)	23/48 (47.9%)
<i>Acinetobacter baumannii</i>	Meropenem	48	24/48 (50.0%)	0/48 (0.0%)	24/48 (50.0%)
<i>Acinetobacter baumannii</i>	Trimethoprim-sulfamethoxazole	48	24/48 (50.0%)	0/48 (0.0%)	24/48 (50.0%)
<i>Klebsiella pneumoniae</i>	Amikacin	33	19/33 (57.6%)	2/33 (6.1%)	12/33 (36.4%)
<i>Klebsiella pneumoniae</i>	Gentamicin	33	17/33 (51.5%)	0/33 (0.0%)	16/33 (48.5%)
<i>Klebsiella pneumoniae</i>	Ciprofloxacin	33	18/33 (54.5%)	0/33 (0.0%)	15/33 (45.5%)
<i>Klebsiella pneumoniae</i>	Ceftriaxone	33	15/33 (45.5%)	3/33 (9.1%)	15/33 (45.5%)
<i>Klebsiella pneumoniae</i>	Ceftazidime	33	13/33 (39.4%)	1/33 (3.0%)	19/33 (57.6%)
<i>Klebsiella pneumoniae</i>	Cefepime	33	17/33 (51.5%)	3/33 (9.1%)	13/33 (39.4%)
<i>Klebsiella pneumoniae</i>	Imipenem	33	14/33 (42.4%)	2/33 (6.1%)	17/33 (51.5%)
<i>Klebsiella pneumoniae</i>	Meropenem	33	21/33 (63.6%)	2/33 (6.1%)	10/33 (30.3%)
<i>Klebsiella pneumoniae</i>	Piperacillin-tazobactam	33	20/33 (60.6%)	0/33 (0.0%)	13/33 (39.4%)
<i>Enterobacter</i> spp.	Amikacin	25	17/25 (68.0%)	1/25 (4.0%)	7/25 (28.0%)
<i>Enterobacter</i> spp.	Gentamicin	25	16/25 (64.0%)	3/25 (12.0%)	6/25 (24.0%)
<i>Enterobacter</i> spp.	Ciprofloxacin	25	16/25 (64.0%)	2/25 (8.0%)	7/25 (28.0%)
<i>Enterobacter</i> spp.	Cefepime	25	17/25 (68.0%)	3/25 (12.0%)	5/25 (20.0%)
<i>Enterobacter</i> spp.	Imipenem	25	19/25 (76.0%)	0/25 (0.0%)	6/25 (24.0%)
<i>Enterobacter</i> spp.	Meropenem	25	21/25 (84.0%)	0/25 (0.0%)	4/25 (16.0%)
<i>Enterobacter</i> spp.	Piperacillin-tazobactam	25	15/25 (60.0%)	4/25 (16.0%)	6/25 (24.0%)
<i>Enterobacter</i> spp.	Trimethoprim-sulfamethoxazole	25	16/25 (64.0%)	1/25 (4.0%)	8/25 (32.0%)
<i>Pseudomonas aeruginosa</i>	Amikacin	29	20/29 (69.0%)	1/29 (3.4%)	8/29 (27.6%)
<i>Pseudomonas aeruginosa</i>	Gentamicin	29	20/29 (69.0%)	0/29 (0.0%)	9/29 (31.0%)
<i>Pseudomonas aeruginosa</i>	Ciprofloxacin	29	14/29 (48.3%)	1/29 (3.4%)	14/29 (48.3%)
<i>Pseudomonas aeruginosa</i>	Ceftazidime	29	11/29 (37.9%)	1/29 (3.4%)	17/29 (58.6%)

Species	Antimicrobial	N	Susceptible	Intermediate	Resistant
<i>Pseudomonas aeruginosa</i>	Cefepime	29	18/29 (62.1%)	1/29 (3.4%)	10/29 (34.5%)
<i>Pseudomonas aeruginosa</i>	Imipenem	29	15/29 (51.7%)	2/29 (6.9%)	12/29 (41.4%)
<i>Pseudomonas aeruginosa</i>	Meropenem	29	15/29 (51.7%)	1/29 (3.4%)	13/29 (44.8%)
<i>Pseudomonas aeruginosa</i>	Piperacillin-tazobactam	29	9/29 (31.0%)	1/29 (3.4%)	19/29 (65.5%)
Other Gram-negative bacilli	Amikacin	9	7/9 (77.8%)	0/9 (0.0%)	2/9 (22.2%)
Other Gram-negative bacilli	Gentamicin	9	4/9 (44.4%)	3/9 (33.3%)	2/9 (22.2%)
Other Gram-negative bacilli	Ciprofloxacin	9	6/9 (66.7%)	0/9 (0.0%)	3/9 (33.3%)
Other Gram-negative bacilli	Ceftazidime	9	4/9 (44.4%)	1/9 (11.1%)	4/9 (44.4%)
Other Gram-negative bacilli	Cefepime	9	4/9 (44.4%)	0/9 (0.0%)	5/9 (55.6%)
Other Gram-negative bacilli	Imipenem	9	5/9 (55.6%)	1/9 (11.1%)	3/9 (33.3%)
Other Gram-negative bacilli	Meropenem	9	3/9 (33.3%)	1/9 (11.1%)	5/9 (55.6%)
<i>Escherichia coli</i>	Amikacin	27	16/27 (59.3%)	0/27 (0.0%)	11/27 (40.7%)
<i>Escherichia coli</i>	Gentamicin	27	13/27 (48.1%)	1/27 (3.7%)	13/27 (48.1%)
<i>Escherichia coli</i>	Ciprofloxacin	27	13/27 (48.1%)	3/27 (11.1%)	11/27 (40.7%)
<i>Escherichia coli</i>	Ceftriaxone	27	12/27 (44.4%)	1/27 (3.7%)	14/27 (51.9%)
<i>Escherichia coli</i>	Ceftazidime	27	11/27 (40.7%)	1/27 (3.7%)	15/27 (55.6%)
<i>Escherichia coli</i>	Cefepime	27	17/27 (63.0%)	0/27 (0.0%)	10/27 (37.0%)
<i>Escherichia coli</i>	Imipenem	27	18/27 (66.7%)	1/27 (3.7%)	8/27 (29.6%)
<i>Escherichia coli</i>	Meropenem	27	13/27 (48.1%)	0/27 (0.0%)	14/27 (51.9%)
<i>Escherichia coli</i>	Trimethoprim-sulfamethoxazole	27	10/27 (37.0%)	1/27 (3.7%)	16/27 (59.3%)

Note. S/I/R were interpreted with CLSI M100, 36th edition. Denominators are organism-drug specific; intrinsic resistance is not displayed as acquired resistance.

Cefoxitin resistance was seen in 41.7% of *S. aureus* and 34.5% of coagulase-negative staphylococci of Gram-positive isolates, indicating a considerable burden of the methicillin-resistant phenotype. There was also significant resistance seen for *S. aureus* to erythromycin (41.7%), ciprofloxacin (38.9%) and clindamycin (36.1%) while linezolid remained the most active (88.9% susceptible). Coagulase-negative staphylococci were still very susceptible to linezolid (93.1%) and fairly susceptible to vancomycin and gentamicin (82.8% each). With a small sample (N=10), there was 100% susceptibility to teicoplanin and 90% to ampicillin, high level gentamicin and vancomycin.

Supplementary Table S2. Susceptibility to antimicrobials of Gram-positive isolates according to their level of drug.

Species	Antimicrobial	N	Susceptible	Intermediate	Resistant
Coagulase-negative staphylococci	Cefoxitin	29	17/29 (58.6%)	2/29 (6.9%)	10/29 (34.5%)
Coagulase-negative staphylococci	Gentamicin	29	24/29 (82.8%)	1/29 (3.4%)	4/29 (13.8%)
Coagulase-negative staphylococci	Ciprofloxacin	29	22/29 (75.9%)	1/29 (3.4%)	6/29 (20.7%)

Coagulase-negative staphylococci	Erythromycin	29	18/29 (62.1%)	1/29 (3.4%)	10/29 (34.5%)
Coagulase-negative staphylococci	Clindamycin	29	19/29 (65.5%)	1/29 (3.4%)	9/29 (31.0%)
Coagulase-negative staphylococci	Linezolid	29	27/29 (93.1%)	1/29 (3.4%)	1/29 (3.4%)
Coagulase-negative staphylococci	Vancomycin	29	24/29 (82.8%)	3/29 (10.3%)	2/29 (6.9%)
Coagulase-negative staphylococci	Trimethoprim-sulfamethoxazole	29	18/29 (62.1%)	1/29 (3.4%)	10/29 (34.5%)
<i>Staphylococcus aureus</i>	Cefoxitin	36	21/36 (58.3%)	0/36 (0.0%)	15/36 (41.7%)
<i>Staphylococcus aureus</i>	Gentamicin	36	22/36 (61.1%)	1/36 (2.8%)	13/36 (36.1%)
<i>Staphylococcus aureus</i>	Ciprofloxacin	36	20/36 (55.6%)	2/36 (5.6%)	14/36 (38.9%)
<i>Staphylococcus aureus</i>	Erythromycin	36	19/36 (52.8%)	2/36 (5.6%)	15/36 (41.7%)
<i>Staphylococcus aureus</i>	Clindamycin	36	21/36 (58.3%)	2/36 (5.6%)	13/36 (36.1%)
<i>Staphylococcus aureus</i>	Linezolid	36	32/36 (88.9%)	1/36 (2.8%)	3/36 (8.3%)
<i>Staphylococcus aureus</i>	Vancomycin	36	27/36 (75.0%)	4/36 (11.1%)	5/36 (13.9%)
<i>Staphylococcus aureus</i>	Trimethoprim-sulfamethoxazole	36	23/36 (63.9%)	1/36 (2.8%)	12/36 (33.3%)
Enterococcus spp.	Ampicillin	10	9/10 (90.0%)	0/10 (0.0%)	1/10 (10.0%)
Enterococcus spp.	Ciprofloxacin	10	5/10 (50.0%)	0/10 (0.0%)	5/10 (50.0%)
Enterococcus spp.	High-level gentamicin	10	9/10 (90.0%)	0/10 (0.0%)	1/10 (10.0%)
Enterococcus spp.	Linezolid	10	8/10 (80.0%)	1/10 (10.0%)	1/10 (10.0%)
Enterococcus spp.	Vancomycin	10	9/10 (90.0%)	0/10 (0.0%)	1/10 (10.0%)
Enterococcus spp.	Teicoplanin	10	10/10 (100.0%)	0/10 (0.0%)	0/10 (0.0%)

Note. Cefoxitin was used as the phenotypic surrogate for methicillin resistance in staphylococci where applicable. Vancomycin and linezolid were interpreted using organism-appropriate CLSI criteria.

The prespecified analysis map matches the research questions to the analysis unit, outcome, predictors, statistical model and effect estimate. Cluster-robust methods are used to account for the non-independence of observations and paired analysis is used for matched planktonic–biofilm comparisons. To interpret the results transparently, reporting adjusted prevalence ratios, odds ratios, adjusted differences, and/or mean paired differences with 95% confidence intervals is helpful. This table is not a results table; it is a methodological table and it will help readers follow the reported results to their planned models.

Supplementary Table S3. Prespecified analysis map

Analysis	Unit	Outcome	Main predictors	Model	Report
Surface positivity	Swab	Culture positive	Hospital; ward; surface; cleaning; visible soil	Modified Poisson with cluster-robust variance	Adjusted prevalence ratio, 95% CI
Surface burden	Swab	log ₁₀ (CFU/100 cm ² + 1)	Hospital; ward; surface; cleaning	Robust summaries; sensitivity regression	Median (IQR); adjusted difference
Biofilm strength	Isolate	Continuous OD; moderate/strong	MDR/XDR; Gram group; hospital	Cluster-robust logistic model	Adjusted OR, 95% CI
Disinfectant killing	Isolate-product-condition	Log ₁₀ reduction	OD; MDR; product; soil; time; organism group	Cluster-aware regression and paired analyses	Adjusted difference / OR, 95% CI
Operational tolerance	Isolate-product	<5-log ₁₀ at 5 min	OD; MDR; Gram group; product; hospital; ward	Cluster-robust logistic regression	Adjusted OR, 95% CI
Biofilm protection	Matched isolate-product	Planktonic LR - biofilm LR	Product; time; MDR	Paired comparison	Mean difference, 95% CI

Discussion

This two-hospital environmental study combined four different culture-positive methods to assess antibiotic-resistance phenotype, quantitative biofilm biomass, and disinfectant killing by neutralizer validation. Over half of the high touch surfaces were culture-positive. The adjusted prevalence of positivity was nearly twice as high in pre-cleaning samples than in post-cleaning samples and faucet handles were independently associated with a higher prevalence of positivity[25]. Nearly two thirds of the isolates were MDR, and nearly three quarters were able to produce detectable biofilm with moderate or strong biofilm being significantly more common in the MDR and XDR isolates[26]. The general surface positivity (55.6%) falls within the broad limits found in environmental studies but care must be taken in comparing directly due to the differences in recovery media, area sampled, sampling time, enrichment, target surfaces, and definitions[27][28][29]. The marked difference between pre-cleaning and post-cleaning (72.8% compared to 38.3% with an adjusted prevalence ratio of 1.88) not only underlines the process value of routine cleaning but also shows that culture positivity was not eradicated[30]. This finding should not be used as an estimate of the efficacy of a specific cleaning program; sampling was observational. The most common organism isolated was *Acinetobacter baumannii*, followed by the isolation of *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*[31]. This distribution is generally in agreement with the results of the many hospital-surface studies conducted in the region and internationally, which all have found non-fermenting Gram-negative bacilli, Enterobacterales and staphylococci[32][33][34][35]. But if the environment is recovered, then that's not proof of patient acquisition. The results can only determine the phenotypically important reservoirs without associated patient isolates and strain typing[36]. The rate of MDR was 64.2% and that of XDR was 8.9%. A pooled percentage is less informative than an organism-specific interpretation as there are differences in the number of antimicrobial categories and intrinsic resistance between taxa[37]. The highest number of MDR were found in *A. baumannii* and *K. pneumoniae*[38]. Current CLSI interpretation and the internationally accepted category-based MDR framework will help increase internal reproducibility[39] without affecting the cross-study comparison, which is dependent on the antimicrobial pressure in the region and the panels tested[40]. Of the isolates 78.5% produced biofilm

and 60.2% were moderate or strong producers. Moderate/strong biofilm was associated with MDR non-XDR isolates (3.62-fold higher odds of moderate/strong biofilm, 95% CI: 1.52–8.66) and there was a higher, but imprecise, estimate for XDR isolates (15.30-fold higher odds of moderate/strong biofilm, 95% CI: 1.84–127.11), in comparison with non-MDR isolates, due to the limited number of XDR isolates available (22). New studies on the hospital devices and the environment have also found a high biofilm capacity in resistant organisms[41]. While this is a biological association, the cross-sectional phenotype does not imply that biofilm formation caused antibiotic resistance, nor does it imply that it is a single mechanism that causes both[42]. Results of disinfectant tests varied by formulation. Median reductions under dirty conditions were 5.28 log₁₀ for sodium hypochlorite, 4.55 log₁₀ for benzalkonium chloride and 5.50 log₁₀ for hydrogen peroxide. Isolate-product pairs with failure to reach 5 log₁₀ were found in 28.5%, 83.3% and 10.6% of the cases, respectively. Heterogeneous disinfectant MIC/MBC and survival phenotypes were observed in environmental *A. baumannii* and other hospital isolates in previous studies[43]. Recently, the same group working with *P. aeruginosa* has corroborated the need to consider the complete formulation and not extrapolate from the chemical class, as some QAC products have failed to perform as well as some hydrogen-peroxide and hypochlorite products against standardized *S. aureus* and *P. aeruginosa* biofilms[44].

The consistently lower killing observed in the mature-biofilm experiment compared to matched planktonic testing is likely due to the increased complexity of the cell-cell interactions in the mature-biofilm environment. For D1, D2 and D3, mean reductions attributed to the presence of the biofilm were 1.83 log₁₀, 2.31 log₁₀ and 1.77 log₁₀ respectively at the labelled contact time. This is of operational importance since matrix protection is not reproduced by the planktonic MIC, MBC and suspension endpoints. The protection index is not a clinical resistance breakpoint, but is an indication of the killing reduction under the experimental biofilm condition. Each 0.10 increase in the biofilm OD was associated with 39% increase in the adjusted odds of operational tolerance in the multivariable model. Product identity also had the greatest effect and was an independent correlate as were MDR and Gram-negative status. Hospital, ward-risk category, cleaning status at the source surface and visible soil at isolation were not significantly associated with the standardized laboratory endpoint. These results differentiate the characteristics of the recovered isolate and test product from environmental factors that may have influenced the surface recovery. The bottom line is not to increase the concentration more than what's recommended on the label, or to combine products. IPC programs should confirm the following: the presence of dilution, preparation, storage, and coverage; neutralization is confirmed when testing recently disinfected surfaces; and environmental culture is used in addition to direct process auditing[45][46].

Conclusion

There was a significant amount of bacterial contamination of high-touch surfaces in both teaching hospitals, especially prior to routine cleaning. MDR organisms were prevalent, and there was an independent correlation between the biomass of the biofilm and decreased killing of disinfectants. The benzalkonium chloride formulation had the lowest median log₁₀ reduction and the highest operational tolerance frequency, while the sodium hypochlorite and hydrogen peroxide formulations had lower operational tolerance frequencies and a higher log₁₀ reduction and did not result in tolerance for every isolate-product combination. The killing of the cells in mature biofilms was about 1.8-2.3 log₁₀ lower than killing of the matched planktonic cells. These data can be used to verify product efficacy, correct dilutions, remove organic soil and provide 100% wet contact time in environmental-cleaning audit programs. They do not define clinically transmitted or warrant the use of the term "disinfectant resistance" in the absence of a standard and a breakpoint.

References

- [1] A. P. Christoff, A. F. R. Sereia, G. N. F. Cruz, D. C. Bastiani, V. L. Silva, C. Hernandez, *et al.*, "One-year cross-sectional study in adult and neonatal intensive care units reveals the bacterial and antimicrobial resistance gene profiles in patients and hospital surfaces," *PLoS One*, vol. 15, no. 6, Art. no. e0234127, 2020, doi: 10.1371/journal.pone.0234127.
- [2] S. Saadi, R. Allem, M. Sebahia, A. Merouane, and M. Bakkali, "Bacterial contamination of

- neglected hospital surfaces and equipment in an Algerian hospital: An important source of potential infection," *Int. J. Environ. Health Res.*, vol. 32, no. 6, pp. 1373–1381, 2022, doi: 10.1080/09603123.2021.1885631.
- [3] H. L. Yosef, M. Yohannes, R. Diriba, and M. H. Legese, "Multidrug-resistant bacterial profiles of inanimate objects at Zewditu Memorial Hospital, Addis Ababa, Ethiopia," *PLoS One*, vol. 20, no. 10, Art. no. e0333361, 2025, doi: 10.1371/journal.pone.0333361.
 - [4] A. Dihmane, R. Aniba, H. Raqraq, A. Ressmi, *et al.*, "Surface contamination by multidrug-resistant Gram-negative bacteria in a healthcare facility: Resistance determinants and biofilm-associated adhesion," *Int. J. Microbiol.*, vol. 2026, Art. no. 5734443, 2026, doi: 10.1155/ijm/5734443.
 - [5] S. M. Ali, T. A. H. Soor, G. A. Ahmed, G. A. Mhdin, G. A. K. Othman, and S. M. Faiq, "Distribution and molecular characterization of antibiotic-resistant *Pseudomonas aeruginosa* in hospital settings of Sulaymaniyah, Iraq," *Pol. J. Microbiol.*, vol. 73, no. 4, pp. 467–473, 2024, doi: 10.33073/pjm-2024-037.
 - [6] K. Bitew, M. Getachew, and A. Abdeta, "Bacterial contamination rates and drug susceptibility patterns of bacteria recovered from medical equipment, inanimate surfaces, and indoor air of a hospital in Ethiopia," *Int. J. Microbiol.*, vol. 2022, Art. no. 5746344, 2022, PMID: 35757831.
 - [7] R. Lordelo, R. Branco, F. Gama, and P. V. Morais, "Assessment of antimicrobial resistance, biofilm formation, and surface modification potential in hospital strains of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*," *Heliyon*, vol. 10, no. 9, Art. no. e30464, 2024, doi: 10.1016/j.heliyon.2024.e30464.
 - [8] I. Cosimato, G. Di Siervi, M. De Prisco, F. Dell' Annunziata, N. Capuano, N. Cafà, *et al.*, "Investigation of biofilm formation and antimicrobial resistance in bacteria isolated from hospital medical devices," *Microorganisms*, vol. 14, no. 7, Art. no. 1429, 2026, doi: 10.3390/microorganisms14071429.
 - [9] H. Ben-Amram, M. Azrad, J. Cohen-Assodi, N. Sharabi-Nov, S. Edelstein, K. Agay-Shay, *et al.*, "Biofilm formation by hospital-acquired resistant bacteria isolated from respiratory samples," *J. Epidemiol. Glob. Health*, vol. 14, no. 2, pp. 291–297, 2024, doi: 10.1007/s44197-024-00215-7.
 - [10] P. Pallós, M. Gajdács, E. Urbán, Y. Szabados, K. Szalai, L. Hevesi, *et al.*, "Characterization of antibiotic and disinfectant susceptibility in biofilm-forming *Acinetobacter baumannii*: A focus on environmental isolates," *Eur. J. Microbiol. Immunol.*, vol. 14, no. 2, pp. 126–133, 2024, doi: 10.1556/1886.2024.00014.
 - [11] P. Rakshit, A. Singh, R. Singh, and T. Banerjee, "An in-depth study on survival mechanisms of bacterial isolates following exposure to disinfectants within the hospital environment," *Front. Cell. Infect. Microbiol.*, vol. 14, Art. no. 1442914, 2024, doi: 10.3389/fcimb.2024.1442914.
 - [12] C. B. Lineback, C. A. Nkemngong, S. T. Wu, X. Li, P. J. Teska, and H. F. Oliver, "Hydrogen peroxide and sodium hypochlorite disinfectants are more effective against *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilms than quaternary ammonium compounds," *Antimicrob. Resist. Infect. Control*, vol. 7, Art. no. 154, 2018, doi: 10.1186/s13756-018-0447-5.
 - [13] C. A. Sanchez, G. G. Vargas-Cuebas, M. E. Michaud, R. A. Allen, K. R. Morrison-Lewis, S. Siddiqui, *et al.*, "Highly effective biocides against *Pseudomonas aeruginosa* reveal new mechanistic insights across Gram-negative bacteria," *ACS Infect. Dis.*, vol. 10, no. 11, pp. 3868–3879, 2024, doi: 10.1021/acsinfecdis.4c00433.
 - [14] S. Tyski, W. Grzybowska, and E. Bocian, "Application of EN 16615 four-field test for evaluation of antimicrobial activity of selected commercial and self-made disinfectant wipes," *Int. J. Environ. Res. Public Health*, vol. 18, no. 11, Art. no. 5932, 2021, doi: 10.3390/ijerph18115932.
 - [15] E. von Elm, D. G. Altman, M. Egger, S. J. Pocock, P. C. Gøtzsche, and J. P. Vandenbroucke, "The Strengthening the Reporting of Observational Studies in Epidemiology statement: Guidelines for reporting observational studies," *PLoS Med.*, vol. 4, no. 10, Art. no. e296, 2007, doi: 10.1371/journal.pmed.0040296.

- [16] Clinical and Laboratory Standards Institute, *Performance Standards for Antimicrobial Susceptibility Testing*, 36th ed., CLSI Supplement M100. Wayne, PA, USA: CLSI, 2026.
- [17] Clinical and Laboratory Standards Institute, *Performance Standards for Antimicrobial Disk Susceptibility Tests*, 14th ed., CLSI Standard M02. Wayne, PA, USA: CLSI, 2024.
- [18] Clinical and Laboratory Standards Institute, *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*, 12th ed., CLSI Standard M07. Wayne, PA, USA: CLSI, 2024.
- [19] A. P. Magiorakos, A. Srinivasan, R. B. Carey, Y. Carmeli, M. E. Falagas, C. G. Giske, *et al.*, "Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance," *Clin. Microbiol. Infect.*, vol. 18, no. 3, pp. 268–281, 2012, doi: 10.1111/j.1469-0691.2011.03570.x.
- [20] S. Stepanović, D. Vuković, V. Hola, G. Di Bonaventura, S. Djukić, I. Ćirković, and F. Růžička, "Quantification of biofilm in microtiter plates: Overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci," *APMIS*, vol. 115, no. 8, pp. 891–899, 2007, doi: 10.1111/j.1600-0463.2007.apm_630.x.
- [21] ASTM International, *ASTM E1054-22: Standard Practices for Evaluation of Inactivators of Antimicrobial Agents*. West Conshohocken, PA, USA: ASTM International, 2022.
- [22] ASTM International, *ASTM E2315-23: Standard Guide for Assessment of Antimicrobial Activity Using a Time-Kill Procedure*. West Conshohocken, PA, USA: ASTM International, 2023.
- [23] International Organization for Standardization, *ISO 18593:2018, Microbiology of the Food Chain — Horizontal Methods for Surface Sampling*. Geneva, Switzerland: ISO, 2018.
- [24] European Committee for Standardization, *EN 16615:2015, Chemical Disinfectants and Antiseptics — Quantitative Test Method for the Evaluation of Bactericidal and Yeasticidal Activity on Non-Porous Surfaces with Mechanical Action Employing Wipes in the Medical Area*. Brussels, Belgium: CEN, 2015.
- [25] J. A. Otter, S. Yezli, and G. L. French, "The role played by contaminated surfaces in the transmission of nosocomial pathogens," *Infect. Control Hosp. Epidemiol.*, vol. 32, no. 7, pp. 687–699, 2011, doi: 10.1086/660363.
- [26] L. Cobrado, A. Silva-Dias, M. M. Azevedo, and A. G. Rodrigues, "High-touch surfaces: Microbial neighbours at hand," *Eur. J. Clin. Microbiol. Infect. Dis.*, vol. 36, no. 11, pp. 2053–2062, 2017, doi: 10.1007/s10096-017-3042-1.
- [27] H. Hu, K. Johani, I. B. Gosbell, A. S. W. Jacombs, A. Almatroudi, G. S. Whiteley, *et al.*, "Intensive care unit environmental surfaces are contaminated by multidrug-resistant bacteria in biofilms: Combined results of conventional culture, pyrosequencing, scanning electron microscopy, and confocal laser microscopy," *J. Hosp. Infect.*, vol. 91, no. 1, pp. 35–44, 2015, doi: 10.1016/j.jhin.2015.05.016.
- [28] A. S. van der Schoor, J. A. Severin, C. H. W. Klaassen, J. P. C. van den Akker, M. J. Bruno, and M. C. Vos, "Environmental sampling of innate hospital surfaces: A survey of current practices and the need for guidelines," *J. Hosp. Infect.*, vol. 127, pp. 92–100, 2022, PMID: 35944791.
- [29] R. E. Thomas, B. C. Thomas, J. Conly, and D. Lorenzetti, "Cleaning and disinfecting surfaces in hospitals and long-term care facilities for reducing hospital- and facility-acquired bacterial and viral infections," *Cochrane Database Syst. Rev.*, vol. 1, no. 1, Art. no. CD012590, 2022, doi: 10.1002/14651858.CD012590.pub2.
- [30] V. Russotto, A. Cortegiani, S. M. Raineri, and A. Giarratano, "Bacterial contamination of inanimate surfaces and equipment in the intensive care unit," *J. Intensive Care*, vol. 3, Art. no. 54, 2015, doi: 10.1186/s40560-015-0120-5.
- [31] D. J. Weber, D. Anderson, and W. A. Rutala, "The role of the surface environment in healthcare-associated infections," *Curr. Opin. Infect. Dis.*, vol. 26, no. 4, pp. 338–344, 2013, doi: 10.1097/QCO.0b013e3283630f04.
- [32] S. J. Dancer, "Controlling hospital-acquired infection: Focus on the role of the environment and

- new technologies for decontamination," *Clin. Microbiol. Rev.*, vol. 27, no. 4, pp. 665–690, 2014, doi: 10.1128/CMR.00020-14.
- [33] P. A. de Campos, S. Royer, D. W. da Fonseca Batistão, B. F. Araújo, L. L. Queiroz, C. S. de Brito, *et al.*, "Multidrug resistance related to biofilm formation in *Acinetobacter baumannii* and *Klebsiella pneumoniae* clinical strains from different pulsotypes," *Curr. Microbiol.*, vol. 72, no. 5, pp. 617–627, 2016, doi: 10.1007/s00284-016-0996-x.
- [34] A. M. Bardbari, M. R. Arabestani, M. Karami, F. Keramat, M. Y. Alikhani, and K. P. Bagheri, "Correlation between the ability of biofilm formation, responsible genes and MDR patterns in clinical and environmental *Acinetobacter baumannii* isolates," *Microb. Pathog.*, vol. 108, pp. 122–128, 2017, doi: 10.1016/j.micpath.2017.04.039.
- [35] H. W. Lee, Y. M. Koh, J. Kim, J. C. Lee, Y. C. Lee, S. Y. Seol, *et al.*, "Capacity of multidrug-resistant clinical isolates of *Acinetobacter baumannii* to form biofilm and adhere to epithelial cell surfaces," *Clin. Microbiol. Infect.*, vol. 14, no. 1, pp. 49–54, 2008, doi: 10.1111/j.1469-0691.2007.01842.x.
- [36] S. J. Kaiser, N. T. Mutters, A. DeRosa, C. Ewers, U. Frank, and F. Günther, "Determinants for persistence of *Pseudomonas aeruginosa* in hospitals: Interplay between resistance, virulence and biofilm formation," *Eur. J. Clin. Microbiol. Infect. Dis.*, vol. 36, no. 2, pp. 243–253, 2017, doi: 10.1007/s10096-016-2792-8.
- [37] P. Espinal, S. Martí, and J. Vila, "Effect of biofilm formation on the survival of *Acinetobacter baumannii* on dry surfaces," *J. Hosp. Infect.*, vol. 80, no. 1, pp. 56–60, 2012, doi: 10.1016/j.jhin.2011.08.013.
- [38] K. Kawamura-Sato, J. Wachino, T. Kondo, H. Ito, and Y. Arakawa, "Reduction of disinfectant bactericidal activities in clinically isolated *Acinetobacter* species in the presence of organic material," *J. Antimicrob. Chemother.*, vol. 61, no. 3, pp. 568–576, 2008, doi: 10.1093/jac/dkm498.
- [39] K. Toté, T. Horemans, D. Vanden Berghe, L. Maes, and P. Cos, "Inhibitory effect of biocides on the viable masses and matrices of *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilms," *Appl. Environ. Microbiol.*, vol. 76, no. 10, pp. 3135–3142, 2010, doi: 10.1128/AEM.02095-09.
- [40] H.-C. Flemming and J. Wingender, "The biofilm matrix," *Nat. Rev. Microbiol.*, vol. 8, no. 9, pp. 623–633, 2010, doi: 10.1038/nrmicro2415.
- [41] A. Bridier, R. Briandet, V. Thomas, and F. Dubois-Brissonnet, "Resistance of bacterial biofilms to disinfectants: A review," *Biofouling*, vol. 27, no. 9, pp. 1017–1032, 2011, doi: 10.1080/08927014.2011.626899.
- [42] J. A. Otter, K. Vickery, J. T. Walker, E. deLancey Pulcini, P. Stoodley, S. D. Goldenberg, *et al.*, "Surface-attached cells, biofilms and biocide susceptibility: Implications for hospital cleaning and disinfection," *J. Hosp. Infect.*, vol. 89, no. 1, pp. 16–27, 2015, doi: 10.1016/j.jhin.2014.09.008.
- [43] J. M. Boyce, "Modern technologies for improving cleaning and disinfection of environmental surfaces in hospitals," *Antimicrob. Resist. Infect. Control*, vol. 5, Art. no. 10, 2016, doi: 10.1186/s13756-016-0111-x.
- [44] W. A. Rutala and D. J. Weber, "Monitoring and improving the effectiveness of surface cleaning and disinfection," *Am. J. Infect. Control*, vol. 44, no. 5, Suppl., pp. e69–e76, 2016, doi: 10.1016/j.ajic.2015.10.039.
- [45] S. Rawlinson, L. Ciric, and E. Cloutman-Green, "How to carry out microbiological sampling of healthcare environment surfaces? A review of current evidence," *J. Hosp. Infect.*, vol. 103, no. 4, pp. 363–374, 2019, doi: 10.1016/j.jhin.2019.07.015.
- [46] W. A. Rutala, H. Kanamori, M. F. Gergen, L. P. Knelson, E. E. Sickbert-Bennett, L. F. Chen, *et al.*, "Enhanced disinfection leads to reduction of microbial contamination and a decrease in patient colonization and infection," *Infect. Control Hosp. Epidemiol.*, vol. 39, no. 9, pp. 1118–1121, 2018, doi: 10.1017/ice.2018.165.