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Evaluation of Disinfectant Efficacy and Antimicrobial Resistance in Nosocomial Pathogens with Emphasis on *Pseudomonas aeruginosa*

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ABSTRACT

Background: This study aimed to assess the efficacy of selected disinfectants in Benghazi Medical Center hospital in Libya.

Materials & Methods: A total of 64 swabs were collected from healthcare workers' hands, and 96 samples were collected from the hospital environment. Five bacterial species isolated from these samples were selected to investigate the bactericidal efficacy of five disinfectants. Bacterial suspensions were prepared in 9 mL of broth medium and mixed with 1 mL of each disinfectant. All inoculated nutrient agar and MacConkey agar plates were incubated overnight at 37 °C. Bactericidal efficacy was assessed based on colony growth. Phenotypic expression of ESBLs (extended spectrum beta lactamases) and MBLs (metallo beta-lactamases) was assessed in all bacterial isolates. Antimicrobial resistance genes were detected by PCR (polymerase chain reaction).

Findings: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Micrococcus* spp., and *Klebsiella pneumonia* were isolated and identified by microbiological and biochemical tests. All isolates were found to be multidrug-resistant (MDR). The tested disinfectants demonstrated high bactericidal efficacy against these isolates. However, *P. aeruginosa* was not affected by hydrogen peroxide and alcohol, and methicillin-resistant *S. aureus* survived in iodine-containing medium. Notably, *P. aeruginosa* isolates were detected as MDR strains in various hospital environments, including floors, wash basins, and even people's hands and disinfectant bottles.

Conclusion: The selection of appropriate disinfectants (or chemical sterilants) is crucial and should be based on their proven efficacy against microbial agents. Proper testing is essential to ensure the effectiveness of disinfectants and antibiotics and to prevent the dissemination of resistant bacteria within hospital environments.

Keywords: Anti-Bacterial agents; Antimicrobial drug resistance; Bactericidal effect; Macrolide; *Pseudomonas aeruginosa*; Sterilization

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Introduction

Pseudomonas aeruginosa is recognized as an opportunistic pathogen, particularly in immunocompromised patients, and is considered one of the leading causes of nosocomial infections worldwide [1]. The World Health Organization (WHO) has categorized this bacterium among 'priority antibiotic-resistant pathogens' because of the diverse antimicrobial resistance mechanisms it has acquired [2]. Resistance to β-lactams is frequently mediated by the production of extended-spectrum β-lactamases (ESBLs) and metallo-β-lactamases (MBLs). ESBL-producing strains often harbor genes such as bla_{TEM}, bla_{SHV}, bla_{VEB}, bla_{PER}, and bla_{GES}, which confer resistance to third-generation cephalosporins (3GCs), like ceftazidime and cefotaxime. However, phenotypic detection of ESBLs in *P. aeruginosa* is particularly challenging, as the presence of *Amp*C β-lactamases and porin mutations could mask ESBL activity. Several studies in regions of high endemicity, including Egypt, have reported ESBL carriage rates of 30-60% in *P. aeruginosa*, with many isolates harboring multiple β-lactamase-encoding genes [3, 4]. While MBLs-producing *P. aeruginosa* strains are of particular concern due to their resistance to almost all β-lactams, including carbapenems, these pathogens pose a major therapeutic challenge. MBLs such as VIM, IMP, NDM, and SPM are especially troublesome because they are encoded on mobile genetic elements that facilitate their rapid dissemination, particularly in healthcare settings. In a 2023 meta-analysis, approximately 16% of *P. aeruginosa* isolates worldwide were MBL positive, with the prevalence exceeding 30% in some countries such as Egypt, which reported the highest detection rate of bla_{VIM} [5]. Although uncommon, the co-production of ESBLs and MBLs in a single isolate has also been documented, which is associated with extensive multidrug resistance and frequent therapeutic failure. In clinical microbiology, phenotypic detection of MBLs using imipenem/EDTA combined disc tests (CDT) or E-test MBL strips is considered reliable. Molecular methods such as PCR (polymerase chain reaction) remain essential for accurate detection of MBLs and epidemiological surveillance of their dissemination [3]. In addition to antimicrobial resistance, *P. aeruginosa* possesses a wide array of virulence factors, including lipopolysaccharide, flagella, alginate/biofilm, pili, lectins, cytotoxin, lytic enzymes, hemolysins, siderophores, and exotoxin, which contribute to its pathogenicity [6]. Antimicrobial-resistant strains, including those resistant to both disinfectants and antibiotics, pose a major threat to healthcare systems and vulnerable patients. The development of acquired and intrinsic resistance in bacteria is largely driven by inadequate surveillance, misuse of antibiotics, inappropriate usage of disinfectants, excessive use of antimicrobials in both livestock and human, and the horizontal transfer of resistance determinants among pathogenic bacteria. Nosocomial (hospital-acquired) infections are prevalent worldwide in both developing and developed countries [7]. Although many pathogens possess virulence factors that enable infection, only a few bacterial species account for most nosocomial infections, notably *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Escherichia coli* [8]. Most bacterial species associated with hospital infections exhibit resistance to antibiotics and disinfectants through both intrinsic and acquired resistance mechanisms. These antimicrobial-resistant bacteria are frequently isolated from immunocompromised patients and are responsible for serious hospital-acquired infections, including bloodstream infections,

urinary tract infections, pulmonary infections, and device-related infections in individuals [9].

Disinfectants are chemical agents containing one or more active ingredients designed to kill or inactivate pathogenic microorganisms on inanimate surfaces. Common active components include chlorine, iodine, ethanol, isopropanol, hydrogen peroxide, silver, chlorhexidine, triclosan, and quaternary ammonium compounds. Their application in all hospital departments is essential to minimize the risk of healthcare-associated infections. By reducing the bioburden on different surfaces, disinfectants play a critical role in comprehensive infection control strategies. Targeted surfaces range from laboratory work surfaces and surgical equipment to operating rooms and patient care areas [10].

The detection of multidrug resistance among Gram-negative bacteria [11], particularly *K. pneumoniae* [12], in patients and the hospital environment at Benghazi Medical Center (BMC) in Benghazi (Libya) has raised serious concerns about the environmental spread of highly resistant pathogens. This highlights the urgent need to prevent the transmission of environmental infections through the effective use of disinfectants.

Objectives: The objectives of the present study were to assess the effectiveness of disinfectants used in BMC in limiting the spread of infections and to detect ESBL and MBL genes in *P. aeruginosa*, given its significant role as an opportunistic pathogen in contaminating hospital environments and infecting immunocompromised patients as well as its high intrinsic and acquired antibiotic resistance.

Materials and Methods

Sample collection: This study was carried out at Benghazi Medical Center, Libya. Swab samples were collected from the hands of male and female healthcare workers (nurses) in medical wards as well as from floors, walls, wash basins, and medical equipment. Sixty-four hand swab samples were collected from healthcare workers, and 96 samples were collected from the hospital environment. All samples were immediately transferred to the laboratory for microbiological investigation. Experimental procedures were carried out in Al-Akeed Laboratory, Benghazi, Libya. Benghazi Medical Center is a 1200-bed hospital providing emergency care and services across medical specialties.

Five common nosocomial bacterial species isolated from the samples were selected to test the bactericidal efficacy of five disinfectant products (alcohol, iodine, hydrogen peroxide, surfanios, and glutaraldehyde). Bacterial suspensions were prepared in 9 mL of broth medium and mixed with 1 mL of each disinfectant according to the concentration recommended by the manufacturer. All inoculated nutrient agar and MacConkey agar plates were incubated overnight at 37 °C.

Inoculation and identification of isolates: Under aseptic conditions, bacterial isolates were identified according to Bergey's Manual of Systematic Bacteriology. Isolates obtained from blood agar, MacConkey agar, and nutrient agar were identified by conventional biochemical tests such as oxidase, catalase, citrate utilization, and urease tests.

Disinfectants: In Benghazi Medical Center (BMC), the most commonly-used and applicable disinfectants were alcohol, iodine, hydrogen peroxide, and glutaraldehyde. They were ready to use without dilution.

Preparation of bacterial suspensions: A fresh suspension was prepared with each of the isolated bacteria (*S. aureus, P. aeruginosa, E, coli, Micrococcus* spp., and *K. pneumonia*). These five bacterial species were selected as presumptive isolates, with one MDR

strain from each species chosen to further investigate the antimicrobial activity of the disinfectants.

Bacterial cultures grown for 24 hours were harvested, washed away with 100 mL of sterile normal saline (0.9%), and adjusted to a turbidity of 0.5 McFarland standard by dilution with sterile normal saline, yielding a suspension of approximately 10^8 CFU/mL. The bacterial species were grown in 9 mL of broth medium mixed with 1 mL of disinfectant, then all cultures were left at room temperature for 2.5 and 5 min. All inoculated nutrient agar and MacConkey agar plates were incubated overnight at 37 °C. Bactericidal efficacy was assessed based on colony growth.

Antibiotic susceptibility testing: According to the Clinical and Laboratory Standards Institute (CLSI) guidelines, all isolates were subjected to antimicrobial susceptibility testing using a modified Kirby–Bauer disk diffusion method.

A bacterial suspension was prepared, adjusted to 0.5 McFarland standard, and then inoculated onto the surface of Müeller–Hinton agar plates using a sterile swab (Oxoid, Basingstoke, England). Antibiotic-impregnated disks (CECON, São Paulo, Brazil) were aseptically placed onto the agar surface using sterile forceps. The inoculated plates were incubated at 35 °C for 18 hours. Following incubation, the diameters of inhibition zones were measured in millimeters and interpreted as susceptible, intermediate, or resistant according to CLSI breakpoints.

The antibiotic disks tested included amikacin (AK, 30 µg), ciprofloxacin (CIP, 5 µg), imipenem (IPM, 10 µg), ceftriaxone (CRO, 30 µg), cefepime (FEP, 10 µg), gentamicin (GN, 10 µg), azithromycin (AZM, 5 µg), trimethoprim/sulfamethoxazole (SXT, 25 µg), ticarcillin/clavulanic acid (TIM, 75/10 µg), and amoxicillin/clavulanic acid (AMC, 20/10 µg).

Detection of ESBL and MBL phenotypes: Phenotypic detection of extended-spectrum β-lactamase (ESBL) production was carried out using the disk diffusion method on Mueller–Hinton agar medium. Disks containing ceftazidime, cefotaxime, and cefepime were placed at a distance of 20–30 mm (center to center) from an amoxicillin–clavulanic acid disk. ESBL phenotype was inferred when an expansion or enhancement in inhibition zones of cephalosporin disks was observed in the presence of clavulanic acid, indicating a synergistic effect. In addition, MBL production was screened using imipenem and EDTA–imipenem (IEH) disks as a simple phenotypic method for detecting MBL-producing clinical isolates [13].

Detection of antibiotic resistance genes: The presence of antibiotic resistance genes in *P. aeruginosa* isolates was investigated using PCR amplification followed by sequencing. The primers and PCR conditions used to detect β-lactamase genes $bla_{\text{CTX-M-15}}$, bla_{SHV}, bla_{TEM}, and bla_{NDM} are summarized in Table 1.

Activity of disinfectants on bacteria: The bactericidal or bacteriostatic effects of five disinfectant products were evaluated against bacterial isolates from healthcare workers' hands, floors, walls, wash basins, and medical equipment in BMC hospital. About 9 mL of nutrient broth was mixed with 1 mL of each disinfectant (tube dilution method), after which one drop of bacterial suspension was added to each tube using a sterile inoculation loop. The tubes were then mixed and incubated at 25 °C for 2.5 and 5 min (as a contact time).

Following incubation, the mixtures were subcultured onto MacConkey agar and/or blood agar plates using the streak plate technique. All plates were incubated overnight at 37 °C. The effectiveness of the disinfectants was evaluated based on the presence or absence of bacterial colonies on culture media.

Table 1) Primer sequences, expected amplicon sizes, and PCR conditions used to detect antibiotic resistance genes

Primer Sequences of Genes	Size	Amplification Conditions		
Bla_{NDM}	620 bp	94 ºC	5 min	1 cycle
		94 ºC	30 s	
F: GGTTTGGCGATCTGGTTTTC		52 ºC	40 sec	36 cycles
R: CGGAATGGCTCATCACGATC		72 ºC	40 sec	
		72 ºC	7 min	1 cycle
Bla_{OXA-48}	743 bp	96 ºC	5 min	1 cycle
		96 ºC	1 min	
F: TTGGTGGCATCGATTATCGG		61 ºC	1 min	35 cycles
R: GAGCACTTCTTTTGTGATGGC		72 ºC	2 min	
		72 ºC	10 min	1 cycle
Bla_{CTX-M}	544 bp	96 ºC	5 min	1 cycle
		96 ºC	15 s	
F: TTT GCG ATG TGC AGT ACC AGT AA		52 ºC	15 s	24 cycles
R: CGA TAT CGT TGG TGG TGC CAT A		72 ºC	2 min	
		72 ºC	5 min	1 cycle
Bla_{SHV}	885 bp	96 ºC	5 min	1 cycle
		96 ºC	15 s	
F: CACTCAAGGATGTATTGTG		52 ºC	15 s	24 cycles
R: TTAGCGTTGCCAGTGCTCG		72 ºC	2 min	
		72 ºC	5 min	1 cycle

Findings

This study was conducted to assess the effectiveness of commonly used disinfectants for cleaning and sterilization in BMC hospital. These disinfectants were tested against bacterial isolates from the hands of healthcare workers (nursing) in medical wards as well as from floors, wash basins, and medical equipment.

Bacterial strains: All isolated bacteria were identified based on their microbiological and biochemical characteristics. A total of 30 bacterial strains were isolated from 64 swab samples of healthcare workers' hands. Among these, *S. aureus* was the most frequently identified pathogen, accounting for 15 isolates (50%), followed by *Micrococcus* spp. with nine isolates (30%). In addition, 79 bacterial strains were obtained from 96 environmental swabs, including 33 *E. coli* (41.8%), 26 *K. pneumoniae* (32.9%), and 20 *P. aeruginosa* (25.3%) isolates.

The recovered species from environmental and hand swab samples included *S. aureus, P. aeruginosa, E. coli, Micrococcus* spp., and *K. pneumonia.*

Antibiotic susceptibility profiles: Antibiotic susceptibility profiles of the five isolated bacteria were determined against ten antibiotic discs (Table 2). Most of the isolates (80%) were resistant to four or more of the ten antibiotics tested, except for *Micrococcus* spp., which was sensitive to all tested antibiotics. Susceptibility patterns varied across species; for example, *P. aeruginosa* was susceptible to imipenem but resistant to amikacin, gentamicin, ceftriaxone, cefepime, ciprofloxacin, azithromycin, trimethoprim/sulfamethoxazole, ticarcillin+clavulanic acid, and amoxicillin+clavulanic acid.

Imipenem (a carbapenem) and amikacin (an aminoglycoside) were the most effective antibiotics against the isolates, with four out of five showing susceptibility to these agents (Table 2).

Based on susceptibility test results, isolated

Table 2) Antibiotic susceptibility of isolated bacteria

Antibiotic Classes	Symbol	*P. aeruginosa*	*S. aureus*	*K. pneumoniae*	*E. coli*	*Micrococcus sp.*
Imipenem (carbapenems)	IMP	S	R	S	S	S
Amikacin (aminoglycosides)	AK	R	S	S	S	S
Gentamicin (aminoglycosides)	GN	I	I	R	R	S
Ceftriaxone (cephalosporins 3G)	CRO	R	R	R	R	S
Cefepime (cephalosporins 4G)	FEP	R	S	R	S	S
Ciprofloxacin (fluoroquinolones)	CIP	R	R	S	S	S
Azithromycin (macrolide)	AZM	R	R	S	S	S
Trimethoprim/sulfamethoxazole (sulfonamide)	SXT	R	R	S	S	S
Ticarcilline + clavulanic acid (carboxypenicillins)	TIM	R	R	R	R	S
Amoxicillin + clavulanic acid (B-lactamase inhibitors)	AMC	R	R	R	R	S

strains were categorized as multidrug-resistant (MDR) and extensively drug-resistant (XDR).

Multidrug resistance (MDR) is defined as acquired resistance to at least one antimicrobial agent in three or more different antimicrobial classes. Extensively drug-resistant (XDR) bacteria are characterized by resistance to at least one agent in all but two or fewer antimicrobial classes, meaning that the isolated bacteria remain susceptible to only one or two classes of antimicrobials [14]. In the present study, *S. aureus* and *P. aeruginosa* exhibited an XDR profile, whereas *E. coli* and *K. pneumoniae* were classified as MDR.

Evaluation of bactericidal activity of disinfectants: The bactericidal activity of alcohol, iodine, hydrogen peroxide, surfanios, and glutaraldehyde was assessed at the concentrations routinely used in BMC hospital. All disinfectants were effective against the bacterial isolates at the recommended concentration and time, except iodine, hydrogen peroxide, and sterillium, which failed to eliminate two isolates.

Iodine was not effective against *S. aureus* after 2.5 min of exposure time but completely inhibited its growth after 5 min. In addition, hydrogen peroxide and sterillium were both ineffective against *P. aeruginosa* at exposure times of 2.5 and 5 min, as evidenced by bacterial growth on culture media. *E. coli*, *K. pneumonia,* and *Micrococcus* spp. were killed by alcohol, iodine, hydrogen peroxide, surfanios, and glutaraldehyde. Overall, variation in response to disinfectants and antibiotics was observed among the isolated strains (Table 3).

Antibiotic resistance characteristics of *P. aeruginosa*: Table 4 summarizes the characteristics and resistance profiles of *P. aeruginosa* isolates. Multidrug-resistant strains were recovered from diverse environmental sites, including hospital floors, wash basins, healthcare workers' hands, and even disinfectant bottles. Notably, all *P. aeruginosa* isolates tested positive for ESBL production, and two of them showed

Table 3) Bactericidal activity of disinfectants against hospital antibiotic-resistant isolates

Bacteria	Site	Hydrogen Peroxide		Glutaraldehyde		Alcohol		Iodine	
		2.5 min	5 min	2.5 min	5 min	2.5 min	5 min	2.5 min	5 min
S. aureus	Hand	NG	NG	NG	NG	NG	NG	G	NG
P. aeruginosa	Floor	G	G	NG	NG	G	G	NG	NG
K. pneumonia	Sink	NG	NG	NG	NG	NG	NG	NG	NG
E. coli	Wall	NG	NG	NG	NG	NG	NG	NG	NG
Micrococcus sp.	Hand	NG	NG	NG	NG	NG	NG	NG	NG

NG = no growth, G = growth.

Table 4) Phenotypic and genotypic characterization of *P. aeruginosa* strains isolated from clinical sources

Strains Code	Isolation Source	Bacterial Strains	ESBL-Producing	MBL-Producing	Antibiotic Resistance Phenotype	Antibiotic Resistance Genes
1	floor	*P. aeruginosa*	+	+	IMP, MEM, ETP, CIP, LEV, CN, AK, CTX, CAZ, CRO, TPZ, DO	$bla_{\text{NDM-1}}$,$bla_{\text{CTX-M-15}}$, bla_{TEM}
2	basin	*P. aeruginosa*	+	-	ETP, CIP, LEV, CN, AK, CTX, CAZ, CRO, AMC, TPZ, AZM	$bla_{\text{NDM-1}}$, $bla_{\text{CTX-M-15}}$
3	Hand	*P. aeruginosa*	+	-	ETP, CIP, LEV, CN, CTX, CAZ, CRO, AMC, TPZ	$bla_{\text{NDM-1}}$, bla_{TEM}, bla_{SHV}
4	Bottle of disinfectant	*P. aeruginosa*	+	+	ETP, MEM , CIP, LEV, CN, CTX, CRO, TPZ, DO	$bla_{\text{NDM-1}}$, bla_{SHV}

positive results for metallo-beta-lactamases (MBL). This suggests a significant potential for resistance against a wide array of beta-lactam antibiotics.

P. aeruginosa isolates exhibited resistance to multiple agents, including carbapenems (imipenem, meropenem, ertapenem), fluoroquinolones (ciprofloxacin, levofloxacin), aminoglycosides (gentamicin, amikacin), and cephalosporins (cefotaxime, ceftazidime, ceftriaxone). Additionally, other agents, including piperacillin-tazobactam and doxycycline, were ineffective. Notably, molecular screening identified several resistance genes, with $bla_{\text{NDM-1}}$ detected in all isolates, alongside $bla_{\text{CTX-M-15}}$, bla_{TEM}, and bla_{SHV}, each detected in two strains. The recovery of resistant *P. aeruginosa* in unexpected sites, such as disinfectant bottles, really highlights the potential of environmental reservoirs to facilitate the spread of antimicrobial resistance within healthcare settings. This situation underscores the urgent need for robust antimicrobial stewardship and stringent infection-control measures to limit dissemination of these highly resistant strains.

Discussion

Disinfectants are essential products used in hospitals and other healthcare settings to control infections. They are available in various forms, including liquid, solid, semi-

solid, or powder. Some products are also used to remove dirt like dust, microorganisms, stains, and foul smell from the body or objects like clothing to maintain health and beauty. Disinfectants act on microorganisms through two different ways: inhibiting growth (bacteriostatic, fungistatic) or exerting lethal effects (bactericidal, fungicidal or virucidal effects) [15, 16].

High resistance rates were observed among environmental isolates of *P. aeruginosa*, *E. coli*, *K. pneumoniae*, and *S. aureus* recovered from healthcare workers, many of which exhibited XDR and MDR profiles. This is consistent with the findings reported in a Palestinian study [17]. In the present study, all environmental isolates of *Pseudomonas* spp., *Enterobacteriaceae*, and *Staphylococcus* spp. obtained from healthcare workers' specimens were classified as multidrug-resistant (MDR). A high percentage of MDR isolates (64.77%) was reported in hospital wastewater samples in a study in Ethiopia [18]. In another study in India, 63.33% (19 of 30) of bacterial isolates from environmental samples were identified as MDR [19]. Likewise, a higher occurrence of MDR bacteria (32 of 82; 39.03%) was detected in hospital environmental samples in another study in Nigeria [20]. This resistance may be attributed to overuse or misuse of antimicrobials, easy access to antibiotics, lack of a clear antibiotic stewardship policy, and horizontal acquisition of resistance genes from other MDR bacteria. These findings suggest a considerable risk of spreading nosocomial pathogens from medical staff and the hospital environment to patients, thereby complicating treatment options due to the limited effectiveness of available drugs against XDR and MDR strains. Continuous and effective disinfection practices could help reduce the persistence of these resistant strains in the hospital environment. In our hospitals, preventive measures should primarily target these pathogens to minimize the risk of infection. The multidrug resistance observed in *P. aeruginosa* isolates to most antimicrobials, even agents like piperacillin-tazobactam and doxycycline, highlights the remarkable adaptive capacity of this bacterium, driven by both chromosomal mechanisms (e.g., efflux pumps, porin alterations) and acquired resistance genes [21]. One of the significant findings of this study is the sensitivity of bacterial species to imipenem and amikacin, indicating that these agents remain an effective treatment option for MDR bacteria in Libyan hospitals.

In the present study, all *P. aeruginosa* isolates tested positive for the ESBL production phenotype. The reported ESBL production rates in *P. aeruginosa* vary across studies, for example, 54% in Egypt [22], 42.2% in China [23], 38.1% in Iraq [24], 36.3% in India [25], and 8.3% in Iran [26].

In this study, 50% of *P. aeruginosa* isolates were positive for the MBL production phenotype. This result is consistent with that reported by Shrestha et al. (2024) [27] in Nepal, who reported a MBL production rate of 40.4%, but lower than that reported (64%) by Najeeb et al. (2025) [28] in India. Notably, 50% of the isolates co-produced ESBLs and MBLs, representing a significant risk in clinical settings. In contrast, studies conducted in countries such as China [23] and Nepal [29] reported lower co-production rates of 10 and 3.1%, respectively. This difference may be attributed to variations in epidemiology and circulating clones across different countries.

The emergence of MBL-mediated resistance in Libya is a serious concern. Carbapenems are among the few effective agents against highly resistant pathogens such as MDR and ESBL-producing *P. aeruginosa* and *Enterobacteriaceae*. The spread of this resistance to other Gram-negative bacteria

could severely limit available therapeutic options [30].

CTX-M, TEM, and SHV enzymes are among the most widely-detected ESBLs in bacterial pathogens worldwide [26]. Numerous studies have documented the occurrence of these enzymes in clinical isolates of *P. aeruginosa* across different regions [31, 32]. The co-occurrence of ESBL genes ($bla_{\text{CTX-M-15}}$, bla_{TEM}, bla_{SHV}) further complicates treatment options, as these enzymes are known to hydrolyze penicillins and cephalosporins [33]. The broad resistance phenotype, together with this diverse array of β-lactamase genes, indicates that both intrinsic mechanisms (e.g., AmpC, efflux pumps) and acquired resistance determinants contribute to the multidrug-resistance profile of these isolates. ESBL determinants, particularly the $bla_{\text{CTX-M-15}}$ gene, are known to disseminate rapidly across the globe. Interestingly, in this study, $bla_{\text{CTX-M-15}}$ was the most predominant ESBL type, which is consistent with the findings of other studies [34, 35]. The extensive spread of this gene is largely attributed to epidemic plasmids capable of horizontal transfer [36].

Detection of the carbapenem-resistance gene $bla_{\text{NDM-1}}$ in our isolates aligns with previous reports of the presence of this gene in *Acinetobacter baumannii*, *P. aeruginosa*, and *K. pneumoniae* strains in Libya [12, 37]. The mechanism of carbapenem resistance often confers co-resistance to multiple antimicrobials, including third-generation cephalosporins [38], and is frequently associated with extensive horizontal gene transfer mediated by mobile genetic elements such as integrons and plasmids. This highlights concerns about the presence of these determinants in Libyan hospitals, which may further complicate infection management.

Disinfectants are widely utilized for a wide range of topical applications in medical facilities such as hospitals. Specifically, they play a crucial role in infection control procedures and help prevent nosocomial infections. In this study, the activity of five commonly used disinfectants (hydrogen peroxide, glutaraldehyde, alcohol, iodine, surfanios) was evaluated against nosocomial strains of *S. aureus*, *P. aeruginosa*, *K. pneumonia*, *E. coli,* and *Micrococcus* spp. In the present study, experimental data revealed that the effects of different disinfectants on bacterial growth varied significantly between the tested species. *K. pneumonia*, *E. coli,* and *Micrococcus* spp. were completely inhibited by all tested disinfectants. This finding suggests that regular and proper disinfection could effectively eliminate pathogenic isolates circulating in clinical environment. All isolates were obtained from nosocomial sites, including hands, floors, and equipment. Cidex (glutaraldehyde) was the most effective disinfectant, inhibiting the growth of all bacterial isolates. This substance exerts its inhibitory effect by denaturing proteins and alkylating nucleic acids [39]. Oxidizing compounds, such as peroxides, play an important role in degrading bacterial cells. In response, bacteria like *E. coli* produce enzymes that neutralize the oxidizing compounds or assist in repair processes, thereby protecting themselves from oxidative damage [40].

In this study, glutaraldehyde (Cidex) at high inoculum levels showed a bactericidal effect within a reasonable time (2.5 and 5 minutes). A previous study identified Cidex (2.4% glutaraldehyde) as the most effective agent among twelve disinfectants, providing high-level (sporicidal) disinfection within 5 minutes [41]. This highlights the importance of glutaraldehyde concentration in semi-critical disinfection, with concentrations ≥2.4% considered effective [42]. The Centers for Disease Control and Prevention (CDC) also emphasizes that dilution may reduce

the efficacy of glutaraldehyde, highlighting the importance of maintaining an adequate minimum effective concentration during use. In addition, formulations combining glutaraldehyde with phenol or phenate compounds are recommended for high-level disinfection [43].

Hydrogen peroxide exhibited bactericidal activity against *K. pneumonia* and *E. coli* but was less effective against *P. aeruginosa*, which was able to grow even in diluted solutions at exposure times of 2.5 and 5 min. These findings are in agreement with those of a recent study, in which oxidizing agents such as sodium hypochlorite and hydrogen peroxide exhibited the weakest MIC activity against *P. aeruginosa* [43]. *P. aeruginosa* resists oxidizing agents such as hydrogen peroxide by inducing antioxidant enzymes, including catalases, peroxidases, and superoxide dismutase. These enzymes help reduce oxidative stress and enhance survival [45].

Additionally, *P. aeruginosa* demonstrated growth in the presence of sterillium (70% alcohol) and betadine (iodine), which is in agreement with the findings of Karpiński et al. (2025) [44]. Hands are considered the primary route of transmission of microbes and infections to individuals [46, 47]. Sterillium is a broad-spectrum microbicidal skin protectant used for regular hand antisepsis [48]. It is effective against Gram-positive bacteria, including *S. aureus,* as well as Gram-negative bacteria, including *E. coli* [49].

In the present study, *P. aeruginosa* exhibited the highest level of resistance to all tested antibiotics (Table 2). In contrast, *S. aureus* was resistant to ceftriaxone and cephalexin but remained susceptible to other antibiotics tested. These findings are consistent with previous reports describing the high intrinsic and acquired resistance mechanisms of *P. aeruginosa*, including reduced membrane permeability, efflux pumps, and biofilm formation [50].

Disinfectants as antimicrobial agents were effective against pathogenic bacteria, including multidrug-resistant strains of *S. aureus*, which is consistent with the findings of Beier et al. (2021) [51]. This study demonstrated that glutaraldehyde was the most effective disinfectant, and some disinfectants were able to eliminate bacterial pathogens within an exposure time of 2.5–5 minutes. These results are supported by previous studies showing the broad-spectrum and rapid biocidal activity of glutaraldehyde against Gram-positive and Gram-negative bacteria [42].

The presence of highly resistant strains in environmental niches, especially in disinfectant bottles, raises serious concerns. Environmental reservoirs of *P. aeruginosa*, including hospital water systems, sink drains, and disinfectant containers, are well-documented and represent persistent sources of nosocomial infections. Biofilm formation in such environmental settings not only protects bacteria from disinfectants and antibiotics but also facilitates horizontal gene transfer due to high cell density and matrix-mediated protection. Indeed, resistance to commonly-used disinfectants, like chlorine and quaternary ammonium compounds, has been documented [52], highlighting potential selective pressures that may even promote co-selection of antibiotic resistance genes in these reservoirs [53].

Future research should focus on microbial communities residing in hospitals, including the susceptibility of biofilm-producing *P. aeruginosa* isolates and the efficiency of disinfectants used in clinical settings.

Conclusion

This study highlights the presence of MDR and XDR bacteria, including *S. aureus*, *E. coli*, *K. pneumoniae*, and particularly *P. aeruginosa*, within hospital environments and even in

disinfectant bottles. The detection of ESBL- and MBL-producing *P. aeruginosa* carrying bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{TEM}, and bla_{SHV} genes underscores the alarming potential of environmental reservoirs to act as sources of antimicrobial resistance dissemination. The findings demonstrated that although most disinfectants were effective at recommended concentrations and exposure times, iodine, hydrogen peroxide, and sterillium failed to eliminate certain resistant strains, particularly *P. aeruginosa*. These results emphasize the importance of careful selection and validation of disinfectants to ensure consistent efficacy against clinically relevant and resistant bacteria.

Given the observed resistance to multiple antibiotic classes, including carbapenems, aminoglycosides, and cephalosporins, robust antimicrobial stewardship programs and strict infection-control measures are urgently needed. Continuous surveillance of hospital environments, coupled with the rational selection of effective disinfectants such as alcohol-based, surfactant-based, or halogen-based agents, is critical to limit the spread of resistant pathogens.

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