Susceptibility patterns of bacteria isolated from the hospital environment towards disinfectants commonly used for surfaces and medical devices

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Abstract This study aimed to evaluate the bactericidal activity of common disinfectants used for surfaces and medical devices. Sodium hypochlorite (D1), disinfectant (D2) composed of N-(3-aminopropyl)-N-dodecylpropane-1,3-diamine, chloride de didecyldimethylammonium, and disinfectant (D3) composed of Didecyldimethylammonium chloride and Polyhexamethylene biguanide hydrochloride, were tested against 15 strains isolated from the hospital environment and four reference bacteria. The microdilution method was performed to assess antimicrobial activity. The susceptibility was evaluated by comparing the minimum inhibitory dilution with the dilution of disinfectant recommended by the manufacture. D1 and D2 were active against *Staphylococcus epidermidis*, *Staphylococcus saprophyticus, Enterobacter cloacae, Escherichia coli, Pseudomonas fluorescens, Methicillin-resistant Staphylococcus aureus, Bacillus spp, Corynebacterium spp, Gram-positive bacillus, Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 3366, and *Pseudomonas aeruginosa* ATCC 27853 strains but not active against Micrococcus spp, and *Staphylococcus aureus* ATCC 29213. D3 was ineffective against Micrococcus spp, Bacillus Gram Positive, *Staphylococcus epidermidis*, and *Escherichia coli* ATCC 25922. Therefore, D1 and D2 can eliminate most pathogenic bacteria in hospitals, in comparison to D3. It is necessary to monitor the antibacterial activity of disinfectants against reference strains but also against those usually present on surfaces. The obtained results could have promising applications in controlling the emergence of nosocomial infections.

1. Introduction

The hospital environment, especially surfaces, is a source of numerous microorganisms that remains not necessarily pathogens, but some, such as those derived from the cutaneous-mucous flora of humans or the environment may be responsible for Health-care Associated Infections (HAIs) [1]. Weber et *al*. [2] reported that 20% to 40% of HAIs have been attributed to cross-infection via the caregiver's hands contaminated by contact with the patient or contact with hospital surfaces. The contribution of the environment in the appearance of HAI has been reported by several studies [3, 4]. Some microorganisms can persist in the hospital environment depending on the nature of the surfaces, the ability of the bacteria to form biofilms (*Pseudomonas aeruginosa*) or to produce spores (*Clostridium difficile*) [5, 6]. Thus, adequate measures are necessary to minimize the risk of cross-contamination between surfaces, patients, and caregivers, such as hand hygiene [7] and disinfection of surfaces and medical devices [8].

Subsequently, surfaces and medical equipment must be cleaned and disinfected regularly several times per day in some services (operating room, burn unit, and intensive care) to reduce the risk associated with HAI [9, 10]. In this context, disinfectants are used in hospitals for many applications on surfaces and equipment. Newer "no-touch" disinfection technologies include aerosol and vaporized hydrogen peroxide, mobile devices that emit continuous ultraviolet light, and the use of high-intensity narrow-spectrum light have been shown to reduce bacterial contamination of surfaces [11]. The main objective of

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disinfection is to eliminate both opportunistic and pathogenic microorganisms present on surfaces in contact with the patients and the caregivers. However, if the disinfection approaches are not correctly undertaken, the process fails to eliminate bacteria. Indeed, the biocide activity is influenced by the disinfectant concentration, the contact time, and the traces of interfering substances (blood, metal ions, etc.) [12,13,14]. Some bacteria such as *methicillin-resistant Staphylococcus aureus* (*MRSA*) or various Gram-negative bacilli can develop resistance to commonly used disinfectants which requires a regular change of the active ingredients of disinfectants [15]. Bacterial resistance against quaternary ammonium, peroxides, phenols, chlorine, and glutaraldehyde has been widely reported [16, 17, 18, 19]. Consequently, the present study was to evaluate the bactericidal activity of common disinfectants used at a hospital in Meknes (Center of Morocco) to 15 bacterial strains from hospital flora and four reference strains. The disinfectants tested are: sodium hypochlorite disinfectant (bleach) 12° (D1), a disinfectant (D2) belonging to quaternary ammonium compound (QAC) composed of 3-aminopropyl-N-dodecylpropane-1,3-diamine (51mg / g) and Didecyldimethylammonuim chloride (25mg / g), and a disinfectant composed of Didecyldimethylammonium chloride (0, 07 mg/g) DDAC (QAC) and Polyhexamethylene biguanide hydrochloride (0,40 mg/g) (D3). The use of these disinfectants for the disinfection of surfaces and medical equipment is due to their antibacterial effects. Disinfectants based on quaternary ammonium constitute an excellent antimicrobial agent due to their significant biocide activity, long-term durability, and compatibility with the environment [20]. Glutaraldehyde is an active biocidal agent for the hard surface in hospitals and industrial areas [21]. Sodium hypochlorite in solution exhibits broad-spectrum antimicrobial activity and is widely used in healthcare facilities [22].

2. Materials and methods

The study was conducted at a hospital in Meknes-Morocco during February and September 2017. Samples were isolated from the surfaces, and medical devices of services belong to areas with a controlled environment (burn unit, operating room, and sterilization service) [23]. Isolates were identified by conventional biochemical techniques such as Gram staining, oxidase (positive oxidase results in a purple coloration), catalase whose reaction results in the evolution of gas in bubbles, anaerobic test, and the detection of deoxyribonuclease (DNase) (The detection of this enzyme is done by placing on the bacterial culture a solution of hydrochloric acid (HCL) diluted to 10%). The presence of a clear area around the stria: DNase (+) strain. The absence of a clear zone: DNase (-).

Confirmation was carried out by Galerie API 20NE® for identification of non-Enterobacteriaceae Gram-negative bacilli, and API 20E® (BioMérieux, French) for identification of Enterobacteriaceae and other Gram-negative bacilli. These identification tests are based either on bacterial growth associated with a metabolism study or on a search for an enzymatic activity that does not require bacterial multiplication. The reactions produced during the incubation period result in spontaneous color changes or are revealed by the addition of reagents.

2.1 Disinfectants

Three disinfectants usually used at a hospital in Meknes (Center of Morocco) are tested on isolated and reference strains: *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 3366, and *Pseudomonas aeruginosa* ATCC 27853.

D1, sodium hypochlorite is the most used amongst chlorine disinfectants. The commercial aqueous solution in Morocco is 10° to 12° sodium hypochlorite called bleach. It's used by caregivers at a dilution of 1/10 in the burn unit for disinfecting beds, tables, carts, etc.

D2 composed of 3-aminopropyl-N-dodecylpropane-1,3-diamine (51mg / g) and Didecyldimethylammonuim chloride (25mg / g). It is quaternary ammonium used at a dilution of 0.25%, according to the manufacturer's recommendations. It's used for disinfecting floors, walls, surfaces, and medical equipment in the operating room and other services.

D3, combination of Didecyldimethylammonium chloride (0, 07 mg/g) DDAC, a quaternary ammonium compound, and Polyhexamethylene biguanide hydrochloride (0,40 mg/g). It's used for disinfecting surfaces, medical equipment, and devices in the operating rooms and intensive care services.

2.2. Bacteria susceptibility test against disinfectants

The susceptibility of bacterial strains against disinfectants is carried out by the micro method described by Rouillon et *al*. (2006) [24]. This method involves the incubation of microbial strains with serial dilutions of the evaluated disinfectant. The susceptibility or not is determined as the minimum inhibitory dilution (MID). Techniques for the assessment of disinfectants against bacteria are standardized (French standard 1276 March 2010). However, the micro method is a preliminary evaluation, more practical, faster, and reliable [16,24].

2.3. Inoculum preparation

The microbial inoculums were prepared by the method described by Rouillon et *al.* [11]. The revivification of bacteria has been performed by subculturing the agar plate surface Luria–Bertani (LB) pre-poured in Petri dishes and incubated at 37°C for 24 hours. A colony was seeded in Brain Heart Infusion (BHI) Broth and incubated at 37 ° C for 18 to 24 hours. Sixty µL of this culture were added to 2 mL of BHI and incubated at 37 ° C for 2 hours to obtain a culture in the exponential growth phase. Two hundred microliters of this culture were added to 1.8 ml of BHI at 37 ° C to get the final suspension [24].

2.4. Test

Plates were inoculated with 180 µl of bacterial suspension and 20 µl of the tested disinfectant at the appropriate dilution. A control was prepared using 180 µL of bacterial suspension or 180 µL of BHI, and 20 µL of sterile distilled water. Finally, the plate was incubated at 37 ° C for 18 hours. The MID was determined as the minimal dilution which doesn't show any bacterial pellet.

3. Results

The identified species were as follow: Staphylococcus epidermidis (n = 3), Micrococcus sp (n = 1), Methicillin-resistant Staphylococcus aureus (n = 1), Bacillus sp (n = 1), Corynebacterium spp (n = 1) and Gram positive bacillus (n = 1) have been isolated from burn unit surfaces and equipment. Enterobacter cloacae (n = 1), Escherichia coli (n = 1), Pseudomonas fluorescens (n = 1) and Methicillin-resistant Staphylococcus (n = 1) have been isolated from operating room surfaces and medical devices. Finally, Staphylococcus saprophyticus (n = 1), Staphylococcus epidermidis (n = 1), Micrococcus sp (n = 1) have been isolated from Sterilization service surfaces.

No bacterial growth was observed for all bacteria at 10^{-1} dilution of D1 except for Micrococcus sp and Staphylococcus aureus ATCC 29213 (Table 1). Only one strain of Staphylococcus epidermidis was able to grow at 10^{-4} dilution.

Table 1: Summary of tested solutions of D1 on bacterial strains

Bacteria	Dilutions tested				
	Without dilution	10^{-1}	10^{-2}	10^{-3}	10^{-4}
Pseudomonas fluorescens	-	-	+	+	+
Staphylococcus epidermidis	-	-	-	-	+
Staphylococcus epidermidis	-	-	+	+	+
Methicillin-resistant Staphylococcus	-	-	+	+	+
Micrococcus sp	-	-	+	+	+
Staphylococcus saprophyticus	-	-	+	+	+
Enterobacter cloacae	-	-	+	+	+
Escherichia coli	-	-	+	+	+
Bacillus sp	-	-	+	+	+
Gram positive bacillus (GPB)	-	-	+	+	+
Corynebacterium spp	-	-	+	+	+
Micrococcus sp	-	+	+	+	+
Staphylococcus epidermidis	-	-	+	+	+
Methicillin-resistant Staphylococcus aureus(*MRSA*)	-	-	+	+	+
Staphylococcus epidermidis	-	-	+	+	+
Staphylococcus aureus ATCC 29213	-	+	+	+	+
Pseudomonas aeruginosa ATCC 27853	-	-	+	+	+
Bacillus subtilis ATCC 3366	-	-	+	+	+
Escherichia coli ATCC 25922	-	-	+	+	+

+ Presence of bacterial growth -No bacterial growth

No bacterial growth was observed for all bacteria at 1/200 dilution of D2 (Table 2). *Staphylococcus epidermidis* (n=2) were viable at 1/400 dilution. *Methicillin-resistant Staphylococcus* was growing at 1/800 dilution. The bacterial growth was noted for *Staphylococcus saprophyticus* and *Escherichia coli* at 1/1600. For Bacillus sp, *methicillin-resistant Staphylococcus aureus*, *Pseudomonas aeruginosa* ATCC 27853, and *Escherichia coli* ATCC 25922, the bacterial growth was noted at 1/3200. The DMI for the other bacteria was noted at 1/3200 dilution except for Corynebacterium spp (MID = 1/6400).

Table 2: Summary of tested solutions of D2 on bacterial strains

Bacteria	Dilutions tested						
	1/200	1/400	1/800	1/1600	1/3200	1/6400	1/12800
Pseudomonas fluorescens	-	-	-	-	-	+	+
Staphylococcus epidermidis	-	-	-	-	-	+	+
Staphylococcus epidermidis	-	+	+	+	+	+	+
Methicillin-resistant Staphylococcus	-	-	+	+	+	+	+
Micrococcus sp	-	-	-	-	-	+	+
Staphylococcus saprophyticus	-	-	-	+	+	+	+
Enterobacter cloacae	-	-	-	-	-	+	+
Escherichia coli	-	-	-	+	+	+	+
Bacillus sp	-	-	-	-	+	+	+
Gram positive bacillus(GPB)	-	-	-	-	-	+	+
Corynebacterium spp	-	-	-	-	-	-	+
Micrococcus sp	-	-	-	-	-	+	+
Staphylococcus epidermidis	-	-	-	-	-	+	+
Methicillin-resistant Staphylococcus aureus	-	-	-	-	+	+	+
Staphylococcus epidermidis	-	+	+	+	+	+	+
Staphylococcus aureus ATCC 29213	-	-	-	-	-	-	+
Pseudomonas aeruginosa ATCC 27853	-	-	-	-	+	+	+
Bacillus subtilis ATCC 3366	-	-	-	-	-	+	+
Escherichia coli ATCC 25922	-	-	-	-	+	+	+

+ Presence of bacterial growth -No bacterial growth

We noted that Micrococcus sp, GPB, *Staphylococcus epidermidis,* and *Escherichia coli* ATCC 25922 were grown without dilution (Table 3). For *Enterobacter cloacae*, *Pseudomonas aeruginosa* ATCC 27853, and *Bacillus subtilis* ATCC 3366, growth was noted at 1/2. While Corynebacterium sp, *Staphylococcus epidermidis* (n=1), and *Staphylococcus aureus* ATCC 29213 grow at 1/4 dilution. *Staphylococcus epidermidis* (n=2), *Staphylococcus saprophyticus,* Bacillus sp, and *MRSA* were grown at 1/8 dilution. Bacterial growth was noted at 1/32 dilution for *Methicillin-resistant Staphylococcus* and Micrococcus. For 1/64 dilution, *Pseudomonas fluorescens* and *Escherichia coli* were grown at this concentration.

Table 3: Summary of tested dilutions of D3 on bacterial strains

Bacteria	Dilutions tested					
	Without dilution	1/2	1/4	1/8	1/32	1/64
Pseudomonas fluorescens	-	-	-	-	-	+
Staphylococcus epidermidis	-	-	-	+	+	+
Staphylococcus epidermidis	-	-	-	+	+	+
Methicillin-resistant Staphylococcus	-	-	-	-	+	+
Micrococcus sp	+	+	+	+	+	+
Staphylococcus saprophyticus	-	-	-	+	+	+
Enterobacter cloacae	-	+	+	+	+	+
Escherichia coli	-	-	-	-	-	+
Bacillus sp	-	-	-	+	+	+
Gram positive bacillus (GPB)	+	+	+	+	+	+
Corynebacterium spp	-	-	+	+	+	+
Micrococcus sp	-	-	-	-	+	+
Staphylococcus epidermidis	+	+	+	+	+	+
Methicillin-resistant Staphylococcus aureus	-	-	-	+	+	+
Staphylococcus epidermidis	-	-	+	+	+	+
Staphylococcus aureus ATCC 29213	-	-	+	+	+	+
Pseudomonas aeruginosa ATCC 27853	-	+	+	+	+	+
Bacillus sublilis ATCC 3366	-	+	+	+	+	+
Escherichia coli ATCC 25922	+	+	+	+	+	+

+ Presence of bacterial growth -No bacterial growth

4. Discussion

The hospital environment is exposed to different types of contamination, especially by resistant microorganisms, including bacteria, which increases the risk of infection to immunocompromised patients [25, 26]. Disinfection represents the main strategy against pathogenic or potentially pathogenic microorganisms in the hospital [27]. Controlling the disinfection process minimizes the risk of cross-contamination between patients, visitors, caregivers, surfaces, and hospital equipment, as well as the risk of healthcare-associated infections (HAIs) [28]. Different microorganisms are frequently isolated from the hospital surfaces, including resistant bacteria, such as *vancomycin-resistant enterococci*, *MRSA*, *Pseudomonas aeruginosa,* and *Acinetobacter baumannii* [29]. Standardized protocols have been developed for cleaning and disinfection of the environment in healthcare settings [11]. Some researchers have shown that bacteria can develop resistance to disinfectants [30, 31, 32]. In this study, we evaluated the bactericidal activity of disinfectants commonly used at a hospital in Meknes (Morocco) against 15 strains isolated from high-risk services like burns, operating room, and sterilization service. D1 Sodium hypochlorite called bleach, a disinfectant (D2) belonging to quaternary ammonium compound (QAC), and disinfectant D3 combination chemistry of QAC and Polyhexamethylene biguanide hydrochloride.

The susceptibility profile of strains to disinfectants is estimated by “bacterial growth (+)” or “non-growth (-)” compared to the two controls: positive fertility control and negative sterility control for each [16]. The plate technique (microdilution) used has the advantage of studying all the strains simultaneously with various disinfectants’ dilutions [16, 24], in contrast to European standards [33, 34]. According to Russel (2003), a biocide was resistant if it does not show an antibacterial effect at the concentration recommended by the manufacturer [35]. Consequently, the determination of a target dilution for the “bacterial species – disinfectant” pair makes it possible to verify when this dilution is less than the use concentration recommended by the manufacturer. Indeed, in this study, we have shown that the antibacterial activity of disinfectants must be carried not only against the reference strains but also against bacteria isolated from the hospital environment. For instance, D2 is active on reference strains but not on *Staphylococcus epidermidis (S.epidermidis)*.

The chlorine compounds have a broad spectrum of antimicrobial activity, do not leave toxic residues, are unaffected by water hardness, remove dried or fixed organisms, and biofilms from surfaces [36]. In our

study, when tested at a dilution of 10^{-1}, D1 has shown antibacterial activity against all treated strains corroborating the dilution used by caregivers except Micrococcus sp and *Staphylococcus aureus* ATCC 29213 (*S. aureus)* were found resistant to this dilution. Based on the literature, hypochlorite acid is known for its potent activity against microorganisms [37]. The compound can (i) penetrate the walls and membranes of bacterial cells, (ii) denature proteins by oxidation, and (iii) inactivates the nucleic acids of bacteria [38, 39, 40]. The resistance of *S. aureus* to hypochlorite acid has been reported by Bekkari et *al.* (2016) [41]. The result could be explained by the efflux pump, the defense mechanism of this bacterium against disinfectant compounds [42].

The D2 belonging to quaternary ammoniums has shown good potency against all bacteria except *S.epidermidis* (n=2). Rouillon et *al.* (2006) showed antibacterial activity of this compound against the environmental strains of staphylococci at lower dilutions to the manufacturer's recommendations [24]. However, the resistance of Staphylococcus species to quaternary ammonium has been demonstrated by several studies [19]. Quaternary ammoniums (synthetic bipolar compounds) are more effective against Gram-positive bacteria than Gram-negative [43]. Despite their properties, concerns have been raised about the irrational use of disinfectants that could fail to eradicate nosocomial pathogens [44, 45].

D3 is combination chemistry of quaternary ammoniums and hydrochloride polyhexamethylene biguanide. This disinfectant did not inhibit the bacterial growth of *Escherichia coli* ATCC 25922, Micrococcus sp, bacillus gram-positive, and *S. epidermidis* at use's dilution. The resistance of Staphylococcus sp and *Escherichia coli* to hydrochloride polyhexamethylene biguanide has been demonstrated by Cowley et *al.* [46]. The association of biguanides and quaternary ammonium increases the biocidal effect [20]. Indeed, these compounds attack the cell membrane and allow the leakage of these constituents [39].

For the three disinfectants D1, D2, and D3, none is 100% effective. The emergence of resistance towards these disinfectants is rapidly increasing [47]. Multiple causes could explain the development of resistance (i) the size of the micrococcus wall and can make up to 50% of its cell mass [48] (ii) the presence of enzymatic systems such as catalase in Staphylococcus or superoxide dismutase in *Escherichia coli* which can destroy the disinfecting agent before bacterial degradation has taken place (iii) the presence of pumps such as efflux pumps which allows the "reject" of disinfecting agents out of the cell [49].

All bacteria in the hospital environment are potential pathogens *S.aureus,* and *Pseudomonas aeruginosa* are dangerous opportunistic microorganisms, capable of growing on inert surfaces and forming biofilms [50]. Their resistance to antimicrobial substances can compromise the therapeutic protocol, hence the necessity of controlling the disinfection process in the care units [51]. *S. epidermidis* can adhere and persist on medical surfaces and equipment [52]. Persistence risks perpetuating microbial reservoirs in the environment close to the patients and can be a source of nosocomial infection. To counter this resistance problem, increasing the concentration of disinfectants to sub-lethal values is not recommended because it can favor the emergence of resistant strains [53]. Moreover, some disinfectants may irritate users or can be toxic if used in high concentrations [54]. It remains the strategy of rotation of the active ingredients in the hospital even if results suggest that the maintenance of high disinfection efficiency can fail by rotation [15, 55]. Other research suggests that susceptibility decreases after repeated exposure to microbicide [46, 56]. Thus, future studies would ideally analyze an increased number of strains, evaluate different analytical methods, and monitor the disinfectant activity over time.

5. Conclusion

We studied the susceptibility of bacterial isolated from the hospital environment as well as reference strains against three disinfectants (D1, D2, and D3) containing various active molecules. The results showed that D1 is effective on all bacterial at a minimum dilution of 10^{-1}, except Micrococcus sp and *Staphylococcus aureus* ATCC 29213. D2 has efficacy on all bacterial strains at a dilution lower than the dilution recommended by the manufacturer (1/400). *Staphylococcus epidermidis* (n=2) were viable at 1/400 dilution. Micrococcus sp, GPB, *Staphylococcus epidermidis,* and *Escherichia coli* ATCC 25922 were resistant to D3 (at the manufacturer's recommended use condition). Therefore, D1 and D2 can eliminate most pathogenic bacteria in hospitals, in comparison to D3.

Conflict of interest statement

The authors declare that no conflicts of interest are associated with this work.

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