

Investigate the Correlation Between Antibiotics Resistance of Bacteria and Quaternary Ammonium Compounds (QACs) Resistance in Maternity Wards

Alaa Mohammed Alali¹, Ayad Almakki², Maysara Mohammed Al-Badran³

Abstract

Nosocomial infections (NIs) provide a considerable challenge in healthcare facilities, as their management challenges contribute to extended hospitalization periods, increased costs, and elevated mortality rates among patients. In order to mitigate the spread of this illness, disinfectants that possess a wide range of antibacterial properties, such as quaternary ammonium compounds (QACs), are employed. This study was conducted to assess the effectiveness of QACs as a disinfectant using a variety of dilution agents. It also investigates the potential correlation between disinfectant resistance and antibiotic resistance. Samples were collected from various areas within maternity wards, including beds, floors, and chairs. These samples were then subjected to diagnosis using the biomérieux vitek®2 system and treated with varying amounts of QACs. A total of 54 samples were tested, distributed as follows: 22 samples representing floors, 24 samples representing beds, and eight samples representing chairs. The analysis revealed the presence of multidrug-resistant bacteria (MDR) in the bacterial samples, indicating resistance to several types of antibiotics. Additionally, a portion of the bacteria isolated from maternity wards exhibited resistance to disinfectants. As with antibiotic resistance, some kinds of bacteria have been found to be resistant to disinfectants. This is because they have developed resistance genes, which have to do with how they are used and how long they are exposed to disinfectants. A potential correlation between bacterial resistance to antibiotics and disinfectants exists, thereby emphasizing the need for proper usage of antiseptics, including appropriate dilution, adherence to disinfectant contact duration, and application to suitable surfaces.

Keywords: *Nosocomial infections, maternity ward, resistance bacteria, disinfectants, quaternary ammonium compounds.*

Introduction

Hospitals have been identified as potential reservoirs for several infectious illnesses, including tuberculosis, diarrhea, and other significant ailments. Additionally, hospitals contribute to the growing concern about antibiotic resistance. [1] Nosocomial infections, also known as hospital-acquired infections, play a crucial role in contributing to avoidable illness and death, as well as imposing substantial economical burdens. The monitoring of nosocomial infection is a crucial component of the infection control program. [2] The World Health Organization (WHO) has repeatedly emphasized that

¹ Department of Laboratory Sciences, College of Pharmacy, University of Basrah, Basrah, Iraq, alaaalali653@gmail.com

² Department of Laboratory Sciences, College of Pharmacy, University of Basrah, Basrah, Iraq, ayad.qasim@uobasrah.edu.iq

³ Department of Obstetrics and Gynecology, College of Medicine, University of Basrah, Basrah, Iraq, maysaram1979@gmail.com

sterile operating rooms are unnecessary for normal deliveries. Nevertheless, that is necessary in some developing countries with poor infection control. [3] It has been found that poor hygiene practices and conditions at birth contribute to life-threatening infections in babies and mothers. According to WHO, sepsis still contributes to maternal deaths, neonatal morbidity, and mortality. [4] The most common germs that cause healthcare-associated infections, which are isolated from inanimate surfaces and equipment in the patient area, are Gram-positive bacteria (*Staphylococcus aureus*, *Enterococcus* species, and *Clostridium difficile*) and Gram-negative bacteria (*Acinetobacter* spp., *Pseudomonas aeruginosa*, *Klebsiella Pneumonia*, and *Escherichia coli*). [5] It is important to remove pathogenic bacteria from contaminated surfaces, equipment, and the medical environment to prevent infections. [6] So there are various methods for preventing contamination by these bacteria, such as physical and chemical processes, but disinfecting surfaces and materials in the hospital setting with antimicrobial agents such as synthetic disinfectants (quaternary ammonium, halogenated chemicals such as sodium hypochlorite, alcohols, and aldehydes) is still the most extensively utilized approach. [5] Disinfectants are chemicals compound that inhibits the growth of microorganisms (Bacteria and viruses) in inanimate surfaces or water.[7]The disinfectant can be classified as high-level, intermediate-level, or low-level disinfection. A high level of disinfection destroys all microbial pathogens, but not bacterial spores example, formaldehyde; an intermediate level of disinfection destroys mycobacteria, most viruses, and most fungi but not bacterial spores example alcohol; and finally, a low level of disinfection destroys most bacteria, some viruses, and some fungi but not bacterial spores' example quaternary ammonium compound(QACs).[7]QACs are the most effective disinfectant with a broad spectrum of antimicrobial properties. [8] Which have been applied in several clinical settings in addition to antimicrobial activities on a hard surface. [7] Due to their little irritation, low toxicity, and low corrosiveness, QACs are often used to control contamination in hospitals over a wide PH range. [9] QACs penetrate the cell wall and cause membrane disintegration and protein and nucleic acid breakdown. As a result, bacteria experience numerous negative impacts on their cell structure and a loss of structural order and cytoplasmic membrane integrity. [7] Occasionally, bacterial pathogens overpopulate certain efflux pumps to resist microbicides. [10] The aim of this study was to assess the antibacterial effectiveness of QACs as a disinfectant, using various dilution agents, against bacterial strains obtained from the maternity ward of hospitals in Basra, Iraq. Furthermore, it is important to evaluate the potential correlation between the subject matter and the phenomenon of antibiotic resistance.

Materials and method

Study site

The study was conducted at the microbiology laboratory of the college pharmacy of Basra University, during the period from January to June 2022. This work consisted of testing the efficacy of QACs with different concentrations frequently used at Basra hospitals.

Samples of the maternity ward

Samples were taken from the maternity ward of three hospitals (Basra Teaching Hospital, Basra Hospital for Women and Children, and Al-Mawanai Teaching Hospital), with sterile swabs from beds, floors, and chairs, as these places had the most contact with the pregnant woman inside the maternity ward. Samples were taken before and after cleaning the ward and transported directly to the laboratory for cultivation.

Culture media

All isolates were cultivated on nutritional agar (OXOID®), and the disinfection efficiency was determined using Muller-Hinton agar (OXOID®).

Microbial identification and antibiotic susceptibility assessment

The biomérieux vitek®2 compact system (AST-N222 and AST-P580 cards) was used to identify bacteria and to test antibiotic susceptibility using Specific sensitivity cards, also known as AST cards, were utilized in order to determine both the minimum inhibitory concentrations (MICs) and the antimicrobial susceptibility tests (ASTs). The VITEK 2 system and the advanced expert system (AES) were used to interpret the susceptibility tests in accordance with the criteria established by the Clinical and Laboratory Standards Institute (CLSI). The AST cards included the Gram-positive sensitivity card (AST-P580) and Gram-negative sensitivity card (AST-N222).[11]

Disinfectants used in hospitals

Inside the maternity ward of hospitals, two types of disinfectants are used: Surfaxn® for hard surfaces like floors, used with dilution, and RD spray® for surfaces of equipment and devices like beds and chairs used without dilution. Table 1 shows the characteristic of QACs used in the study.

Preparation of disinfectant dilutions

Dilutions were made for each type of disinfectant used in hospitals from a ratio of 1:1 to 1:10, in addition to making a positive(disinfectant) and negative (Sterile distilled water) control for each bacterial sample. [6]

Preparation of the suspension for isolated bacterial

The bacteria were identified by the vitek®2 Compact system, a sterile swab was dipped in bacterial suspension that was calibrated with a turbidimeter to 0.5 McFarland Standard (Approximately 1.5 x CFU/ml) and then streaked on Mueller-Hinton Agar.[8] Three replications were then made for each dilution ratio after the disinfectant had been added, and the plates were incubated for 24 hours at 37°C. The following day, a ruler was used to measure the zone inhibition for each dilution ratio. [5]

Results

A total of 54 samples were tested, distributed as follows: 22 samples representing floors, 24 samples representing beds, and eight samples representing chairs. Of these, 25 Gram-positive bacteria, 25 Gram-negative bacteria. And four bacteria could not be identified.

Distribution of isolated bacteria

Vitek®2 Compact system data reveal 25 Gram-positive and 25 Gram-negative microorganisms. *Staphylococcus haemolyticus* and *Staphylococcus hominis* were the most common species (5 samples (10%)) in a Gram-positive sample. *Enterobacter cloacae* were the most common Gram-negative bacteria (5 samples (10%)). as demonstrated in Table2

Antimicrobial susceptibility pattern of Gram-positive and Gram-negative bacteria

Fourteen antibiotic drugs were screened for in Gram-positive isolates (Table 3). Twenty five Gram-positive isolates were examined and found to be completely resistant (100%) to OX1. *Aerococcus viridans* and *Staphylococcus xylosus* were discovered to be resistant to 14 antibiotics(OX1, GM, TM, LEV, MXF, R, CM, LNZ, VA, TE, FT, FA, RA, and SXT), followed by *Kocuria varians*, which were resistant to 12 drugs(OX1, GM, LEV, MXF, R, CM, LNZ, VA, TE, FA, RA, and SXT), and *Streptococcus thoraltensis*, which was resistant to 11 antibiotics(OX1, GM, R, CM, LNZ, VA, TE, FA, RA, and SXT). In the case of Gram-negative Bacteria (Table 3), 15 antibiotics were tested on 25 isolates and found substantial resistance to IPM (68%) and FEP (60%). *Acinetobacter lwoffii* and *Enterobacter cloacae* were resistant to ten antibiotics (TIC, TCC, PIP, FEP, IPM, GM, TM, CIP, MNO, and SXT), with another serotype of *Acinetobacter lwoffii* and another

serotype of *Enterobacter cloacae* resistant to nine drugs (TIC, PIP, FEP, IPM, GM, TM, CIP, MNO, and SXT). *Acinetobacter baumannii*, on the other hand, was resistant to one drug (IPM).

Sensitivities of the isolates from the maternity ward to disinfectant (QACs)

A total of 20 samples were collected from the floor of the maternity ward, with 13 identified as Gram-negative bacteria and the remaining seven as Gram-positive bacteria. *Enterobacter cloacae* exhibited resistance to (QACs) with a diameter of 10mm at a dilution ratio of 1:1. Conversely, *Aerococcus viridians* showed sensitivity to QACs, displaying a diameter of 20mm at a dilution ratio of 1:10. As illustrated in Figure 1. About the specimens obtained from the maternity ward bedding, 22 samples were collected, consisting of 10 Gram-negative bacteria and 12 Gram-positive bacteria. *Enterobacter cloacae*, *Escherichia coli*, and *Staphylococcus hominins* exhibited resistance (QACs) at a dilution ratio 1:1, with corresponding inhibition zone diameters of 10mm, 11mm, and 10mm, respectively. Conversely, *Staphylococcus lentus* and another serotype of *Staphylococcus hominins* demonstrated sensitivity to QACs at a dilution ratio 1:10, resulting in inhibition zone diameters of 15mm and 19mm, respectively. As demonstrated in Figure 2. In finality, a comprehensive sample obtained from the chair of the maternity ward consisted of 8 bacterial isolates, with two identified as Gram-negative bacteria and six as Gram-positive bacteria. *Aeromonas hydrophila* exhibited a resistance of 10mm to (QACs) when exposed to concentrated QACs, serving as the positive control. In contrast, *Staphylococcus haemolyticus* demonstrated resistance of 10mm to QACs at a dilution ratio of 1:6. Shown in Figure 3.

Table 1. Names of disinfectants used in the study, its manufacturer, and origin

NO.	disinfectant	composition	Manufacturer	Origin
1	Surfaxn®	Multilayered Quaternary Ammonium compound (QAC)(4%),blended with Tributylorganotin (TBTO)(0.286%),surfactant(20%),and inert ingredients(75.714%)	Sterimed	Lebanon
2	RD spray®	Multilayered Quaternary Ammonium compound (QAC)(0.06%),blended with Tributylorganotin (TBTO)(0.0011%),perfume(0.08%),and inert ingredients(99.859%)	Sterimed	Lebanon

Table 2. Frequency and percentage of Gram-positive and Gram-negative bacterial isolates from the maternity ward floor, bed, and chair.

Environment bacteria in a delivery ward (N=50)			
Genus or species (Gm+ve)	Frequency(%)	Genus or species (Gm-ve)	Frequency(%)
<i>Staphylococcus haemolyticus</i>	5(10%)	<i>Enterobacter cloacae</i>	5(10%)
<i>Staphylococcus hominins</i>	5(10%)	<i>Aeromonas hydrophila</i>	4(8%)
<i>Aerococcus viridians</i>	2(4%)	<i>Acinetobacter lwoffii</i>	3(6%)
<i>Enterococcus faecium</i>	2(4%)	<i>Pantoea spp</i>	3(6%)
<i>Kocuria Kristina</i>	1(2%)	<i>Acinetobacter baumannii</i>	2(4%)
<i>Kocuria rosea</i>	1(2%)	<i>E.coli</i>	2(4%)
<i>Kocuria varians</i>	1(2%)	<i>Sphingomonas paucimbilis</i>	2(4%)
<i>Streptococcus thoralensis</i>	1(2%)	<i>Shewanella putrefaciens</i>	1(2%)
<i>Staphylococcus vitulinus</i>	1(2%)	<i>Rhizobium radiobacter</i>	1(2%)
<i>Staphylococcus warneri</i>	1(2%)	<i>Acinetobacter junii</i>	1(2%)

Staphylococcus lentus	1(2%)	Pseudomonas fluorescens	1(2%)
Staphylococcus captis	1(2%)		
Staphylococcus xylosus	1(2%)		
Leuconostoc mesenteroides	1(2%)		
Staphylococcus saprophyticus	1(2%)		

Table 3. Resistance percentage profile of Gram-positive and Gram-negative bacteria isolates from the maternity ward to antibiotic drugs.

Resistance of Gram-positive bacteria (N=25)		Resistance of Gram-negative bacteria (N=25)	
Antibiotic	N(%)	Antibiotic	N(%)
Oxacillin(OX1)	25(100%)	Ticarcillin (TIC)	10(40%)
Gentamicin(GM)	10(40%)	Ticarcillin/clavulanic acid (TCC)	7(28%)
Tobramycin(TM)	6(24%)	Piperacillin (PIP)	8(32%)
Levofloxacin(LEV)	5(20%)	Piperacillin/tazobactam (TZP)	1(4%)
Moxifloxacin(MXF)	3(12%)	Ceftazidime (CAZ)	13(52%)
Erythromycin(R)	21(84%)	Cefepime (FEP)	15(60%)
Clindamycin(CM)	15(60%)	Aztreonam (ATM)	14(56%)
Linezolid(LZN)	10(40%)	Imipenem (IPM)	17(68%)
Vancomycin(VA)	9(36%)	Meropenem (MEM)	1(4%)
Tetracycline(TE)	6(24%)	Amikacin (AN)	4(16%)
Nitrofurantoin(FT)	8(32%)	Gentamicin (GM)	9(36%)
Fusidic acid (FA)	10(40%)	Tobramycin (TM)	5(20%)
Rifampicin(RA)	9(36%)	Ciprofloxacin (CIP)	5(20%)
Trimethoprim/sulfamethoxazole (SXT),	6(24%)	Minocycline (MNO)	5(20%)
		Trimethoprim/sulfamethoxazole (SXT),	13(52%)

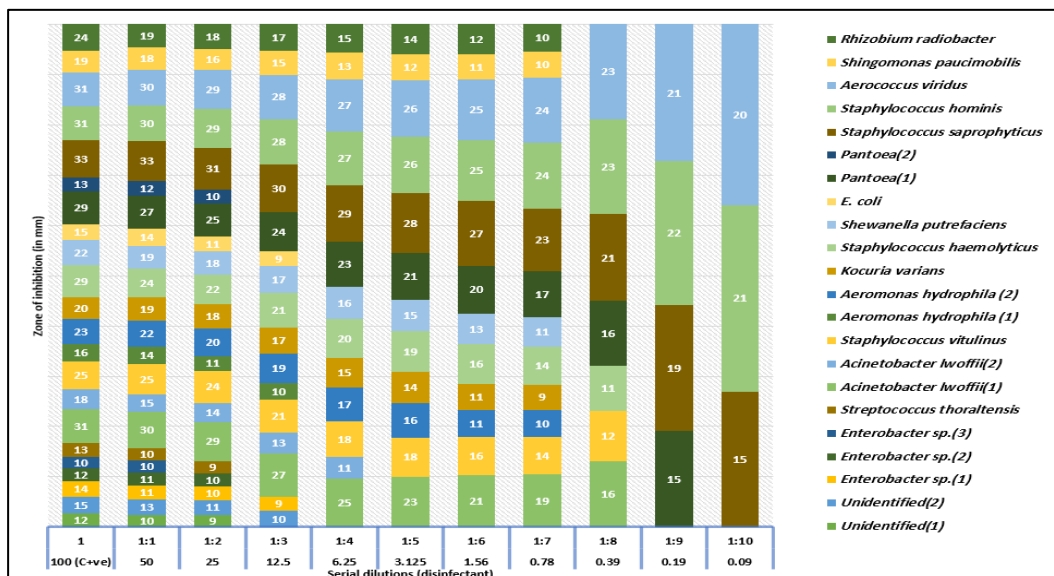


Figure 1. Show the extent of strain variety within the floor located in the maternity ward, employing serial dilutions of disinfectant and measuring the resulting zone of inhibition in millimeters.

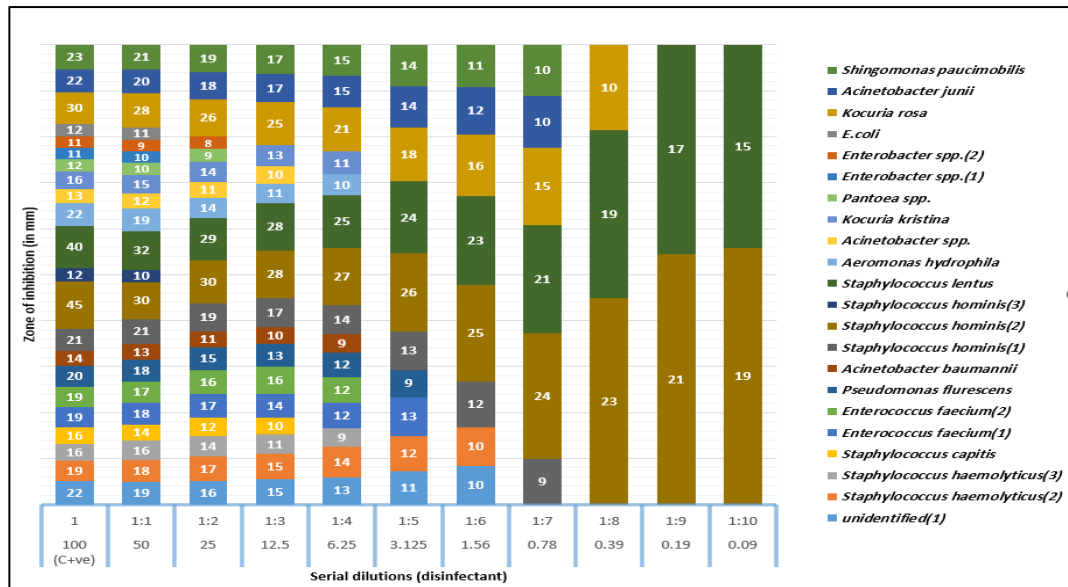


Figure 2. Show the extent of strain variety within beds located in the maternity ward, employing serial dilutions of disinfectant and measuring the resulting zone of inhibition in millimeters.

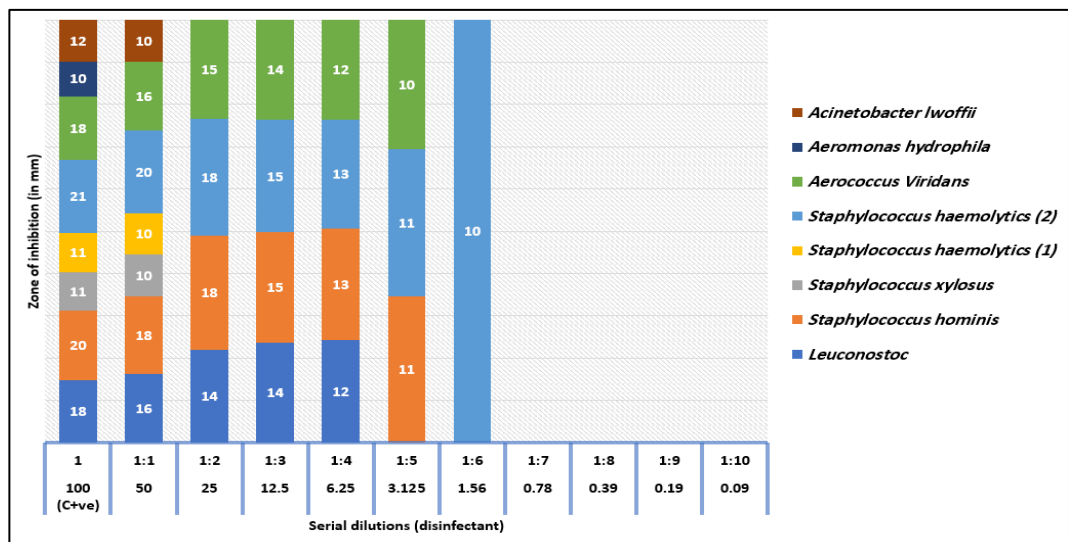


Figure 3. Show the extent of strain variety within chairs located in the maternity ward, employing serial dilutions of disinfectant and measuring the resulting zone of inhibition in millimeters

Discussion

Confirmed pathogenic microorganisms in the maternity ward pose a significant risk to patients and infants. These infections can readily develop antibiotic resistance, posing a significant risk to the patient's life. This study observed the presence of multidrug-resistant bacteria (MDR), specifically *Aerococcus viridians* and *Staphylococcus xylosus*, which exhibited resistance to various antibiotics. [12] So it is important to disinfect the environmental surface and medical devices in this ward to prevent the transmission of microorganisms and minimize the risk of infection.[5] In our study, two distinct categories of QACs were employed. The first kind, Surfaxn®, necessitated dilution before application, while the second type, RD spray®, was directly administered onto surfaces. QACs-based disinfectants have notable antimicrobial properties due to their

substantial biocidal efficacy, extended longevity, and environmental compatibility. [5] The emergence of resistance to QACs is intricately linked to these compounds' practical implementation and extended environmental exposure. [13] In this study, Certain organisms exhibited resistance to QACs, including *Enterobacter cloacae*, *Escherichia coli*, some serotypes of *Staphylococcus hominins*, and *Aeromonas hydrophila*. According to Figures 1,2, and 3, found Gram-negative bacteria are more resistant to disinfectants compared to Gram-positive bacteria. This is mostly attributed to the structural differences in their outer membrane, namely the asymmetric lipopolysaccharide composition. Consequently, the entry of disinfectants into Gram-negative bacteria is more challenging than in Gram-positive bacteria. [14] Hospital-acquired infections caused by *Acinetobacter* spp. provide a significant challenge in terms of treatment, mostly due to the rapid evolution of strains that exhibit resistance to many drugs. [10] Some previous studies mentioned some serotypes of *Acinetobacter* SPP. have resistance to B-lactams and carbapenems. In our study, *Acinetobacter lwoffii* also resisted this family of antibiotics. [15] The objective of this study was to investigate the potential correlation between antibiotic resistance and disinfectant resistance. Previous research has indicated that certain disinfectants can impact the viability of resistant bacteria, such as *Acinetobacter* SPP., which demonstrated resistance to multiple antibiotics (TIC, TCC, PIP, CAZ, FEP, ATM, IPM, CIP, and SXT) while remaining susceptible to disinfectants, as shown in Figure 1. [5] Nevertheless, other previous research has indicated a correlation between antibiotic resistance and antiseptic resistance. [16] It has been noted that there exists a resemblance in the mechanisms of resistance to antibiotics and disinfectants. This similarity encompasses the overexpression of efflux pumps, the creation of biofilms, and the occurrence of spontaneous mutations, all of which are observed in the presence of (QACs). This observation was made on *Staphylococcus xylosus*, which exhibited resistance to a range of antibiotics including (OX1, GM, TM, LEV, MXF, R, CM, LNZ, VA, TE, FT, FA, RA, and SXT). Furthermore, this strain showed reduced susceptibility to QACs, as evidenced by a measured diameter of 10mm. As seen in Figure 3. [17] Therefore, multiple parameters contribute to the enhancement of disinfection efficacy. The effectiveness of a disinfectant may be compromised if the application method is not executed correctly. This includes improper dilution of the disinfectant, failure to adhere to the recommended contact time, repeated use of the same disinfectant at the same dosage, potentially resulting in resistance, and the application of certain disinfectants on unsuitable surfaces. [5]

Conclusion

Few or no studies in Basra's hospitals have studied the antimicrobial effect of disinfectant on the bacteria isolated from maternity wards. The issue of microbicide resistance continues to be a significant subject in the field of healthcare, potentially existing as a distinct phenomenon from antimicrobial resistance. But inappropriate or excessive disinfectant use might potentially lead to complications. Therefore, it is important to acknowledge the factors that impact the efficacy of these products within the context of hospital disinfection procedures, as well Workers in maternity wards need to be taught how to use disinfectants correctly because it was found that the wrong use of disinfectants can lead to the spread and persistence of infection inside the hospital and the development of strains resistant to disinfectants. However, more research is necessary to assess the antibacterial capabilities of alternative disinfectants in conjunction with other bioactive compounds, as well as to investigate the synergistic effects of combining certain disinfectants in order to enhance their antimicrobial effectiveness.

References

- [1] S. A. Alkinani, A. J. Mohammad, and I. A. Al-Gizzi, "INDOOR MICROBIAL AIR CONTAMINATION IN SOME COLLEGE ROOMS AND HOSPITALS IN BASRA CITY," *Biochem. Cell. Arch*, vol. 21, no. 2, pp. 5019–5025, 2021, [Online]. Available: <https://connectjournals.com/03896.2021.21.5019>
- [2] A. Abdullah, "Isolation and identification of bacteria causing nosocomial infections in Al-Shafaa General Hospital in Basrah Surrogate markers for GHB exposure in cell lines View project Clinical Pharmacology View project." [Online]. Available: <https://faculty.uobasrah.edu.iq/faculty/815>
- [3] L. Charrier, P. Serafini, V. Chiono, M. Rebor, G. Rabacchi, and C. M. Zotti, "Clean and sterile delivery: Two different approaches to infection control," *J Eval Clin Pract*, vol. 16, no. 4, pp. 771–775, Aug. 2010, doi: 10.1111/j.1365-2753.2009.01191.x.
- [4] S. Cross et al., "Hygiene on maternity units: lessons from a needs assessment in Bangladesh and India," *Glob Health Action*, vol. 9, no. 1, Dec. 2016, doi: 10.3402/gha.v9.32541.
- [5] A. Ramzi, B. Oumokhtar, Y. Ez Zoubi, T. Filali Mouatasse, M. Benboubker, and A. El Ouali Lalami, "Evaluation of Antibacterial Activity of Three Quaternary Ammonium Disinfectants on Different Germs Isolated from the Hospital Environment," *Biomed Res Int*, vol. 2020, 2020, doi: 10.1155/2020/6509740.
- [6] F. Amini Tapouk, R. Nabizadeh, N. Mirzaei, N. Hosseini Jazani, M. Yousefi, and M. A. Valizade Hasanloei, "Comparative efficacy of hospital disinfectants against nosocomial infection pathogens," *Antimicrob Resist Infect Control*, vol. 9, no. 1, Jul. 2020, doi: 10.1186/s13756-020-00781-y.
- [7] G. McDonnell, A. D. Russell, L. Operations, and S. Louis, "Antiseptics and Disinfectants: Activity, Action, and Resistance," 1999. [Online]. Available: <http://cmr.asm.org/>
- [8] X. F. He et al., "A novel method to detect bacterial resistance to disinfectants," *Genes Dis*, vol. 4, no. 3, pp. 163–169, Sep. 2017, doi: 10.1016/j.gendis.2017.07.001.
- [9] G. Wu et al., "Evaluation of agar dilution and broth microdilution methods to determine the disinfectant susceptibility," *Journal of Antibiotics*, vol. 68, no. 11, pp. 661–665, Nov. 2015, doi: 10.1038/ja.2015.51.
- [10] M. Betchen et al., "Evaluating the Effectiveness of Hospital Antiseptics on Multidrug-Resistant *Acinetobacter baumannii*: Understanding the Relationship between Microbicide and Antibiotic Resistance," *Antibiotics*, vol. 11, no. 5, May 2022, doi: 10.3390/antibiotics11050614.
- [11] "Instrument User Manual EC REP [04]," 2426. [Online]. Available: <http://www.biomerieux.com>
- [12] F. H. Kamel, F. HKamel, K. Qader Salh, and Y. Abdulmajeed Shakir, "Isolation of Potential Pathogenic Bacteria from Pregnant Genital Tract and Delivery Isolation of Potential Pathogenic Bacteria from Pregnant Genital Tract and Delivery Room in Erbil Hospital," 2014.
- [13] K. Hegstad, S. Langsrud, B. T. Lunestad, A. A. Scheie, M. Sunde, and S. P. Yazdankhah, "Does the Wide Use of Quaternary Ammonium Compounds Enhance the Selection and Spread of Antimicrobial Resistance and Thus Threaten Our Health?"
- [14] C. Tong, H. Hu, G. Chen, Z. Li, A. Li, and J. Zhang, "Disinfectant resistance in bacteria: Mechanisms, spread, and resolution strategies," *Environmental Research*, vol. 195. Academic Press Inc., Apr. 01, 2021. doi: 10.1016/j.envres.2021.110897.
- [15] L. Chaoui, R. Mhand, F. Mellouki, and N. Rhallabi, "Contamination of the Surfaces of a Health Care Environment by Multidrug-Resistant (MDR) Bacteria," *Int J Microbiol*, vol. 2019, 2019, doi: 10.1155/2019/3236526.
- [16] S. Buffet-Bataillon, B. Branger, M. Cormier, M. Bonnaure-Mallet, and A. Jolivet-Gougeon, "Effect of higher minimum inhibitory concentrations of quaternary ammonium compounds in clinical *E. coli* isolates on antibiotic susceptibilities and clinical outcomes," *Journal of Hospital Infection*, vol. 79, no. 2, pp. 141–146, Oct. 2011, doi: 10.1016/j.jhin.2011.06.008.

- [17] S. Buffet-Bataillon, P. Tattevin, J. Y. Maillard, M. Bonnaure-Mallet, and A. Jolivet-Gougeon, "Efflux pump induction by quaternary ammonium compounds and fluoroquinolone resistance in bacteria," *Future Microbiology*, vol. 11, no. 1. Future Medicine Ltd., pp. 81–92, Jan. 01, 2016. doi: 10.2217/fmb.15.131.