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An Assessment of the Efficacy of Chlorhexidine in Nebulizer Disinfectant to Prevent Contaminated Aerosol Administration

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ABSTRACT: Nebulization therapy is an essential method of drug administration that enables effective delivery of medications to the respiratory tract in aerosol form; however, contaminated nebulizer components may serve as a source of pathogenic microorganisms associated with nosocomial pneumonia. This in vitro experimental study aimed to evaluate the effectiveness of chlorhexidine as a nebulizer disinfectant in eliminating pathogenic bacteria. The study employed a post-test control group design using six groups of nebulizer chambers, consisting of two control groups and four intervention groups. Two clinically relevant bacteria, *Pseudomonas aeruginosa* and *Acinetobacter baumannii,* were inoculated into the nebulizer chambers. The intervention groups were disinfected using chlorhexidine gluconate at concentrations of 2.5%, 4%, and 5% diluted in 70% alcohol, and 2.5% chlorhexidine diluted in distilled water, while the control groups received sterile water and 70% alcohol, in accordance with existing guidelines. After a standardized exposure period, bacterial growth was assessed using Colony Forming Unit (CFU) counts. Data were analyzed descriptively and comparatively to evaluate bacterial eradication across groups. The results demonstrated that 5% chlorhexidine diluted in 70% alcohol achieved complete bacterial elimination (0 CFU) for both bacterial strains, whereas lower concentrations showed residual growth. These findings indicate that chlorhexidine, particularly at higher concentrations, demonstrates strong disinfectant activity against common nosocomial pneumonia pathogens in nebulizer chambers. Nevertheless, the results are limited to in vitro conditions; therefore, further studies involving a wider range of microorganisms, standardized exposure times, and assessments of aerosol contamination during clinical nebulization are warranted to support its practical application.

Keywords: **chlorhexidine; disinfectant; nebulizer; nosocomial infection.**

Introduction

Nebulization therapy is the conversion of medications into fine aerosol particles for efficient delivery to the respiratory tract and lungs. This process is generated with a drug solution by nebulizer, through the mouth, nose, or artificial airway, including endotracheal and tracheotomy tubes [1–3]. It is important for managing a range of respiratory conditions, such as asthma, bronchitis, and chronic obstructive pulmonary disease (COPD) [4]. However, the efficacy can be compromised due to the potential contamination of aerosols administered during the procedure. The release of contaminated aerosols can originate from both patient and medical sources, into the environment and potentially expose caregivers and people around the patient to infectious agents [5]. This contamination causes significant health risks to patients and worsens the respiratory illnesses [6]. The major concern is the occurrence of nosocomial pneumonia due to contaminated aerosols caused by the therapy [7,8].

Nosocomial infection is a term used in pneumonia cases that manifest in 48 hours or after patient is admitted to hospital but is not consistent with the incubation period during admission [9]. Pneumonia is a condition with potentially life-threatening complications, such as respiratory failure, sepsis, metastatic infection, empyema, lung abscess, and multi-organ dysfunction [10]. A previous study showed that nosocomial pneumonia is a substantial contributor to pneumonia-related fatalities, accounting for 1.5 million or 55% of deaths worldwide across all age groups [11]. Bacteria predominantly instigate nosocomial infection, with fungi and viruses seldom implicated, specifically in immunocompetent patients [12].

The exploration of effective strategies is essential to prevent aerosol contamination during nebulization therapy and to ensure

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its efficacy and safety. One critical factor in minimizing contamination is maintaining proper hygiene of nebulization equipment [7,13]. However, previous studies have demonstrated that nebulizer contamination may persist despite routine cleaning and disinfection practices. An observational study reported that a substantial proportion of nebulizers remained contaminated even when regular hygiene measures were applied, suggesting that commonly used disinfection methods may be insufficient to completely eliminate microorganisms from nebulizer components [14–16]. These findings highlight the need for more effective and reliable disinfectants for nebulizer equipment.

One potential approach involves the use of disinfectants with established antimicrobial properties, such as chlorhexidine. Chlorhexidine has long been recognized as an effective antiseptic and disinfectant in healthcare settings [17]. Nevertheless, its application as a disinfectant for semi-critical medical devices has not been widely adopted. Chlorhexidine solutions are relatively safe for direct human contact when used at appropriate concentrations and are available at a comparatively low cost. A study by Hutauruk et al. (2021) demonstrated that chlorhexidine at a concentration of 2.5% was effective in decontaminating tracheostomy cannulas, which are classified as semi-critical instruments [18].

Accordingly, this study aimed to evaluate the effectiveness of chlorhexidine as a disinfectant for nebulizer chambers, with a specific focus on different chlorhexidine concentrations beyond those previously reported and their ability to eliminate Pseudomonas aeruginosa and Acinetobacter baumannii. This study addresses the existing knowledge gap by providing experimental evidence on chlorhexidine-based disinfection for nebulizer equipment, thereby contributing to the development of safer and more effective infection prevention strategies in nebulization therapy.

Methods

Study Design and Experimental Unit

This study employed an in vitro experimental post-test control group design. The experimental unit in this study was the nebulizer chamber, with each chamber treated as an independent unit of analysis. Each nebulizer chamber was inoculated and disinfected only once to ensure independence of measurements between groups. Given the exploratory nature of this investigation, the study was conducted as a pilot in vitro study and was not powered for definitive statistical inference. Ethical approval was obtained from the Ethics Commission of the Faculty of Medicine, Universitas Brawijaya. No human participants or animal subjects were involved. The study exclusively used clinical bacterial isolates obtained from hospitalized patients, without any identifiable patient data.

Intervention and Control Groups

The study consisted of four intervention groups and two control groups. The intervention groups underwent disinfection using chlorhexidine gluconate at concentrations of 2.5%, 4%, and 5% diluted in 70% alcohol, as well as 2.5% chlorhexidine diluted in distilled water, following previously reported formulations [18]. Two control groups were defined as follows: Negative control: sterile water, representing minimal disinfection without antimicrobial activity. Positive control: 70% alcohol, representing a commonly recommended disinfectant in current guidelines [19,20]. The selection of control groups was based on their widespread use and recommendation in existing nebulizer hygiene guidelines.

Bacterial Inoculation in The Nebulizer Chamber

Inoculation was performed within the nebulizer chamber, using isolated *Pseudomonas aeruginosa* (2068) and *Acinetobacter baumannii* (1702) bacteria from the hospital in Malang and cultured on agar media for preservation. The proliferation process begins by extracting 1000 bacteria/ml and placing it in a liquid growth medium. This is followed by a 24-hour incubation period, the bacterial concentration was quantified using spectrophotometry at a wavelength of 625 nm. The bacteria used for the experiment exhibited a concentration of log 10^7, with *P. aeruginosa* at 1.45 x 10^7 and *A. baumannii* at 1.14 x 10^7 [21–23]. Furthermore, the liquid containing bacteria was separately introduced into the nebulizer chamber and then incubated for 24 hours at 37°C. Before the initiation of the washing intervention, a subsequent measurement of bacterial concentration was conducted, showing an increase to 1.01 x 10^9 (1.010.000.000 bacteria/ml) and 2.61 x 10^8 (260.000.000 bacteria/ml) for *P. aeruginosa* and *A. baumannii*, respectively.

Preparation of Chlorhexidine Solutions

Chlorhexidine gluconate stock solution was diluted aseptically to obtain final concentrations of 2.5%, 4%, and 5% using either 70% alcohol or distilled water, depending on group allocation. Serial dilutions were prepared using calibrated volumetric instruments to ensure accuracy and consistency across all experimental units.

Nebulizer Chamber Disinfection Process

Nebulizer chamber is a storage for medicine evaporated into an aerosol which is prone to contamination and can spread contaminated aerosols [22]. The disinfection procedure commence with the introduction of the liquid containing bacteria into the biological specimen disposal container. This is followed by the introduction of chlorhexidine with varying concentrations into the chamber and agitated with a rotational movement before disposal. A second wash with chlorhexidine disinfectant is applied and allowed to sit for 10 minutes. The disinfectant solution is then drained for a second time and nebulizer chamber is meticulously rinsed using sterile water to eliminate any residual washing fluid. Distilled water is introduced once more for the purpose of obtaining samples to be cultured on agar plates. An identical methodology was adopted in the control group, using sterile water and a 70% alcohol solution.

Bacterial Culture and Colony Counting

In this procedure, a single dose was extracted from the sample and streaked onto nutrient agar for bacterial cultivation before disinfection intervention, and the agar plates were incubated for 24 hours at 37°C. In the culture examination, a meticulous method was used including the use of two agar plates for each sample. The examiner remained blinded to the categorization of the samples into the control or intervention group, ensuring an unbiased assessment.

Statistical Analysis

Data analysis was performed using SPSS version 26. The primary outcome variable was the reduction in bacterial colony counts (CFU/mL) following disinfection. Normality of data distribution was assessed prior to analysis. An independent t-test was used to compare bacterial reduction between intervention and control groups. A p-value of < 0.05 was considered statistically significant. Given the pilot nature of the study, statistical analyses were interpreted descriptively to explore trends rather than to draw definitive inferential conclusions.

Result and Discussion

A total of 12 nebulizer chambers from GEA medical were used and were contaminated by two gram-negative bacteria that cause nosocomial pneumonia, *P. aeruginosa*, and *A. baumannii*. After the disinfection procedure, effectiveness was assessed using bacterial culture and colony counting. In the control group, which used sterile water and 70% alcohol for washing and soaking, it was observed that *P.aeruginosa* bacterial colonies remained densely populated (>300 CFU/plate, according to O'Toole, 2016). Additionally, *A. baumannii* showed persistent growth, with colonies measuring 158 CFU/plate (1.58×10^5 CFU/mL) after washing and soaking in sterile water, and 202 CFU/plate (2.02×10^5 CFU/mL) after exposure to 70% alcohol. The results of the microbiological examination are shown in Table 1.

In the intervention groups, disinfection with 2.5% chlorhexidine diluted in sterile water resulted in persistent *P. aeruginosa* growth that remained too numerous to count (>300 CFU/plate; $>3.0 \times 10^5$ CFU/mL). However, *A. baumannii* counts were reduced to 39 CFU/plate (3.9×10^5 CFU/mL). When 2.5% chlorhexidine was diluted with 70% alcohol, *P. aeruginosa* colonies were reduced to 58 CFU/plate (5.8×10^5 CFU/mL), while *A. baumannii* colonies were reduced to 82 CFU/plate (8.2×10^5 CFU/mL).

Further reduction in bacterial counts was observed with higher concentrations of chlorhexidine. Disinfection with 4% chlorhexidine diluted in 70% alcohol reduced *P. aeruginosa* to 36 CFU/plate (3.6×10^5 CFU/mL), while no detectable growth of *A. baumannii* was observed (0 CFU/plate). Complete absence of bacterial growth for both *P. aeruginosa* and *A. baumannii* was observed following disinfection with 5% chlorhexidine diluted in 70% alcohol (0 CFU/plate).

In the context of nebulization therapy, ensuring the purity of administered aerosols is important to prevent potential harm to patients, particularly those with respiratory conditions. Nebulizer is recognized as potential source that facilitates significant nosocomial infections when colonized by various types of bacteria. Another study also identified *Pseudomonas, B. cepacia, Klebsiella sp, Acinetobacter sp*, and polymicrobial flora as contaminants in nebulizer [24]. This study focused on evaluating the viability of chlorhexidine as an effective disinfection agent for nebulizer chambers, by addressing the pressing concern of bacterial contamination during nebulization.

The investigation led to a significant breakthrough, particularly with chlorhexidine showing potent efficacy in eradicating bacterial growth. Furthermore, the use of chlorhexidine as an antiseptic and skin disinfectant has been substantiated as highly efficacious in averting nosocomial infections [25–27]. A previous study used the 1% chlorhexidine solution for disinfecting sputum specimens containing *Mycobacterium tuberculosis* before the disposal [28]. Recently, Chlorhexidine has demonstrated efficacy in disinfecting semi-critical equipment [18].

Table 1. Colony counting results after disinfection intervention in the control and treatment groups.

No	Group	Intervention	P. aeruginosa	A. baumannii
1	Control Group 1	Washing and soaking with sterile water	Too numerous to count (>300 CFU/plate; >3.0 × 10^5 CFU/mL)	158 CFU/plate (1.58 × 10^5 CFU/mL)
2	Control Group 2	Washing and soaking with 70% alcohol	Too numerous to count (>300 CFU/plate; >3.0 × 10^5 CFU/mL)	202 CFU/plate (2.02 × 10^5 CFU/mL)
3	Intervention Group 1	Washing and soaking with 2.5% chlorhexidine (diluted with sterile water)	Too numerous to count (>300 CFU/plate; >3.0 × 10^5 CFU/mL)	39 CFU/plate (3.9 × 10^4 CFU/mL)
4	Intervention Group 2	Washing and soaking with 2.5% chlorhexidine (diluted with 70% alcohol)	58 CFU/plate (5.8 × 10^4 CFU/mL)	82 CFU/plate (8.2 × 10^4 CFU/mL)
5	Intervention Group 3	Washing and soaking with 4% chlorhexidine (diluted with 70% alcohol)	36 CFU/plate (3.6 × 10^4 CFU/mL)	0 CFU/plate (below detection limit)
6	Intervention Group 4	Washing and soaking with 5% chlorhexidine (diluted with 70% alcohol)	0 CFU/plate (below detection limit)	0 CFU/plate (below detection limit)

*CFU: Colony Forming Unit

This study evaluated the efficacy of chlorhexidine in cleansing contaminated nebulizer chambers, focusing on two bacteria associated with nosocomial pneumonia as a starting point. Specifically, *P. aeruginosa* and *A. baumannii*, which fall under the ESKAPE comprising the scientific names of six highly virulent and antibiotic-resistant bacterial pathogens, are prominent pathogens in health care [29]. In nosocomial infections, *P. aeruginosa* and *A. baumannii* are formidable adversaries, recognized for the capacity to instigate infections [9,30,31]. These Gram-negative bacteria showed an alarming threat due to the intrinsic resistance mechanisms and ability to survive in diverse environments [29].

P. aeruginosa, a ubiquitous pathogen, poses a substantial risk, particularly in immunocompromised individuals and those with underlying respiratory conditions [32]. It leads to severe pneumonia, urinary tract infections, sepsis, and wound infections. The inherent resistance of this pathogen to antibiotics with the ability to form biofilms poses exceptional challenges for effective eradication and therapy [33]. *Pseudomonas aeruginosa* commonly instigates cases of nosocomial pneumonia and is associated with unfavorable clinical outcomes [32]. The results of this study are consistent with Ben-Knaz Wakshlak et al. (2019). The antibacterial impact of chlorhexidine against *P. aeruginosa*, with an initial population of 108 CFU/mL, showed a significant reduction in bacterial population by a factor of 5.4, equivalent to a 99.999% [34].

A. baumannii is recognized as a significant nosocomial pathogen commonly affecting critically ill patients. It is well-known for causing ventilator-associated pneumonia, bloodstream, and surgical site infections [35,36]. The ability of *A. baumannii* to develop resistance to multiple classes of antibiotics, including carbapenems, exacerbates its clinical management [37]. Its reduction by chlorhexidine is consistent with many other investigations using chlorhexidine to reduce the viability of *A. baumannii* on environmental surfaces in hospitalized patients [38].

The major concern is the potential dissemination of these multidrug-resistant organisms through contaminated nebulizer during aerosol administration. This contamination leads to nosocomial infections, making the investigation of effective disinfection strategies for nebulizer chambers imperative. In this study, the successful eradication of both *P. aeruginosa* and *A. baumannii* with a 5% concentration of chlorhexidine shows great potential in mitigating the associated risk.

The effectiveness of chlorhexidine is shown in the extensive antimicrobial activity, ability to disrupt bacterial cell membranes, and critical cellular processes, leading to the eradication of bacteria. Lower concentrations impact membrane integrity, while higher concentrations induce cytoplasmic coagulation [39]. Biswas et al. (2019) provided a more comprehensive explanation, showing that the antibacterial activity of chlorhexidine is due to a high production of Reactive Oxygen Species (ROS) and elevated lipid peroxidation. These biochemical alterations lead to membrane damage and modifications in proteins, phospholipids, carbohydrates, and nucleic acids [40].

The results of this study showed the substantial potential of chlorhexidine, particularly at a 5% concentration, in effectively nebulizer disinfectant. This application significantly reduces the risk of administering contaminated aerosols during nebulization therapy, thereby ensuring patient safety and mitigating nosocomial infection risks. Further inquiry into the potential allergic risks associated with a 5% concentration of chlorhexidine is important. A previous study showed that allergic

reactions manifest, particularly concerning the risk during nebulizer chamber disinfection [41]. Ensuring safety is undeniably an important consideration for the widespread use of chlorhexidine.

Despite the promising outcomes, further studies and comprehensive clinical trials are required to validate these results across diverse healthcare contexts. Expanding the scope to include broader demographics and investigating the long-term implications of chlorhexidine-based disinfection on durability and patient outcomes is crucial for seamless clinical integration..

Conclusion

This study demonstrated that chlorhexidine is effective as a disinfectant for nebulizer chambers contaminated with Pseudomonas aeruginosa and Acinetobacter baumannii, with higher concentrations showing greater bacterial reduction. The findings suggest that chlorhexidine has potential to reduce bacterial contamination in nebulizer equipment used during respiratory therapy. Given that this study was conducted as a preliminary in vitro investigation, the results should be interpreted cautiously. Further studies involving larger sample sizes, diverse clinical settings, and comprehensive safety evaluations are required to confirm the effectiveness and feasibility of chlorhexidine for routine nebulizer disinfection. Future research should also examine the long-term effects of repeated chlorhexidine use on nebulizer materials and patient safety to support its potential clinical application.

Conflict of Interest

The authors have no conflicts of interest regarding this investigation.

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References

[1]. Chinese College of Emergency Physicians (CCEP). Expert consensus on nebulization therapy in pre-hospital and in-hospital emergency care. Ann Transl Med [Internet]. 2019 Sep;7(18):487–487. Available from: http://atm.amegroups.com/article/view/29746/25813

[2]. Le Brun PPH, de Boer AH, Frijlink HW, Heijerman HGM. A review of the technical aspects of drug nebulization. Pharmacy World and Science. 2000;22(3):75–81.

[3]. Longest W, Spence B, Hindle M. Devices for Improved Delivery of Nebulized Pharmaceutical Aerosols to the Lungs. J Aerosol Med Pulm Drug Deliv. 2019 Oct 1;32(5):317–39.

[4]. Talwar D, Ramanathan R, Lopez M, Hegde R, Gogtay J, Goregaonkar G. The emerging role of nebulization for maintenance treatment of chronic obstructive pulmonary disease at home. Lung India. 2021;38(2):168.

[5]. Mac Giolla Eain M, Cahill R, MacLoughlin R, Nolan K. Aerosol release, distribution, and prevention during aerosol therapy: a simulated model for infection control. Drug Deliv [Internet]. 2022 Dec 31;29(1):10–7. Available from: https://www.tandfonline.com/doi/full/10.1080/10717544.2021.2015482

[6]. Terry PD, Dhand R. Maintenance Therapy with Nebulizers in Patients with Stable COPD: Need for Reevaluation. Pulm Ther. 2020 Dec 20;6(2):177–92.

[7]. Bell J, Alexander L, Carson J, Crossan A, McCaughan J, Mills H, et al. Nebuliser hygiene in cystic fibrosis: evidence-based recommendations. Breathe. 2020 Jun;16(2):190328.

[8]. Vassal S, Taamma R, Marty N, Sardet A, d'Athis P, Brémont F, et al. Microbiologic contamination study of nebulizers after aerosol therapy in patients with cystic fibrosis. Am J Infect Control. 2000 Oct;28(5):347–51.

[9]. Shebl E, Gulick PG. Nosocomial Pneumonia. StatPearls [Internet]. 2022 Jul 18 [cited 2023 Feb 8]; Available from: https://www.ncbi.nlm.nih.gov/books/NBK535441/

[10]. Jain V, Vashisht R, Yilmaz G, Bhardwaj A. Pneumonia Pathology. StatPearls [Internet]. 2022 Aug 1 [cited 2023 Feb 9]; Available from: https://www.ncbi.nlm.nih.gov/books/NBK526116/

[11]. Lim WS. Pneumonia—Overview. Encyclopedia of Respiratory Medicine [Internet]. 2022 Jan 1 [cited 2023 Feb 8];4:185. Available from: /pmc/articles/PMC7241411/

[12]. Morrow LE, Kollef MH. Hospital-Acquired Pneumonia. In: Netter's Infectious Diseases [Internet]. Elsevier; 2012. p. 137–45. Available from: https://linkinghub.elsevier.com/retrieve/pii/B9781437701265000276

[13]. O'Malley CA. Device cleaning and infection control in aerosol therapy. Respir Care. 2015;60(6):917–30.

[14]. Yegit CY, Ergenekon P, Duman N, Mursaloglu H, Cenk M, Uzunoglu BS, et al. Microbial Contamination of Nebulizers in Patients With Cystic Fibrosis. Turkish Archives of Pediatrics. 2025 Jan 2;60(1):22–8.

[15]. Biney IN, Ari A, Barjaktarevic IZ, Carlin B, Christiani DC, Cochran L, et al. Guidance on Mitigating the Risk of Transmitting Respiratory Infections During Nebulization by the COPD Foundation Nebulizer Consortium. Chest. 2024 Mar;165(3):653–68.

[16]. Riquena B, Monte L de FV, Lopes AJ, Silva-Filho LVRF da, Damaceno N, Aquino E da S, et al. Microbiological contamination of nebulizers used by cystic fibrosis patients: an underestimated problem. Jornal Brasileiro de Pneumologia. 2019;45(3).

[17]. Knobloch MJ, Musuuza JS, McKinley L, Zimbric ML, Baubie K, Hundt AS, et al. Implementing daily chlorhexidine gluconate (CHG) bathing in VA settings: The human factors engineering to prevent resistant organisms (HERO) project. Am J Infect Control [Internet]. 2021;49(6):775–83. Available from: https://www.sciencedirect.com/science/article/pii/S0196655320310658

[18]. Hutauruk SM, Hermani B, Monasari P. Role of chlorhexidine on tracheostomy cannula decontamination in relation to the growth of Biofilm-Forming Bacteria Colony- a randomized controlled trial study. Annals of Medicine and Surgery [Internet]. 2021;67(June):102491. Available from: https://doi.org/10.1016/j.amsu.2021.102491

[19]. Shen K, Hong J, El Beleidy A, Furman E, Liu H, Yin Y, et al. International expert opinion on the use of nebulization for pediatric asthma therapy during the COVID-19 pandemic. J Thorac Dis. 2021 Jul;13(7):3934–47.
[20]. Swarnakar R, Gupta NM, Halder I, Khilnani GC. Guidance for nebulization during the COVID-19 pandemic. Lung India. 2021 Mar;38(Suppl 1):S86–91.
[21]. Hohenwarter K, Prammer W, Aichinger W, Reychler G. An evaluation of different steam disinfection protocols for cystic fibrosis nebulizers. Journal of Cystic Fibrosis [Internet]. 2016;15(1):78–84. Available from: http://dx.doi.org/10.1016/j.jcf.2015.07.005
[22]. Towle D, Baker V, Schramm C, O'Brien M, Collins MS, Feinn R, et al. Ozone disinfection of home nebulizers effectively kills common cystic fibrosis bacterial pathogens. Pediatr Pulmonol. 2018;53(5):599–604.
[23]. Towle D, Callan DA, Lamprea C, Murray TS. Baby bottle steam sterilizers for disinfecting home nebulizers inoculated with non-tuberculous mycobacteria. Journal of Hospital Infection [Internet]. 2016;92(3):222–5. Available from: http://dx.doi.org/10.1016/j.jhin.2015.08.030
[24]. Nitin K, Santlal Hassani U. Microbial Colonization Profile of Respiratory Devices. 2016 Apr;165–7.
[25]. Edmiston CE, Bruden B, Rucinski MC, Henen C, Graham MB, Lewis BL. Reducing the risk of surgical site infections: Does chlorhexidine gluconate provide a risk reduction benefit? Am J Infect Control. 2013 May;41(5):S49–55.
[26]. Hasegawa T, Tashiro S, Mihara T, Kon J, Sakurai K, Tanaka Y, et al. Efficacy of surgical skin preparation with chlorhexidine in alcohol according to the concentration required to prevent surgical site infection: meta-analysis. BJS Open. 2022 Sep 2;6(5).
[27]. Reynolds SS, Woltz P, Keating E, Neff J, Elliott J, Hatch D, et al. Results of the CHlorhexidine Gluconate Bathing implementation intervention to improve evidence-based nursing practices for prevention of central line associated bloodstream infections Study (CHanGing BathS): a stepped wedge cluster randomized trial. Implementation Science [Internet]. 2021 Dec 26;16(1):45. Available from: https://implementationscience.biomedcentral.com/articles/10.1186/s13012-021-01112-4
[28]. Asmar S, Drancourt M. Chlorhexidine decontamination of sputum for culturing Mycobacterium tuberculosis. BMC Microbiol. 2015 Dec 5;15(1):155.
[29]. Wieland K, Chhatwal P, Vonberg RP. Nosocomial outbreaks caused by Acinetobacter baumannii and Pseudomonas aeruginosa: Results of a systematic review. Am J Infect Control. 2018 Jun;46(6):643–8.
[30]. Reynolds D, Kollef M. The Epidemiology and Pathogenesis and Treatment of Pseudomonas aeruginosa Infections: An Update. Drugs. 2021 Dec 7;81(18):2117–31.
[31]. Yang YS, Lee YT, Huang TW, Sun JR, Kuo SC, Yang CH, et al. Acinetobacter baumannii nosocomial pneumonia: is the outcome more favorable in non-ventilated than ventilated patients? BMC Infect Dis. 2013 Dec 19;13(1):142.
[32]. Lu Q, Eggimann P, Luyt CE, Wolff M, Tamm M, François B, et al. Pseudomonas aeruginosa serotypes in nosocomial pneumonia: prevalence and clinical outcomes. Crit Care. 2014;18(1):R17.
[33]. Wolska K, Kot B, Jakubczak A. Phenotypic and genotypic diversity of Pseudomonas aeruginosa strains isolated from hospitals in siedlce (Poland). Brazilian Journal of Microbiology. 2012 Mar;43(1):274–82.
[34]. Ben-Knaz Wakshlak R, Pedahzur R, Avnir D. Antibacterial Activity of Chlorhexidine-Killed Bacteria: The Zombie Cell Effect. ACS Omega. 2019 Dec 17;4(25):20868–72.
[35]. Huang Y, Zhou Q, Wang W, Huang Q, Liao J, Li J, et al. Acinetobacter baumannii Ventilator-Associated Pneumonia: Clinical Efficacy of Combined Antimicrobial Therapy and in vitro Drug Sensitivity Test Results. Front Pharmacol. 2019 Feb 13;10.
[36]. Lynch J, Zhanel G, Clark N. Infections Due to Acinetobacter baumannii in the ICU: Treatment Options. Semin Respir Crit Care Med. 2017 Jun 4;38(03):311–25.
[37]. Ibrahim S, Al-Saryi N, Al-Kadmy IMS, Aziz SN. Multidrug-resistant Acinetobacter baumannii as an emerging concern in hospitals. Mol Biol Rep. 2021 Oct 30;48(10):6987–98.
[38]. Martinez-Resendez MF, Cruz-López F, Gaona-Chávez N, Camacho-Ortiz A, Mercado-Longoria R, Flores-Treviño S, et al. The effect of chlorhexidine on Acinetobacter baumannii in intensive care units. Iran J Microbiol. 2022 Feb 21;
[39]. McDonnell G, Russell AD. Antiseptics and Disinfectants: Activity, Action, and Resistance. Clin Microbiol Rev. 1999 Jan;12(1):147–79.
[40]. Biswas D, Tiwari M, Tiwari V. Molecular mechanism of antimicrobial activity of chlorhexidine against carbapenem-resistant Acinetobacter baumannii. PLoS One. 2019 Oct 29;14(10):e0224107.
[41]. Chiewchalermsri C, Sompornrattanaphan M, Wongsa C, Thongngarm T. Chlorhexidine Allergy: Current Challenges and Future Prospects. J Asthma Allergy [Internet]. 2020 Mar;Volume 13:127–33. Available from: https://www.dovepress.com/chlorhexidine-allergy-current-challenges-and-future-prospects-peer-reviewed-article-JAA.

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