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Evaluating the Role of Microbicides in Antibiotic Resistance Development in Clinical Pathogens

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ABSTRACT

A group of microbicides such as disinfectants and antiseptics are commonly used in healthcare settings to control cross contamination, outbreak of diseases, and hospital-acquired infections. Yet, concerns have been raised in recent years regarding co-selection for antibiotic resistance among pathogenic bacteria genre after repeated exposure to microbicide. The aim of this study was therefore to evaluate the effect repeated exposure of povidone-iodine (PVP-I) and surfanios premium at sub-inhibitory concentrations on antibiotic resistance in *K. pneumoniae*. **Methods & Material**: Twenty clinical pathogens of K. *pneumoniae* strains were obtained from various clinical samples of patients admitted at Aljala Trauma hospital, Benghazi, Libya were identified by standard microbiological and chemical methods at microbiology laboratory of hospital. MICs of biocides were determined by the broth dilution method; antibiotics susceptibility was performed were tested using Kirby and Bauer disk diffusion method. MICs and antibiotic susceptibilities were determined before and after repeated exposure to to sub-inhibitory concentrations of biocides (sub-MICs) to test for changes in biocide tolerance and Co-selection resistance to antibiotics. **Result**: Our results show that 5 out of 20 *K. pneumoniae* isolates (25%) exhibited an acquired tolerance to PVP-I. In this study was observed subtle of susceptibility changes to ceftriaxone, cefotaxime, ciprofloxacin, levofloxacin, and amikacin in *K. pneumoniae* isolates after exposure to sub-MIC concentrations of biocides. **Conclusions**: Use low suboptimal concentrations biocides may increase antibiotic resistance in clinically relevant bacteria such as *K. pneumoniae*. Therefore, further studies are needed to evaluate whether these associations are causal.

تقييم دور المبيدات الميكروبية في تطور مقاومة المضادات الحيوية في مسببات الأمراض السريرية

نادية العبدلي و أمال بوليفة

علم الأحياء الدقيقة، قسم المختبرات، مستشفى العيون، بنغازي

كلمات المفتاحية
كلبسيلا
الحد الأدنى من التثبيط
الميكروبات المثبطة
المبيدات الميكروبية
الاختيار المشترك

الملخص

تُستخدم مجموعة من مبيدات الميكروبات مثل المطهرات والمعقمات بشكل شائع في بيئات الرعاية الصحية للسيطرة على التلوث المتبادل وتفشي الأمراض والعدوى المكتسبة من المستشفيات. ومع ذلك، أثيرت مخاوف في السنوات الأخيرة بشأن الاختيار المشترك لمقاومة المضادات الحيوية بين أنواع البكتيريا المسببة للأمراض بعد التعرض المتكرر للمبيدات الميكروبية. لذلك كان الهدف من هذه الدراسة هو تقييم تأثير التعرض المتكرر لبوفيدون اليود (PVP-I) وسيرفانيوس بريميوم بتركيزات دون المثبطة على مقاومة المضادات الحيوية في .K. pneumoniae الطرق والمواد: تم الحصول على عشرين مسببًا للأمراض السريرية من سلالات K. pneumoniae من عينات سريرية مختلفة من المرضى الذين تم إدخالهم إلى مستشفى الجلاء للصدمات، بنغازي، ليبيا، وتم التعرف عليها بالطرق الميكروبيولوجية والكيميائية القياسية في مختبر علم الأحياء الدقيقة بالمستشفى. تم تحديد الحد الأدنى المثبط للمبيدات الحيوية بطريقة تخفيف المرق، وتم إجراء اختبار حساسية المضادات الحيوية باستخدام طريقة انتشار القرص كيربي وباور. تم تحديد MICs وحساسية المضادات الحيوية قبل وبعد التعرض المتكرر لتركيزات دون المثبطة من المبيدات الحيوية (sub-MICs) لاختبار التغيرات في تحمل المبيدات الحيوية ومقاومة الاختيار المشترك للمضادات الحيوية. النتيجة: تظهر نتائجنا أن 7 من أصل 20 عزلة (35٪) أظهرت تحملًا مكتسبًا لـ PVP-I وأظهرت 5 من أصل 20

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عزلة (25٪) تحملًا مكتسبًا لـ .surfanios premium في هذه الدراسة، كشفت النتائج أن التعرض لفترات طويلة لـ PVP-Iأو surfanios بتركيزات دون المثبطة أدى إلى تغييرات كبيرة في تغيرات حساسية المضادات الحيوية لسيفترياكسون وسيفوتاكسيم وسيبروفلوكساسين وليفوفلوكساسين وأميكاسين في عزلات .K. pneumoniae الاستنتاجات: قد يؤدي استخدام تركيزات منخفضة دون المستوى الأمثل من المبيدات الحيوية إلى زيادة مقاومة المضادات الحيوية في البكتيريا ذات الصلة السريرية مثل .K. pneumoniae ومن ثم، هناك حاجة إلى مزيد من الدراسات لتقييم ما إذا كانت هذه الارتباطات سببية.

1. Introduction

The emergence of antimicrobial resistance is associated with high morbidity and mortality rates. Thus, antibiotics resistance is a public health threat and a growing global concern [1, 2].

Antibiotic resistance can be acquired through bacterial random mutations, or exchange of resistance genes between different species of bacterial[3]. The misuse of antibiotics and over-overuse have been considered the contributing factors to bacterial resistance [4, 5]. Microbicides can act as a selective pressure that encourages the acquisition of resistance traits in bacterial cells and indirectly select for antibiotic resistance, through by co-selection process [6, 7]. Thus, repeated exposure of very low concentrations of microbicides over long periods of time may increase resistance to antibiotic and microbicides, even in the absence of antibiotic selection pressure [8,9]. Antimicrobial resistant bacteria in the hospital setting is extremely dangerous, where can cause diseases serious, these bacteria can also spread into the community[10].

Klebsiella pneumoniae is one of an opportunistic pathogens associated with both community-acquired and healthcare-associated infections [8,11]. *K. pneumonia* is a Gram-negative, within the "ESKAPE" groups (Enterococcus faecium, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp*) and is of concern, due to multidrug and extended-spectrum β-lactamase (ESBL) resistance. These bacteria show resistance to most *β-lactamase* inhibitor combinations and carbapenems. These carbapenems are typically the last line of defense against *K. pneumoniae* infections [9,10,11].

Some microbicides may be used antiseptics on skin as wound cleaners and can use as disinfectants on equipment and surfaces in hospitals [12,13]. Povidone-iodine (PVP-I) and surfanios premium are among the most extensively used biocides in hospitals [2]. A special place among antimicrobial substances is occupied by the widely used antiseptic PVP-I [14–16], where has broad antimicrobial activity against Gram-positive and Gram-negative bacteria [17,18]. Has been proven to be effective both before and after surgery as an antiseptic and disinfectant [19], while surfanios premium is used as cleaner and disinfection of floors, walls, medical equipment and non-invasive medical devices (a detergent and disinfectant action at once) [20].

The potential of the emergence of bacterial tolerance to microbicides and the possible association between the effect of microbicides use advent of antibiotic resistance is now topic of many discussions with conflicting evidence, and it appears to be more of a concern [21]. Therefore, the aim of the present study is evaluating the effect of bacterial resistance development to PVP-I and surfanios premium on antibiotic co-selection by repeated exposure, and the effect of sub-inhibitory concentrations of these biocides on clinical *K. pneumoniae* isolated from Aljala Trauma Hospital, Benghazi, Libya.

Material and Methods

2.1. Bacteria strains

This study involved twenty clinical of *K. pneumoniae* strains were collected from the different departments of Aljala Trauma hospital, Benghazi, Libya. The samples were pus, sputum, and urine. The collected isolates were identified by standard methods to study the phenotypic characteristics including Gram staining, morphology, culture characteristics and biochemical reactions [22] at microbiology laboratory of hospital.

2.2. Microbicides

Two commonly used microbicides in Benghazi hospitals, were tested in this study using their commercially available preparations; namely Betadine solution(povidone iodine 10% (betadine®; Nile pharm, Egypt), and surfanios premium (Laboratoires Anios, France) containing (N-(3-aminopropyl)-N-dodecylpropane-1,3-diamine – CAS N° 2372-82-9 (51 mg/g), di decyldim ethyl ammonium chloride– CAS N° 7173-51-5 (25 mg/g), excipients.) diluted at 0025%, and ready-to-use product.

2.3. Bacterial inoculum preparation

Briefly, the inoculum was prepared from a colony grown on MacConkey agar for 24 h, in a 0.9% sterile saline solution (0.85% NaCl w/v in water) and adjusted to the density of a *McFarland 0.5* standard, the suspension was measured with a spectrophotometer(BD Phoenixspec).

All inoculum suspensions were used within 60 min. of preparation.

2.4. Determination MIC of Microbicides

The minimum inhibitory concentrations (MICs) of PVP-I and surfanios premium were determined for each strain by using the Mueller Hinton broth (MHb) dilution method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines and the report of several studies [23–26]. Briefly, it was determining the minimum inhibitory concentration (MIC) for PVP-I and surfanios premium, through a series of broths had mixed with serially diluted biocides. In twelve numbered screw tubes, sterile MHB was added to each tube in aseptic conditions, except for the tube number 1. For the first and the second tubes of the series, 1 ml of tested biocides was added; tube 2 was stirred and 1 ml was withdrawn and transferred for tube 3 repeated until tube 11. It is called serial dilution. It was added 0.1 ml of inoculation bacteria to all tubes, except for tube number 12, All tubes were incubated for 24 and 48 hours. Tube 11 was the growth control (MHB + bacterial suspension). Tube12 was the broth sterility control (MHB + disinfectant).

The MIC was determined as the highest dilution of the disinfectant that visually inhibited bacterial growth, as demonstrated by turbidity. Minimum bactericidal concentration (MBC) of these biocides was determined by 5µl of each tube was sub-cultured onto Mueller Hinton agar(MHA), which were then incubated aerobically at 37°C for 24–48h. The highest dilution of biocide at which no growth was obtained on MHA was taken as MBC.

2.5. Exposure to sub-MICs of Microbicides

Microbicides resistant mutants were generated by repeated exposure to a sub-inhibitory concentration of the tested biocides with a gradual increase beginning with a concentration of 0.5× MIC of (PVP-I or surfanios premium) for five consecutive passages. The modification method that according to several studies was adopted [26–28]. Briefly, after determining the MIC and MBC, bacterial suspensions were withdrawn from tubes containing the highest concentration of biocides allows bacteria to grow (sub-MIC), and were cultured onto (MHA) plates and incubated overnight at 37°C to isolate the survived organisms, then bacterial suspensions (the second sub-culture) exposed to a new series of concentration (sub-MICs) of the previously mentioned biocides. by mixing of aliquots of bacterial suspensions with concentration of biocides within 1/2, 1/4, 1/8 and 1/16 of previously determined MIC of biocides. Tubes were incubated at 37°C for 24 to 48 hours, followed by centrifugation for 5 minutes at 4500 rpm and washed once with washing buffer (1 mol l)1 NaCl, 10 mmol l)1 EDTA, pH 8Æ0). Samples from sediment of each tube were sub-cultured on (MHA) plate. One colony with the typical size and morphology of the original strain was chosen randomly and sub-cultured onto antiseptics free broth and incubated at 37°C for 24 hours. All isolates were tested in triplicate, cultures

that failed to restore the initial susceptibility in the absence of the biocides were considered as biocide-resistant mutants.

2.6. Determination of Antibiotic Susceptibilities

The antibiotic susceptibility testing of each bacterial strain Pre and Post repeated exposure to sub-inhibitory concentrations of microbicides using modified Kirby–Bauer disk diffusion method as per the Clinical and Laboratory Standards Institute (CLSI) guidelines [23].

The tested antibiotics were as follows: colistin (10 µg), meropenem (10 µg), imipenem (10 µg), aztreonam (30 µg), amikacin (30 µg), ceftriaxone (30 µg), cefotaxime (30 µg), levofloxacin (5 µg), and ciprofloxacin (5 µg) from (Oxoid, UK). Isolates were categorized as susceptible, intermediate or resistant, according to the (CLSI) guidelines.

2.7. Statistical Analysis

Means MICs were determined by averaging MIC values from all isolates and percentage using the calculator. The strains that were following initial exposed to PVP-I or surfanios is designated P0. The strains that were not exposed to sub- inhibitory concentrations of PVP-I or surfanios concentrations, were designated as controls.

3. Result

The antibiotic susceptibility pattern of *K. pneumonia* isolates using the disk diffusion method are shown in (Fig. 1).

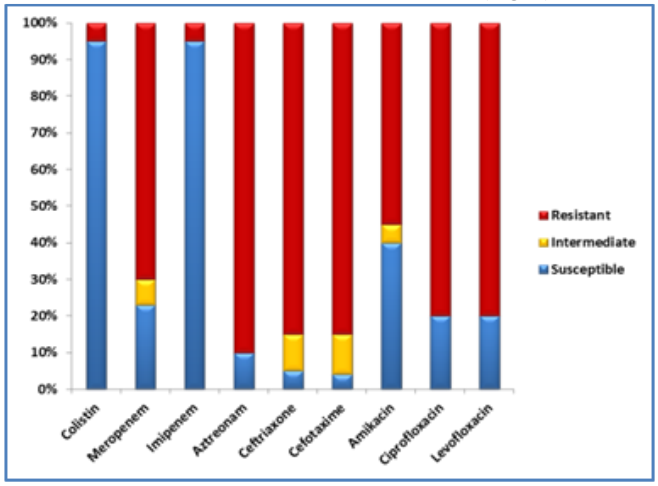

Fig. 1: antibiotic susceptibility of *K. pneumoniae* isolates.

To assess the antimicrobial activity of microbicides, we determined the MICs for PVP-I or surfanios against *K. pneumoniae*. Percentage MICs following initial PVP-I or surfanios exposure are identified as P0 MICs in Table(1). P0 MICs (>50%) of the collected isolates PVP-I were 256 µg/mL, while (50%) of the collected isolates surfanios premium were 256 µg/ml.

Table 1: P0 MICs for PVP-I and surfanios

MIC (µg/ml)	PVP-I No (%)	Surfanios No (%)
8 – 16	2 (10)	0 (0.0)
32– 64	7 (35)	8 (40)
128 – 256	11 (55)	10 (50)
≥512	0 (0.0)	2 (10)

(>50%) of the collected isolates had PVP-I MBC of 64 µg/mL while (45%) of the collected isolates had surfanios premium MBC of 256 µ g/ml and (45%) ⩾512 µ g/ml. Table (2).

Table 2: MBC values of biocides against *K. pneumoniae* .

MBC (µg/ml)	PVP-I No (%)	Surfanios No (%)
8 – 16	0 (0.0)	0 (0.0)
32– 64	10 (50)	2 (35)
128 – 256	9 (45)	9 (45)
≥512	3 (15)	9 (45)

5 out of 20 isolates (25%) showed an acquired tolerance to PVP-I and a subtle increase in antibiotic resistance Post exposure to sub-MICs of PVP-I and surfanios premium. Figure (2).

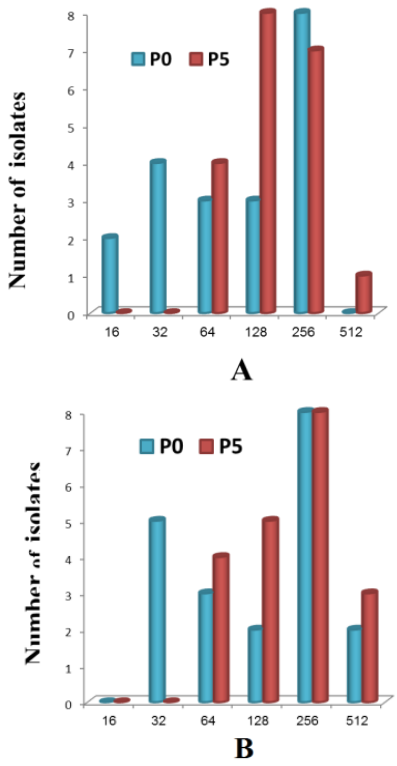

Figure 2. MICs before and after repeated exposure to PVP-I and surfanios . (A) PVP-I mean MICs. (B) surfanios mean MICs. P0, initial exposure to PVP-I or surfanios; P5, bacteria were cultured in sub- inhibitory concentrations of PVP-I or surfanios for 5 consecutive passages.

We assessed antibiotic susceptibilities in *K. pneumoniae* isolates before and after repeated exposure to sub-MICs of PVP-I and surfanios premium. Table (3) shows the number and percentage of isolates that showed antibiotic resistance to susceptible and intermediate isolates post- exposure (sub-MIC) to PVP-I. Briefly, resistance was acquired to aztreonam, cefotaxime, and ceftriaxone (100%), amikacin (56%), ciprofloxacin, and levofloxacin (50%), and colistin (5%), whereas susceptible of imipenem, and meropenem was similar to that of parent strain of all isolated.

Table 3: Antibiotic susceptibility before and after repeated exposure to sub-MICs of PVP-I 10% .

Antibiotic(s)	Pre-exposure Susceptible No (%)	Post- exposure Resistance. No (%)
Colistin	20 (100)	1 (5)
Imipenem	19 (95)	0 (0.0)
Amikacin	9 (45)	5 (56)
Meropenem	6 (30)	0 (0,0)
Ciprofloxacin	4 (20)	2 (50)
Levofloxacin	4 (20)	2 (50)
Cefotaxime	3 (15)	3 (100)
Ceftriaxone	3 (15)	3 (100)
Aztreonam	2 (10)	2 (100)

Table 4) shows the number and percentage of isolates that showed antibiotic resistance to susceptible and intermediate isolates post-exposure (sub-MIC) to surfanios premium . Briefly, resistance was acquired to aztreonam , cefotaxime, and ceftriaxone (100%), amikacin (22%), ciprofloxacin, and levofloxacin (50%), meropenem (33%) and colistin (10%), whereas susceptible of imipenem was similar to that of parent strain of all isolated.

Table 4: Antibiotic susceptibility before and after repeated exposure to sub-MICs of surfanios premium

Antibiotic(s)	Pre-exposure Susceptible No (%)	Post- exposure Resistance No (%)
Colistin	20 (100)	2 (10)
Imipenem	19 (95)	0 (0.0)
Amikacin	9 (45)	2 (22)
Meropenem	6 (30)	2 (33)

Ciprofloxacin	4 (20)	1 (25)
Levofloxacin	4 (20)	1 (25)
Cefotaxime	3 (15)	3 (100)
Ceftriaxone	3 (15)	3 (100)
Aztreonam	2 (10)	2 (100)

4. Discussion

Many scientific publications described the antibiotic resistance mechanisms, but there are only limited studies addressing the molecular mechanisms of biocide resistance and there is a considerable lack of scientific reports regarding (MIC) values of typical biocides against clinically important bacteria isolates [29, 30].

Some studies are shown that there is some evidence that biocides may have an impact on the antibiotic susceptibility, but other studies found no association between exposure to biocides and antibiotic resistance [1, 22, 30, 31].

An inappropriate use of biocides may lead to bacterial exposure to sub- MIC [32]. Sub-MIC levels of biocides can increase rates of mutation which may influence the rate of de novo biocide, and antibiotic resistance development [33].

Sub inhibitory concentrations of PVP-I may be clinically present due to residues of diluted PVP-I may remain on the skin after use [2, 34,35].

Some vitro studies shown the selection of bacteria an increased tolerance to a biocide following exposure with a low concentration of a biocide [36, 37, 38, 39, 40,41,42].

Microbicides can co-select for antibiotic resistance when bacteria harbour resistance or tolerance genes towards both types of compounds provides an increased likelihood of this resistance passing between environmental and clinically relevant bacterial strains[1,43]. Relevant increases in MIC, genetic changes of several bacterial species were observed in vitro studies in the presence of biocides at different concentrations [44, 45,46].

Some would suspect the existence of a relationship between the use of microbicides and the emergence of antibiotic resistance, but in vitro reports indicate that repeated exposure to microbicides at sub-inhibitory concentrations can promote development of antibiotic resistance[43, 44,47,48,49].

Microbicides tolerance is associated with antibiotic cross-tolerance in microbicides-acclimated bacteria to determine if tolerance to PVP-I or surfanios premium could co-select for antibiotic cross-resistance, we assessed antibiotic susceptibilities in bacterial isolates before and after repeated exposure to sub-MICs of PVP-I and surfanios premium[49]. Our study are pointing toward that sub - MIC of biocides can prime bacteria to become increased tolerance to biocide and resistant to antibiotics Table (3, 4) and Figure (2,3). Our findings are consistent with those of [43, 44].

Unlike other antiseptics, there have been no reports of PVP-I inducing selection of antibiotic resistance [16,50]. In contrast, in this study the susceptibility to ceftriaxone, cefotaxime, ciprofloxacin, levofloxacin, and amikacin has changed after exposure to sub-MIC concentrations of PVP-I. No studies are available on possible resistance development to surfanios premium. In this study was observed subtle of susceptibility changes to meropenem, ceftriaxone, cefotaxime, and aztreonamin in *K. pneumoniae* isolates after exposure to sub-inhibitory concentrations of surfanios premium.

5. Conclusion

Although in vitro studies show a correlation between biocides use and antibiotic resistance, such there is no conclusive evidence that the use of biocides leads to an increase in antibiotic resistance in everyday life settings. However, even small susceptibility changes in laboratory may trigger a higher frequency of high-level resistance development over time. Thus, comprehensive studies are needed to assess the mechanisms of resistance, and how biocides horizontal gene transfer and how this may increase the spread of antibiotic resistance determinants.

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7. References

[1]- Murray, L. M., Hayes, A., Snape, J ., Kasprzyk-Hordern, B., Gaze, W. H., Murray, A. K., (2024), Co-selection for antibiotic resistance by environmental contaminants., npj Antimicrobials & Resistance., 2:9, 1–13.https://doi.org/10.1038/s44259-024-00026-7.

[2]- Barakat, N. A., Rasmy, S. A., Hosny, A. M. S., Kashef, M. T., (2022), Effect of povidone-iodine and propanol-based mecetronium ethyl sulphate on antimicrobial resistance and virulence in *Staphylococcus aureus*., Antimicrobial Resistance & Infection Control ; 11:139, 2–14. https://doi.org/10.1186/s13756-022-01178-9.

[3]- Wales, A. D., Davies, R. H., (2015), Co-Selection of Resistance to Antibiotics, Biocides and Heavy Metals, and Its Relevance to Foodborne Pathogens., Antibiotics ; 4, 567-604; doi:10.3390/antibiotics4040567.

[4]- Ali, N. E., Morsi, S. S., Elgohary, E. A., (2014), Association Between Antibiotics And Disinfectants Resistance profiles Among Acinetobacter Baumannii Isolates In Zagazig University Hospital's Intensive Care Unit., Life Sci J. 11(10):1–8. http://www.lifesciencesite.com.

[5]- Daniel S.A, Shawky M.S, Omar H.M.G, Abou-Shleib H.M, El-Nakeeb M.A ., (2014), Antibiotic resistance and its association with biocide susceptibilities among microbial isolates in an Egyptian hospital. Int Arab J Antimicrob Agents ;4(4):1–11.doi:10.3390/antibiotics4040567.

[6]- Chapman, J. S. (2003). Disinfectant resistance mechanisms, cross resistance, and co-resistance. Int. Biodeterior. Biodegrad. 51, 271–276 .

[7]- Baker-Austin, C., Wright, M. S., Stepanauskas, R. & McArthur, J. V. (2006). Co-selection of antibiotic and metal resistance. Trends Microbiol. 14, 176–182.

[8]- Wand, M. E., Bock, L. J., Bonney, L. C. & Sutton, J. M. (2017). Mechanisms of increased resistance to chlorhexidine and cross-resistance to colistin following exposure of *klebsiella pneumoniae* clinical isolates to chlorhexidine. Antimicrob. Agents Chemother. 61.

[9]- Hérault, H.S. (2019). Impact of commonly used antimicrobial biocides on resistance and cross-resistance in carbapenemase-producing Enterobacteriaceae., PhD Thesis, School of Pharmacy and Pharmaceutical Sciences, Cardiff University.

[10]- Pandey R., Mishra Sh.K., Shrestha A. (2021). Characterisation of ESKAPE Pathogens with Special Reference to Multidrug Resistance and Biofilm Production in a Nepalese Hospital. Infection and Drug Resistance. ;14: 2201–2212. doi: 10.2147/IDR.S306688.

[11]- Liu H., Xiang Y., Xiong M., Xiao X., Zhou J., Tian H., Chen Q & Li Y. (2024). Prevalence of ST1049-KL5 carbapenem resistant *Klebsiella pneumoniae* with a blaKPC-2 and blaNDM-1 co-carrying hyper transmissible IncM1 plasmid. communications biology. ;695(7):1–13. https://doi.org/10.1038/s42003-024-06398-w.

[12]- Kawamura-Sato K., Wachino J., Kondo T., Ito H., Arakawa Y. (2010). Correlation between reduced susceptibility to disinfectants and multidrug resistance among clinical isolates of *Acinetobacter species*. *J Antimicrob Chemother* ; 65: 1975 – 1983. doi:10.1093/jac/dkq227.

[13]- SCENIHR. (2009). Assessment of the Antibiotic Resistance Effects of Biocides; Scientific Committee on Emerging and Newly Identified Health Risks, European Commission: Brussels, Belgium, pp. 1–87.

[14]- Monstrey S. J., Govaers K., Lejuste P., Lepelletier D, Ribeiro de Oliveirae P. (2023). Evaluation of the role of povidone-iodine in the prevention of surgical site infections. *Surg Open Sci*.; 13: 9–17. doi: 10.1016/j.sopen.2023.03.005

[15]- Eggers M. (2019). Infectious Disease Management and Control with Povidone Iodine. *Infect Dis Ther* ; 8(4): 581–593. doi: 10.1007/s40121-019-00260-x.

[16]- Lepelletier D., Maillard J.Y., Pozzetto B., Simon A . (2024). Povidone Iodine: Properties, Mechanisms of Action, and Role in Infection Control and *Staphylococcus aureus* Decolonization.

Antimicrobial Agents and Chemotherapy ; 64(9):1–13. https://doi.org/10.1128/AAC00682-20.

[17]- Jayaraja Kumar.K, Hemanth Kumar Reddy.C, Gunashakaran.V, Ramesh.Y, Kalayan Babu.P, Pawan Narasimha.N, Venkatewarulu.A, Lakshmikanth Reddy.P,. (2019). Application of broad spectrum antiseptic povidone iodine as powerful action: A Review. *J of Pharmaceutical Science and Technology* ; 1: (2), 48-58. https://www.researchgate.net/publication/41653465.

[18]- Kunisada T, Yamada K, Oda S, Hara O. (1997). Investigation on the efficacy of povidone-iodine against antiseptic-resistant species. Dermatology 195(Suppl 2):14 –18. https://doi.org/10.1159/000246025.

[19]- Lachapelle J-M, Castel O, Casado AF, Leroy B, Micali G, Tennstedt D, Lambert J. (2013). Antiseptics in the era of bacterial resistance: a focus on povidone iodine. Clin Pract 10:579–592. https://doi.org/10.2217/cpr.13.50.

[20]- Laboratoires Anios .(02-01-2019). Surfanios Premium 1917_FIRG_FR-EN_13-11-2014 v2 Version 2. https://www.free-med.com/cache/documents/product/surfaniospremium_md-fr-583.pdf.

[21]- Coombs, K., Rodriguez-Quijada, C., Clevenger, J. O., and Sauer-Budge, A. F .(2023). Current Understanding of Potential Linkages between Biocide Tolerance and Antibiotic Cross-Resistance. https://doi.org/10.3390/ microorganisms11082000.

[22]- Cheesbrough M, 2009. District Laboratory Practice in Tropical Countries. Cambridge University, UK, 2(2): 65-67.

[23]- CLSI Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. 11th ed. CLSI standard M07 Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

[24]- Jorgensen H, Faller MAP, Carroll K.C. (2015). Manual of clinical microbiology. Edition : Print book: English: 11th edition/Publisher: Washington, DC: ASM Press, pp.1258-1270.

[25]- Balouiri M, Sadiki M, Ibnsouda S.K. (2016). Methods for in vitro evaluating antimicrobial activity. J Pharm Anal,, 6(2): 71–79: PMC5762448.

[26]- Ebrahimi A, Arvaneh Z, Mahzounieh M, Lotfalian S,.(2017). Antibiotic Resistance Induction by Benzalkonium Chloride Exposure in Nosocomial Pathogens. Int J Infect, 4(2): 1-5: e40296.

[27]- Levinson W, Jawetz E. (2004). Medical Microbiology and Immunology. Examination and board Review. 8th ed Lang Medical Brooks McGraw- Hill New York, pp. 143-187.

[28]- Alsaady KA. (2011). The Selective Pressure Effect of Antiseptics on the Patterns of Resistance in *Staphylococcus aureus*. J Kerbala Univ, 9(4): 299–304.

[29]- Vijayakumar R , Sandle T. (2019). A review on biocide reduced susceptibility due to plasmid-borne antiseptic-resistant genes-special notes on pharmaceutical environmental isolates. Review J Appl Microbiol ; 126(4):1011-1022. doi: 10.1111/jam.14118.

[30]- Fox, L. J., Kelly, P. P., Humphreys, G. J., Waigh, T. A., Lu, J. R., and McBain, A. J. (2022). Assessing the risk of resistance to cationic biocides incorporating realism-based and biophysical approaches. J Ind Microbiol Biotechnol. 49(1).

[31]- Kampf, G. Acquired resistance to chlorhexidine – is it time to establish an 'antiseptic stewardship' initiative? J. Hospital Infect. 94, 213–227 (2016).

[32]- Capita, R., Riesco-Peláez, F., Alonso-Hernando, A. & Alonso-Calleja, C. (2014). Exposure of Escherichia coli ATCC 12806 to sub lethal concentrations of food-grade biocides influences its ability to form biofilm, resistance to antimicrobials, and ultrastructure. Appl. Environ. Microbiol. 80(4), 1268–1280.

[33]- Muñoz, M., El Bakali, N.B., Ennahar S., Abriouel, H. (2016). Comparative proteomic analysis of a potentially probiotic Lactobacillus pentosus MP-10 for the identification of key proteins involved in antibiotic resistance and biocide tolerance. Review International Journal of Food Microbiology ; 222, 8–15. doi: 10.1016/j.ijfoodmicro.2016.01.012.

[34]- Eggers M. (2019). Infectious disease management and control with povidone iodine. Infect Dis Ther ;8(4):581–93.

[35]- Barreto R, Barrois B, Lambert J, Malhotra-Kumar S, Santos-Fernandes V, Monstrey S. (2020). Addressing the challenges in antisepsis: focus on povidone iodine. Int J Antimicrob Agents;56(3):106064. https:// doi. org/ 10. 1016/j. ijant imicag. 2020. 106064.

[36]- Nasr A.M, Mostafa MS, Arnaout H.H, Elshimy A.A. (2018). The effect of exposure to sub-inhibitory concentrations of hypochlorite and quaternary ammonium compounds on antimicrobial susceptibility of *P. aeruginosa.* AJIC 46 e57-e63.

[37]- Al-abdli, N., Boulifa, A., and Trabelsi, I. (2020). Effect of sub-minimum inhibitory concentrations of biocides on the selective pressure towards antibiotic resistance of *Staphylococcus aureus* strains. Microbiology Research International, 8(4): 76-82.

[38]- Thomas L. Maillard J-Y, Lambert R.J, Russell A.D. (2000). Development of resistance to chlorhexidine diacetate in *P. aeruginosa* and the effect of 'residual' concentration. J Hosp Infect; 46:297-303.

[39]- Tattawasart U, Maillard J-Y, Furr J.R, Russell A.D. (1999). Development of resistance to chlorhexidine diacetate and cetylpyridinium chloride in Pseudomonas stutzeri and changes in antibiotic susceptibility. J Hosp Infect; 42:219-29.

[40]- Walsh SE, Maillard J-Y, Russell AD, Charbonneau DL, Bartolo RG, Catrenich C. (2003). Development of bacterial resistance to several biocides and effects on antibiotic susceptibility. J Hosp Infect ; 55:98-107.

[41]- Noel, D.J. (2023). The Antibacterial Efficacy of Disinfectants used for Infection Control, PhD Thesis, Faculty of Environmental and Life Sciences School of Biological Sciences, University of Southampton of England, United Kingdom.

[42]- Gaze W.H, Abdouslam N, Hawkey P.M, Wellington E.M. (2005). Incidence of class-1 integrons in a quaternary ammonium compound- polluted environment. Antimicrob Agents Chemother; 49:1802-7.

[43]- Abdel Malek S.M, Al-Adham I.S, Winder CL, Buuljens T.E, Gartland K.M, Collier P.J. (2002). Antimicrobial susceptibility changes and T-OMP shifts in pythione-passaged planktonic cultures of *Pseudomonas aeruginosa* PAO1. J Appl Microbiol; 92:729-36.

[44]- Jian Z, Zeng L, Xu T, Sun Sh, Yan Sh, Yang L, Huang Y, Jia J, Dou T. (2021). Antibiotic resistance genesin bacteria: Occurrence, spread, and control. J Basic Microbiol; 61:1049-70. DOI: 10.1002/jobm.202100201.

[45]- Petersen, M.S. (2013). Development of bacterial resistance to biocides and antimicrobial agents as a consequence of biocide usage, PhD Thesis, Institute Technical University of Denmark Kemitorvet Kongens Lyngby, Denmark.

[46]- Rozman U, Pušnik M, Kmetec S, Duh D and Turk S Š . (2021). Reduced Susceptibility and Increased Resistance of Bacteria against Disinfectants: A Systematic Review. J Microorganisms.; 9(12): 2550. doi: 10.3390/microorganisms9122550.

[47]- Sheldon AT Jr. (2005). Antiseptic 'resistance': real or perceived threat? Clin Infect Dis ;40(11):1650–6.

[48]- Adkin, P., Hitchcock, A., Smith, L.J., and Walsh, S.E. (2022). Priming with biocides: A pathway to antibiotic resistance?. *J Appl Microbiol.* 00:1–12. DOI: 10.1111/jam.15564.

[49]- Pena, Sh. A., Salas, J.G., Gautam, N., Ramos, A.M., Frantz, A.L. (2023). Sublethal Exposure to Common Benzalkonium Chloride Leads to Antimicrobial Tolerance and Antibiotic Cross-Resistance in Commensal and Opportunistic Bacterial Species. Appl. Microbiol. , 3, 580–591. https://doi.org/10.3390/applmicrobiol3020041.

[50]- Bigliardi P.L, Alsagoff SAL, El-Kafrawi HY, Pyon JK, Wa CTC, Villa MA. (2017). Povidone iodine in wound healing: a review of current concepts and practices. Int J Surg 44:260 –268. https://doi.org/10.1016/j.ijsu..06.073.