ZANCO Journal of Pure and Applied Sciences
The official scientific journal of Salahaddin University-Erbil
https://zancojournals.su.edu.krd/index.php/JPAS

ISSN (print):2218-0230**, ISSN (online):** 2412-3986**, DOI:** **http://dx.doi.org/10.21271/zjpas**

RESEARCH PAPER

In Vitro Control of Multidrug-Resistant Klebsiella pneumoniae Infection by Some Biocides used in Erbil Hospitals

[1]Halima Mohammed Saber, [2]Payman Akram Hamasaeed

[1&2] Department of Biology, College of Education, Salahaddin University–Erbil, Kurdistan Region, Iraq.

A B S T R A C T:

Aim: Screening of the multidrug-resistant *Klebsiella pneumoniae* isolates in Erbil hospitals, which are the main causative agent of nosocomial infections, additionally, the effectiveness of some of the most widely used biocides was monitored in our hospitals on these isolates and the most effective one was selected to be used in infection control in these hospitals. **Methods:** From August 1 to November 1, 2021, the present study 81 *Klebsiella spp*. isolates were collected from various clinical specimens from different hospitals in Erbil City. The disc-agar method and the cup plate agar diffusion method were used to test antibiotic and biocide susceptibility against 50 isolates of *Klebsiella pneumoniae*. **Results:** Of the eighty-one isolates, only 50 (%62) were identified as *Klebsiella pneumonia*. Two (4%) isolates that were not biofilm producers and 48 (96%) isolates were biofilm producers. Their antibiotic resistance profile showed all isolates (% 100) were resistant to ampicillin, while (%74) of isolates were showed susceptibility to Imipenem and Meropenem, and (%57.1) to ciprofloxacin. Statistical analysis showed that the higher and lower mean of biocide was Virkon and Alcohol-Base which was 51.54 mm and 12.91 mm, respectively. **Conclusion:** Our observations imply that side by side bacteria increase the ability to resist biocides. In addition, Virkon was the most effective biocide against *K. pneumoniae* isolates. To prevent the growth of pathogenic bacteria and control infections in hospitals, we propose paying close attention to selecting the finest biocide.

KEY WORDS: Biocide, Biofilm, Klebsiella pneumoniae, Multidrug Resistance.
DOI: http://dx.doi.org/10.21271/ZJPAS.34.6.18
ZJPAS (2022) , 34(6);160-168 .

1.INTRODUCTION :

One of the main causative agents of hospital-acquired infections (HAI) is with the widespread of multidrug-resistant (MDR) *Klebsiella pneumoniae*, which causes many diseases, such as pneumonia, urinary tract infections, bacteremia, burns and wound infections, septicemia, and meningitis (Lenchenko et al., 2020) (Farhadi et al., 2021). The MDR and biocides resistant isolates readily spread in hospital environments. Therefore, hospital disinfection policies have a major role in controlling infection and preventing the transmission of infectious pathogens in hospitals and control of HAIs (Lenchenko et al., 2020). Biocides have a variety of effects compared to antibiotics with comprehensive and non-specific effectiveness.

It is significant to note that many of these biocides can be used alone or with a variety of products, that differ widely in their activity against microbes. Antiseptics differ according to their efficiency toward the vital cell. Some of them target cell membranes, plasma membranes, and nucleic acids, or they may be oxidizing agents (Hassan *et al*., 2021).

2. Material and Method

2.1. Sample collection, Isolation and Identification of *K. pneumoniae*

Eighty one samples were collected from different sources such as (urine, sputum, high vaginal swab and blood) from hospitals in Erbil City during the period 1 August to 1 November 2021. All isolates were identified according to cultural characteristics, microscopic examination, and some biochemical tests. Moreover, the identification of bacterial isolates was confirmed using an automated bacterial identification system, VITEK® 2 compact system (BioMérieux).

2.2. Biofilm assay

* **Corresponding Author:**
Mukhlis Hamad Aali
E-mail: saberhalima09@gmail.com
Article History:
Received: 15/05/2022
Accepted: 24/07/2022
Published: 20/12/2022

Congo red agar (CRA) method: This CRA is prepared by mixing 37 g of brain heart infusion broth, 50 g sucrose, 0.8 g Congo red dye, and 10 g of agar in 1 L of distilled water. Then the isolates were inoculated and incubated aerobically at 35°C for 18–24 hours. The strains that produced biofilm formed black colonies, while non-forming biofilm isolates developed red colonies (Shrestha et al., 2018).

2.3. Antibiotic susceptibility testing

The susceptibilities of 50 isolates to different antibiotic discs were tested by the disc diffusion method (Hombach et al., 2012). After the inoculums had been dried on the surface of the Mueller Hinton Agar, the Discs were applied to the agar with sterile forceps. The discs were pressed firmly to ensure contact with the agar, within 15 min. of disc placement; plates were incubated at 35 ^{o}C for 16-18 hours. The diameters of inhibition zones were measured and recorded.

2.4. Biocide susceptibility testing

The sensitivity of *K. pneumonia* to biocides was evaluated by the cup plate agar diffusion method. Some the biocides such as (Big-Extra AF, Virkon, and Surfanios) were prepared based on the protocol of biocides on the gallon. While, other biocides such as (AseptaNios AD, Konix AF, Povix, Povidin, H_2O_2-Sanosil solo, Alcohol-Base and Anios spray) were ready to use. After the bacterial inoculums had been dried on the surface). of the Mueller Hinton Agar, cups of 8 mm in diameter were made in the agar using a sterile cork borer. Then biocides were inoculated into cups by using a sterile micro-pipette. The plates were then incubated at 37 ℃ for 24 hours. The diameters of inhibition zones were measured and recorded (Idowu et al., 2017).

2.5. Statistical analysis

Graph Pad Prism 8 software was used to analyze the data. A one-way ANOVA test was used to determine the statistical significance of the data. A *P* value of < 0.05 was considered significant.

3. Results

A total of 81 isolates of *Klebsiella spp.* were collected from different hospitals in Erbil city. The Characteristics of these isolates were studied according to their cultural morphology, microscopically, some biochemical properties and Vitek. According to these, only 50 isolates carry the properties of *K. pneumoniae* (Fig. 1). As shown in Fig. 2, the high percentage of *Klebsiella spp.* was 43 (53 percent) in sputum, 32 (40%) in urine, 3 (4%) in wounds, 2 (2%) in blood, and 1 (1%) in HVS. Table 1 also summarized the biochemical features. In the hypermucoviscosity test, 9 (18%) were hyper-virulent and 41 (82%) were classic (Table 2 Fig. 3).

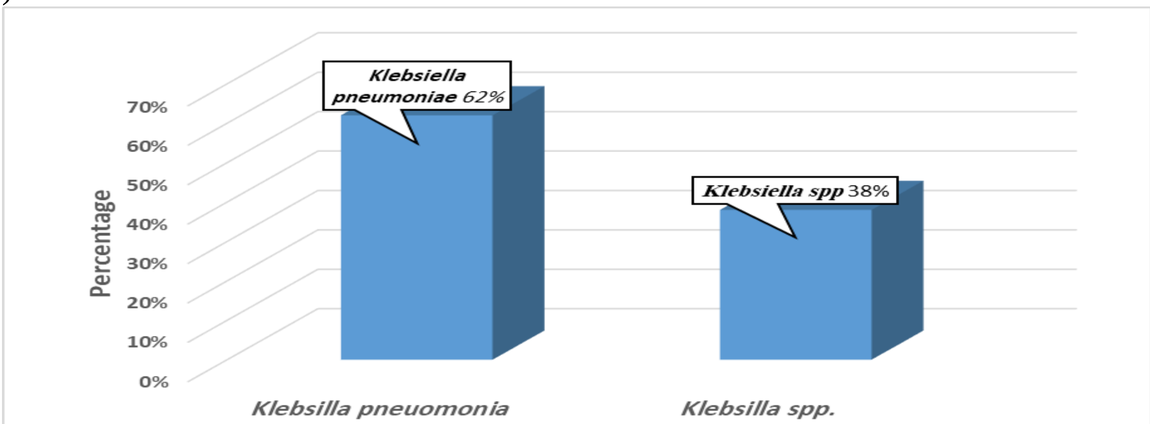


Figure1: Percentage of *Klebsiella pneuomoniae* and *Klebsiella spp*

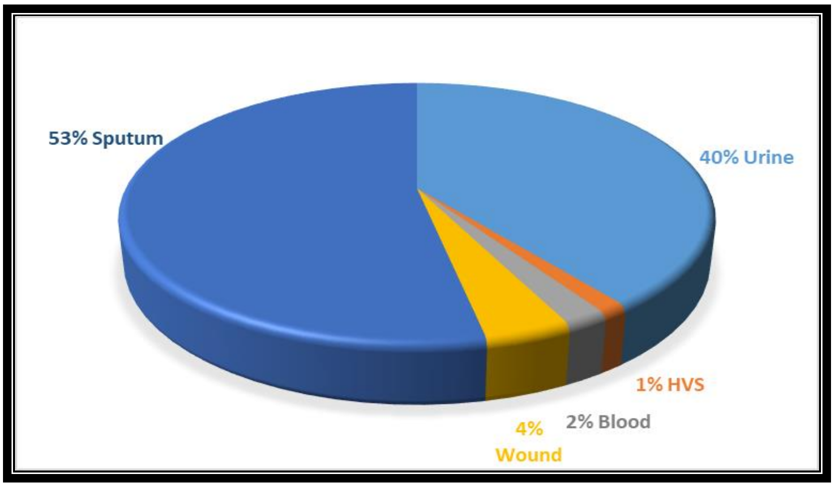


Figure2: Percentage of isolates according to clinical sources.

Table1: Phenotypic characteristics of *K. pneumoniae*

Test	Results
Gram stain	-ve*
Indole	-ve
H_2S	-ve
Oxidase	-ve
Citrate	+ve*
Urease	+ve

* -ve: negative, +ve: positive

Table2: Percentage of hypermucoviscosity profile of *K. pneumoniae* isolates.

Characteristics	Number (%)
Hyper-virulent	9 (18)
Classic	41(82)
Total	50

Figure3: Stretching of *K. pneumoniae* colonies (formation of a string >5 mm in length)

In addition, biofilm formation testing revealed that 2 (4%) of isolates were not biofilm producers and 48 (96%) were biofilm producers. Additionally, among biofilm producers, the rates of weak, moderate, and strong were 30 (60%), 6 (12%), and 12 (24%), respectively (Table 3 Fig. 4).

Table3: Biofilm producing capacity of *K. pneumoniae* isolates.

Properties	Number (%)
Non-biofilm producer	2 (4)
Weak biofilm producer	30 (60)
Moderate biofilm producer	6 (12)
Strong biofilm producer	12(24)
Total	(50)

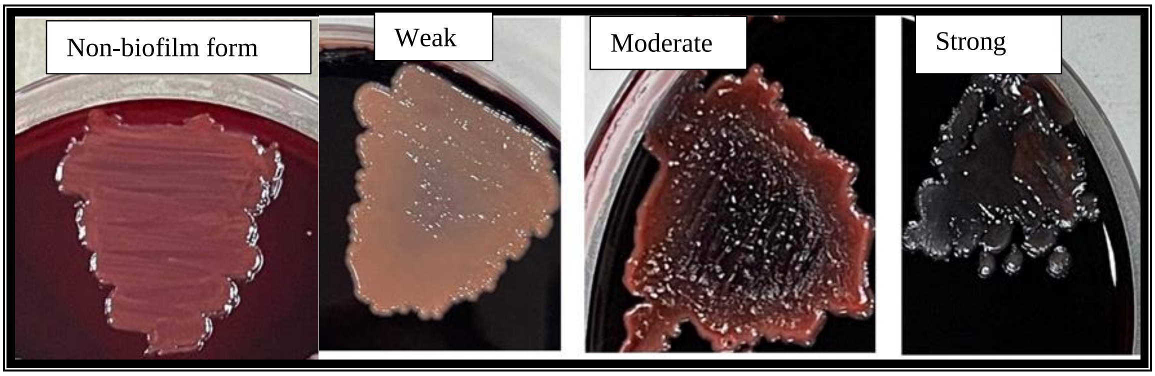


Figure 4: Biofilm forming ability of *K. pneumoniae* isolates.

In antibiotic susceptibility testing, all isolates were resistant to ampicillin and 64% to amoxicillin clavulanic acid. Furthermore, the rate of resistance of isolates to ciprofloxacin was 36.73%. While, the percentage of resistance to tetracycline, tobramycin and amikacin in clinical isolates were 56%, 34.7%, and 24%, respectively. Furthermore, the percentage of isolates resistant to azithromycin and nitrofurantoin was 44.9% and 48%, respectively; the percentages for imipenem and meropenem were the same, at 26 percent (Table 4). The antimicrobial resistance pattern showed that 32% of isolates were not MDR and the percentages of isolates that were MDR, XDR, and PDR were 42%, 18%, and 8%, respectively (Table 5).

Table 4: The antibiotic profile of *K. pneumoniae* isolates.

Antibiotics	Percentage of isolates		
	Resistance%	Intermediate%	Sensitive%
Ciprofloxacin (Cip)	36.74	6.12	57.14
Tetracycline (Te)	56	6	38
Azithromycin (Azm)	44.9	10.2	44.9
Amikacin (Ak)	24	18	58
Imipenim(Ipm)	26	-	74
Meropenem (Mem)	26	-	74
Nitrofurantoin (F)	48	10	42
Tobramycin (Tob)	34.7	10.2	55.1
Amoxicillin Clavulanic Acid (Amc)	64	-	36
Ampicillin (Am)	100	-	-

Table 5: The resistance profile of *K. pneumoniae*

Category	Number (%)
Not MDR	16 (32)
MDR	21(42)
XDR	9 (18)
PDR	4 (8)
Total	50

The cup plate agar diffusion method was used to expose all 50 bacterial isolates to 10 biocides that are regularly used in hospitals included in the study. The statistical study revealed that Virkon and Alcohol- Base had the highest and lowest mean of inhibition zone of biocide, respectively 51.54 mm and 12.91 mm. Big-Extra AF, AseptaNios AD, Konix Af, Povix, Povidin, H_2O_2-Sanosil Solo, Surfanios, and Anios- Spray 29 had mean values of 24.70 mm, 28.56 mm, 12.92 mm, 20.44 mm, 24.09 mm, 32.28 mm, 20.34 mm, and 16.18 mm, respectively (Fig 5 Fig 6).

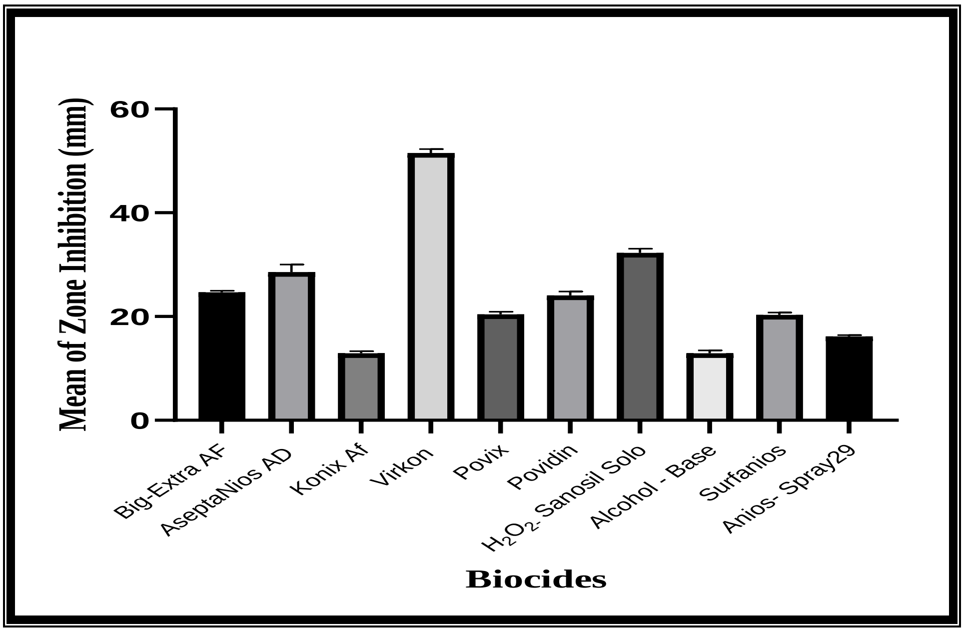
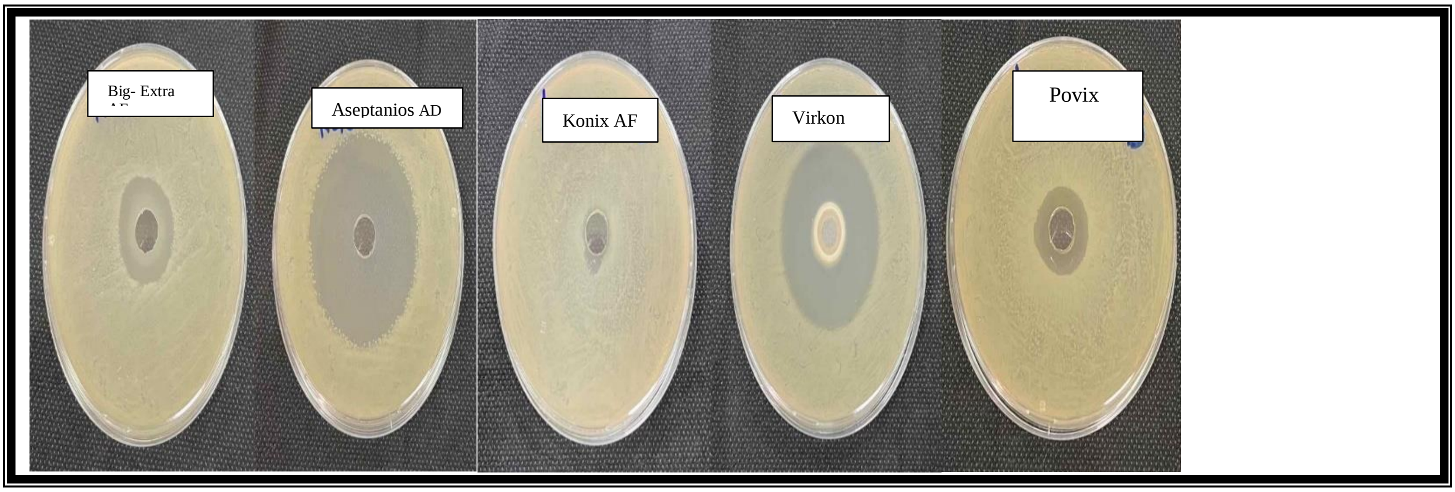


Figure5: Mean values of inhibition zones of biocides in millimeters (mm).



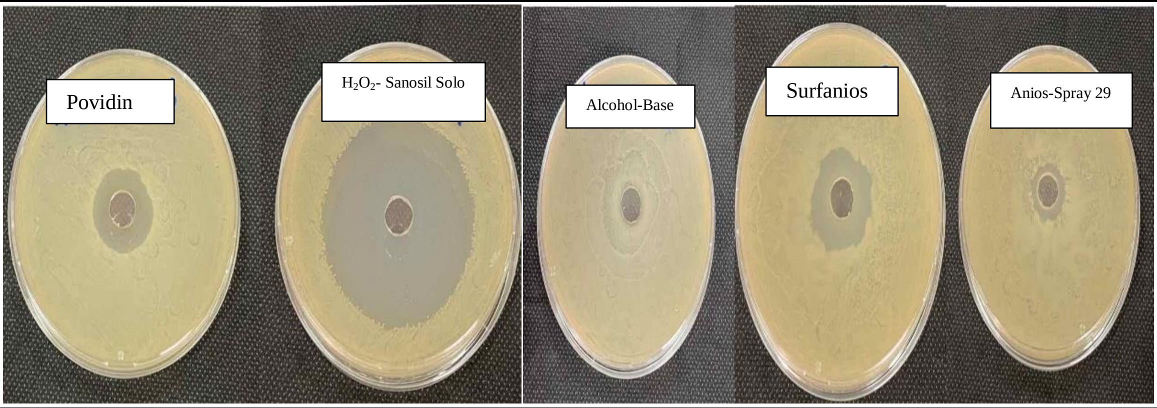


Figure 6: Inhibition zones of biocides using cup plate agar diffusion method.

4. DISCUSSION

The high rate of MDR *K. pneumoniae* isolates is a global problem because these pathogens are recognized as the main hazard to cause both hospital-acquired and community-acquired diseases. Hence, the factors which could contribute to the distribution of resistance among *K. pneumoniae* clinical isolates should be broadly explained and studied to be prohibited as much as possible. The repeated exposure of *K. pneumoniae* isolates to different biocides at sub-lethal doses in society and health care locales could be one of these reasons (Elekhnawy et al., 2021). In our study, (96%) of isolates were biofilm producers, and (4%) were non-biofilm forms. This findings is comparable to that of (Hassan et al., 2021) how found that (93.6%) of isolates were biofilm producers and only 6.4% were not, and (Kuinkel et al., 2021) reported that 94.8% of isolates were biofilm forms and 5.2% were confirmed as not biofilm producers.

Antibiotic susceptibility patterns revealed that all isolates were completely resistant to Ampicillin. Another study from Erbil (Tawgozy and Amin, 2018), Duhok (Naqid et al., 2020), Baghdad (Al-Hashimy and Al-Musawy, 2020), Russia (Khaertynov et al., 2018), and Iran (Kashefieh et al., 2021) found comparable results. While imipenem and meropenem, followed by amikacin and Ciprofloxacin, have demonstrated good activity and effectiveness against *K. pneumoniae*, this finding is consistent with a study conducted in Erbil by (Ali and Ismael, 2017). Furthermore, another review from China (Effah et al., 2020) observed a similar issue. The antimicrobial resistance pattern demonstrated that the majority of the isolated bacteria were MDR (42%) and extreme drug-resistant (XDR) (18%), whereas pan drug-resistant (PDR) accounted for 8% of the isolates. Another study from Egypt came to the same conclusion (El-Domany et al., 2021).

However, another study by (Nirwati et al., 2019) observed that drug resistance was higher in biofilm-producing *K. pneumoniae* than in non-biofilm-producing *K. pneumoniae*. The protective covering of the adhesive biomaterial, which leads to poor antibiotic penetration, adaptive responses to stress, and the formation of persister cells is thought to form a multilayered defense in biofilms, making eradication more difficult, especially when combined with the bacteria's resistance.

On the other hand, a study by (Alcántar-Curiel et al., 2018) suggested that while it appears that antibiotic resistance and the bacterial ability to form biofilm play a significant role in the worldwide spread of *K. pneumoniae*, the unambiguous relationship between these elements has not been fully recognized and expanded.

Biocides, with proper use, have a crucial role in preventing the colonization and infection of pathogenic microorganisms (Alizadeh et al., 2021). In clinical practice, a variety of disinfectants and antiseptics are routinely utilized and support current health care (Wand et al., 2017). Furthermore, microorganisms lower biocide susceptibility. Cross-resistance between antibiotics and biocides may occur via various and common mechanisms between them, including efflux pump systems, permeability alterations, and biofilm formation (Alizadeh et al., 2021).

The diameter of the microbiological inhibition zones was used to determine the efficacy of each antiseptic. When the inhibitory zone diameter was greater than eight millimeters, the microorganisms were termed sensitive (Montagna et al., 2019). Our data on disinfectants used in our hospitals confirmed that effective biocides were Virkon (51.54 mm) and H_2O_2- Sanosil Solo (32.28 mm). These biocides contain active ingredients which inhibit the growth of bacteria. Virkon, for instance, is made up of three salts: sodium salt, potassium hydrogen-sulphate, and dipotassium disulphate. Our findings are consistent with those of prior Indian study (Chakraborty et al., 2014). Virkon is broad-spectrum disinfectant that is efficient against a wide range of viruses, bacteria and fungi (Bartlett et al., 2021) (Geraldes et al., 2021) (Tedesco et al., 2019). Virkon is an oxidizing agent with an anionic surfactant and a low pH. The oxidizing agent in Virkon is potassium peroxymonosulphate and it antibacterial action is suggested to be that it acts on bacteria by oxidation (Osland et al., 2020).

Hydrogen peroxide disinfectants eliminate all pathogenic bacteria, biofilms, fungi, mold, viruses, amoeba, etc. without side effects. The two main components are hydrogen peroxide (H_2O_2) as the oxidizing agent and silver (Ag+) (Ganjoor and Mehrabi, 2017). The sterilization mechanism of hydrogen peroxide depends on the release of oxygen free radicals, which causes genetic material damage (Totaro et al., 2020), proteins and lipids cleavage and cell death (Lin et al., 2020) in bacterium cells.

Our finding showed that all bacterial isolates were less susceptible to alcohol-based compared to virkon and hydrogen peroxide, which wereboth at 12.91 mm. Alcohol influences the fluidity of cellular membranes by altering their lipid structure. As a result of the thick outer layer of lipopolysaccharide and the inner phospholipidic membrane of *K. pneumonaie*, it is predicted that alcohols will attack this bacterial class effectively and rapidly. The alcohols interestingly, stopped *K. pneumoniae* from growing. The polysaccharide capsule is the defining structure for this bacterial action, on which the alcohols have a precipitation impact, although the severity of this effect depends on the alcohol concentration. The capsule also protects against dehydration. (Man et al., 2017).

5. CONCLUSION

The isolates reduced susceptibility to disinfectants and antiseptics, according to our findings. On the other hand, Virkon was the most effective biocide against of *K. pneumoniae* isolates. To minimize microorganism dissemination and infection control in hospitals, we propose that the antimicrobial activity of biocides be continuously monitored.

Acknowledgments: We would like to express our gratitude to Salahaddin University – Erbil (SUE) for their help.

REFERENCE

AL-HASHIMY, A. B. & AL-MUSAWY, W. K. Molecular Study and Antibiotic susceptibility patterns of some Extended Spectrum Beta-Lactamase Genes (ESBL) of Klebsiella pneumpniae in Urinary Tract Infections. Journal of Physics: Conference Series, 2020. IOP Publishing, 012017.

ALCÁNTAR-CURIEL, M. D., LEDEZMA-ESCALANTE, C. A., JARILLO-QUIJADA, M. D., GAYOSSO-VÁZQUEZ, C., MORFÍN-OTERO, R., RODRÍGUEZ-NORIEGA, E., CEDILLO-RAMÍREZ, M. L., SANTOS-PRECIADO, J. I. & GIRÓN, J. A. 2018. Association of antibiotic resistance, cell adherence, and biofilm production with the endemicity of nosocomial Klebsiella pneumoniae. *BioMed research international,* 2018.

ALI, F. A. & ISMAEL, R. M. 2017. Dissemination of Klebsiella pneumonia and Klebsiella oxytoca Harboring bla TEM genes isolated from different clinical samples in Erbil City. *Diyala Journal of Medicine,* 12**,** 40-51.

ALIZADEH, S. A., JAVADI, A., NIKKHAHI, F., ROSTAMANI, M., BAKHT, M., AZIMI, A., KELISHOMI, F. Z. & KAZEMZADE, R. 2021. Biocide's susceptibility and frequency of biocide resistance genes and the effect of exposure to sub-inhibitory concentrations of sodium hypochlorite on antibiotic susceptibility of Stenotrophomonas maltophilia. *bioRxiv*.

BARTLETT, J. C., RADCLIFFE, R. J., CONVEY, P., HUGHES, K. A. & HAYWARD, S. A. 2021. The effectiveness of Virkon® S disinfectant against an invasive insect and implications for Antarctic biosecurity practices. *Antarctic Science,* 33**,** 1-9.

CHAKRABORTY, B., PAL, N., MAITI, P. K., PATRA, S. K. & RAY, R. 2014. Action of newer disinfectants on multidrug resistant bacteria. *Journal of Evolution of Medical and Dental Sciences,* 3**,** 2797-2813.

EFFAH, C. Y., SUN, T., LIU, S. & WU, Y. 2020. Klebsiella pneumoniae: an increasing threat to public health. *Annals of clinical microbiology and antimicrobials,* 19**,** 1-9.

EL-DOMANY, R. A., AWADALLA, O. A., SHABANA, S. A., EL-DARDIR, M. A. & EMARA, M. 2021.

Analysis of the correlation between antibiotic resistance patterns and virulence determinants in pathogenic klebsiella pneumoniae isolates from Egypt. *Microbial Drug Resistance,* 27**,** 727-739.

ELEKHNAWY, E. A., SONBOL, F. I., ELBANNA, T. E. & ABDELAZIZ, A. A. 2021. Evaluation of the impact of adaptation of Klebsiella pneumoniae clinical isolates to benzalkonium chloride on biofilm formation. *Egyptian Journal of Medical Human Genetics,* 22**,** 1-6.

FARHADI, M., AHANJAN, M., GOLI, H. R., HAGHSHENAS, M. R. & GHOLAMI, M. 2021. High Frequency of Multidrug-resistant (Mdr) Klebsiella Pneumoniae Harboring Several β-lactamase and Integron Genes Collected From Several Hospitals in the North of Iran.

GANJOOR, M. & MEHRABI, M. 2017. Inhibitory Effect of Hydrogen Peroxide (H2O2) and Ionic Silver (Sanosil-25Â®) on Growth of a Pathogenic Bacterium (Vibrio harveyi) Isolated From Shrimp (Litopenaeus vannamei). *Oceanography & Fisheries Open Access Journal,* 2**,** 56-59.

GERALDES, C., VERDIAL, C., CUNHA, E., ALMEIDA, V., TAVARES, L., OLIVEIRA, M. & GIL, S. 2021. Evaluation of a Biocide Used in the Biological Isolation and Containment Unit of a Veterinary Teaching Hospital. *Antibiotics,* 10**,** 639.

HASSAN, N. S., AL–MARJANI, M. F. & HUSSAIN, N. H. 2021. Detection of Antiseptic Resistant Genes in Colistin-Resistant Pseudomonas aeruginosa and MDR Klebsiella penumoniae. *Indian Journal of Forensic Medicine & Toxicology,* 15.

HOMBACH, M., BLOEMBERG, G. V. & BÖTTGER, E. C. 2012. Effects of clinical breakpoint changes in CLSI guidelines 2010/2011 and EUCAST guidelines 2011 on antibiotic susceptibility test reporting of Gram-negative bacilli. *Journal of antimicrobial chemotherapy,* 67**,** 622-632.

IDOWU, E. T., JA'AFARU, M. I., AJAYI, A. O. & AKINTERINWA, A. 2017. Preliminary Investigation on the Resistance of Some Environmental Bacteria in Yola Metropolis, Adamawa State, Nigeria, to Biocides and Antibiotics. *Avicenna Journal of Clinical Microbiology and Infection,* 4**,** 61825-61825.

KASHEFIEH, M., HOSAINZADEGAN, H., BAGHBANIJAVID, S. & GHOTASLOU, R. 2021. The Molecular Epidemiology of Resistance to Antibiotics among Klebsiella pneumoniae Isolates in Azerbaijan, Iran. *Journal of Tropical Medicine,* 2021.

KHAERTYNOV, K. S., ANOKHIN, V. A., RIZVANOV, A. A., DAVIDYUK, Y. N., SEMYENOVA, D. R., LUBIN, S. A. & SKVORTSOVA, N. N. 2018. Virulence factors and antibiotic resistance of Klebsiella pneumoniae strains isolated from neonates with sepsis. *Frontiers in medicine***,** 225.

KUINKEL, S., ACHARYA, J., DHUNGEL, B., ADHIKARI, S., ADHIKARI, N., SHRESTHA, U. T., BANJARA, M. R., RIJAL, K. R. & GHIMIRE, P. 2021. Biofilm Formation and Phenotypic Detection of ESBL, MBL, KPC and AmpC Enzymes and Their Coexistence in Klebsiella spp. Isolated at the National Reference Laboratory, Kathmandu, Nepal. *Microbiology Research,* 12**,** 683-697.

LENCHENKO, E., BLUMENKRANTS, D., SACHIVKINA, N., SHADROVA, N. & IBRAGIMOVA, A. 2020. Morphological and adhesive properties of Klebsiella pneumoniae biofilms. *Veterinary world,* 13**,** 197.

LIN, Q., LIM, J. Y., XUE, K., YEW, P. Y. M., OWH, C., CHEE, P. L. & LOH, X. J. 2020. Sanitizing agents for virus inactivation and disinfection. *View,* 1**,** e16.

MAN, A., GÂZ, A. Ş., MARE, A. D. & BERŢA, L. 2017. Effects of low-molecular weight alcohols on bacterial viability. *Revista Romana de Medicina de Laborator,* 25**,** 335-343.

MONTAGNA, M. T., TRIGGIANO, F., BARBUTI, G., BARTOLOMEO, N., DE GIGLIO, O., DIELLA, G., LOPUZZO, M., RUTIGLIANO, S., SERIO, G. & CAGGIANO, G. 2019. Study on the in vitro activity of five disinfectants against nosocomial bacteria. *International journal of environmental research and public health,* 16**,** 1895.

NAQID, I. A., HUSSEIN, N. R., BALATAY, A. A. & ABDULLAH, K. 2020. The Antimicrobial Resistance Pattern of Klebsiella pneumonia Isolated from the Clinical Specimens in Duhok City in Kurdistan Region of Iraq. *J Kermanshah Univ Med Sci,* 24.

NIRWATI, H., SINANJUNG, K., FAHRUNISSA, F., WIJAYA, F., NAPITUPULU, S., HATI, V. P., HAKIM, M. S., MELIALA, A., AMAN, A. T. & NURYASTUTI, T. Biofilm formation and antibiotic resistance of Klebsiella pneumoniae isolated from clinical samples in a tertiary care hospital, Klaten, Indonesia. BMC proceedings, 2019. BioMed Central, 1-8.

OSLAND, A. M., VESTBY, L. K. & NESSE, L. L. 2020. The effect of disinfectants on quinolone resistant E. coli (QREC) in biofilm. *Microorganisms,* 8**,** 1831.

SHRESTHA, L. B., BHATTARAI, N. R. & KHANAL, B. 2018. Comparative evaluation of methods for the detection of biofilm formation in coagulase-negative staphylococci and correlation with antibiogram. *Infection and drug resistance,* 11**,** 607.

TAWGOZY, F. H. A. & AMIN, B. K. 2018. Molecular analysis of Klebsiella Pneumoniae isolated from UTI patients in Erbil city. *ZANCO Journal of Pure and Applied Sciences,* 30**,** 1-9.

TEDESCO, P., FIORAVANTI, M. L. & GALUPPI, R. 2019. In vitro activity of chemicals and commercial products against Saprolegnia parasitica and Saprolegnia delica strains. *Journal of fish diseases,* 42**,** 237-248.

TOTARO, M., CASINI, B., PROFETI, S., TUVO, B., PRIVITERA, G. & BAGGIANI, A. 2020. Role of hydrogen peroxide vapor (HPV) for the disinfection of hospital surfaces contaminated by multiresistant bacteria. *Pathogens,* 9**,** 408.

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. *Antimicrobial agents and chemotherapy,* 61**,** e01162-16.